Year : 2015

Keeping the balance ? Management of oxidative stress, body mass and reproduction under energetic constraints by dispersing and philopatric collared flycatchers

Récapet Charlotte

UNIL | Université de Lausanne
Faculté de biologie et de médecine

Keeping the balance ?

Management of oxidative stress, body mass and reproduction under energetic constraints by dispersing and philopatric collared flycatchers

Thèse de doctorat ès sciences de la vie (PhD)

Présentée en co-tutelle à la Faculté de biologie et de médecine
de l'Université de Lausanne (Suisse) et à l'Université Claude Bernard, Lyon 1, (France)

par

Charlotte Récapet
Titulaire d'un Master en écologie de l'Université Pierre et Marie Curie, Paris 6, France

Jury

Professeur Emmanuel Desouhant, Président du jury
Docteur Pierre Bize, Directeur de thèse
Docteur Blandine Doligez, Directrice de thèse
Professeur Alexandre Roulin, Co-directeur de thèse

Professeur Philippe Christe, expert
Docteur Jean Clobert, expert
Professeur Bruno Faivre, expert

Villeurbanne 2015

UNIL | Université de Lausanne
Faculté de biologie et de médecine

Ecole Doctorale

Doctorat ès sciences de la vie en cotutelle avec
Université Lyon 1 - Claude Bernard

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Président · e	Monsieur Prof. Emmanuel **Desouhant**
Directeur · rice	Monsieur Prof. Pierre **Bize**
	Madame Prof. Blandine **Doligez**
Co-directeur · rice	Monsieur Prof. Alexandre **Roulin**
Experts · es	Monsieur Prof. Bruno **Faivre**
	Monsieur Prof. Philippe **Christe**
	Monsieur Prof. Jean **Clobert**

le Conseil de Faculté autorise l'impression de la thèse de

Madame Charlotte Récapet

Master Université Pierre et Marie Curie - Paris VI, France

intitulée

KEEPING THE BALANCE ?
MANAGEMENT OF OXIDATIVE STRESS, BODY MASS AND REPRODUCTION UNDER ENERGETIC CONSTRAINTS BY DISPERSING AND PHILOPATRIC COLLARED FLYCATCHERS

Lausanne, le 3 décembre 2015

pour le Doyen
de la Faculté de biologie et de médecine

Prof. Niko GELDNER
Directeur de l'Ecole Doctorale

Université Lyon 1 – Claude Bernard
N° d'ordre: 272-2015

Faculté de Biologie et de Médecine

KEEPING THE BALANCE?

MANAGEMENT OF OXIDATIVE STRESS, BODY MASS AND REPRODUCTION UNDER ENERGETIC CONSTRAINTS BY DISPERSING AND PHILOPATRIC COLLARED FLYCATCHERS

Thèse de doctorat (PhD)
de l'Université de Lyon et de l'Université de Lausanne

Présentée devant l'Université Lyon 1 - Claude Bernard

pour l'obtention

du DIPLÔME DE DOCTORAT
(arrêté du 7 Août 2006)

par

Charlotte RÉCAPET

Biologiste Master de l'Université Paris 6 – Pierre et Marie Curie

Co-directrice de thèse: Blandine DOLIGEZ
Co-directeur de thèse: Pierre BIZE
Co-directeur de thèse: Alexandre ROULIN

Jury:	Emmanuel DESOUHANT	Président du jury
	Jean CLOBERT	Rapporteur
	Bruno FAIVRE	Rapporteur
	Philippe CHRISTE	Examinateur
	Blandine DOLIGEZ	Co-directrice de thèse
	Pierre BIZE	Co-directeur de thèse
	Alexandre ROULIN	Co- directeur de thèse
	Anna QVARNSTRÖM	Rapporteur hors jury

Villeurbanne 2015

UNIVERSITE CLAUDE BERNARD - LYON 1

Président de l'Université	**M. François-Noël GILLY**
Vice-président du Conseil d'Administration	M. le Professeur Hamda BEN HADID
Vice-président du Conseil des Etudes et de la Vie Universitaire	M. le Professeur Philippe LALLE
Vice-président du Conseil Scientifique	M. le Professeur Germain GILLET
Directeur Général des Services	M. Alain HELLEU

COMPOSANTES SANTE

Faculté de Médecine Lyon Est – Claude Bernard	Directeur : M. le Professeur J. ETIENNE
Faculté de Médecine et de Maïeutique Lyon Sud – Charles Mérieux	Directeur : Mme la Professeure C. BURILLON
Faculté d'Odontologie	Directeur : M. le Professeur D. BOURGEOIS
Institut des Sciences Pharmaceutiques et Biologiques	Directeur : Mme la Professeure C. VINCIGUERRA
Institut des Sciences et Techniques de la Réadaptation	Directeur : M. le Professeur Y. MATILLON
Département de formation et Centre de Recherche en Biologie Humaine	Directeur : Mme. la Professeure A-M. SCHOTT

COMPOSANTES ET DEPARTEMENTS DE SCIENCES ET TECHNOLOGIE

Faculté des Sciences et Technologies	Directeur : M. F. DE MARCHI
Département Biologie	Directeur : M. le Professeur F. FLEURY
Département Chimie Biochimie	Directeur : Mme Caroline FELIX
Département GEP	Directeur : M. Hassan HAMMOURI
Département Informatique	Directeur : M. le Professeur S. AKKOUCHE
Département Mathématiques	Directeur : M. le Professeur Georges TOMANOV
Département Mécanique	Directeur : M. le Professeur H. BEN HADID
Département Physique	Directeur : M. Jean-Claude PLENET
UFR Sciences et Techniques des Activités Physiques et Sportives	Directeur : M. Y.VANPOULLE
Observatoire des Sciences de l'Univers de Lyon	Directeur : M. B. GUIDERDONI
Polytech Lyon	Directeur : M. P. FOURNIER
Ecole Supérieure de Chimie Physique Electronique	Directeur : M. G. PIGNAULT
Institut Universitaire de Technologie de Lyon 1	Directeur : M. le Professeur C. VITON
Ecole Supérieure du Professorat et de l'Education	Directeur : M. le Professeur A. MOUGNIOTTE
Institut de Science Financière et d'Assurances	Directeur : M. N. LEBOISNE

SUMMARY

Dispersal, i.e. individuals' movement between breeding sites, is a key process for metapopulation dynamics and gene flow. Differences in phenotypic and life-history traits between dispersing and philopatric (i.e. non-dispersing) individuals, also called dispersal syndromes, have been put forward as an important determinant of the success of dispersal strategies. However, the mechanisms underlying such syndromes remain poorly known in most species. In particular, the relative role of environmental (external) and physiological (internal) constraints in shaping differences between dispersing and philopatric individuals deserves more attention. This project aimed at clarifying the impact of environmental variation and oxidative constraints, linked to the production of reactive oxygen species during mitochondrial respiration, on phenotypes associated to dispersal in a migratory passerine bird, the collared flycatcher *Ficedula albicollis*. To disentangle the direct effect of environment from fixed phenotypic differences, energetic constraints were experimentally (i) increased through a wing load manipulation or (ii) relieved through food supplementation. The different experiments were performed during breeding to control for habitat selection. The oxidative balance of breeding collared flycatchers, including both the levels of pro-oxidants and antioxidants and their correlation, was influenced by complex interactions of intrinsic (dispersal status) and extrinsic (breeding density, year, experimental treatments) factors. Interestingly, antioxidant capacity was influenced both by permanent individual differences and by food availability, whereas measures of pro-oxidants were highly variables within individuals. Environmental variation and energetic constraints also modulated the differences in reproductive success and parental behaviour between dispersing and philopatric individuals. Our results confirm that dispersing and philopatric birds differ in their management of the oxidative balance when it is competing with reproductive investment. These differences could have longer-term consequences on fitness and balance out differences between dispersing and philopatric individuals in reproductive success in low quality habitats. This thesis highlights that reaction norms rather than fixed differences often shape traits associated to dispersal, and improves our understanding of dispersal syndromes.

RÉSUMÉ

La dispersion, c'est-à-dire le déplacement d'un individu entre deux sites de reproduction, est un processus clé pour la dynamique des métapopulations et les flux de gènes. Les différences de phénotype et de traits d'histoire de vie entre individus dispersants et philopatriques (non-dispersants), ou syndromes de dispersion, peuvent avoir une importance majeure pour le succès des stratégies de dispersion. Cependant, les mécanismes qui sous-tendent ces syndromes restent mal connus chez la plupart des espèces. En particulier, les rôles respectifs des contraintes environnementales (externes) et physiologiques (internes) dans les différences entre individus dispersants et philopatriques mériteraient d'être mieux décrits. Ce projet vise à clarifier l'impact des variations environnementales et des contraintes oxydatives (liées à la production d'espèces réactives de l'oxygène durant la respiration mitochondriale) sur les phénotypes associés à la dispersion chez un passereau migrateur, le gobemouche à collier *Ficedula albicollis*. Afin de dissocier l'effet direct de l'environnement de différences fixées de phénotype, les contraintes énergétiques ont été expérimentalement (i) augmentées par une manipulation de la surface alaire ou (ii) diminuées par une supplémentation en nourriture. Ces expériences ont été menées durant la reproduction pour contrôler l'effet de la sélection d'habitat. L'équilibre oxydo-réducteur des gobemouches à collier en reproduction, incluant à la fois les niveaux de pro- et antioxydants ainsi que leurs corrélations, est influencé par des interactions complexes entre facteurs intrinsèques (statut de dispersion) et extrinsèques (densité de couples reproducteurs, année, traitement expérimental). La capacité antioxydante est influencée à la fois par des différences permanentes entre individus et par la disponibilité en nourriture, alors que les pro-oxydants présentent de grandes variations intra-individu. Les variations environnementales et les contraintes énergétiques modulent aussi les différences de succès reproducteur et de comportement parental liées au statut de dispersion. Nos résultats confirment que les oiseaux dispersants et philopatriques diffèrent dans leur gestion de l'équilibre oxydo-réducteur lorsqu'il est en compétition avec l'investissement reproducteur. Ces différences pourraient avoir des conséquences à long terme sur la valeur sélective et compenser les différences de succès reproducteur entre individus dispersants et philopatriques dans les habitats de faible qualité. Ce travail souligne que les traits associés à la dispersion sont souvent déterminés par des normes de réaction à l'environnement et non des différences fixées entre individus, et améliore notre compréhension des syndromes de dispersion.

RÉSUMÉ DÉTAILLÉ EN FRANÇAIS

La dispersion, c'est-à-dire le déplacement d'un individu depuis son site de naissance ou de reproduction vers un nouveau site de reproduction, est un processus clé pour la dynamique des métapopulations et les flux de gènes. Les différences de phénotype et de traits d'histoire de vie entre individus dispersants et philopatriques (non-dispersants), ou syndromes de dispersion, peuvent avoir une importance majeure pour le succès des stratégies de dispersion. En particulier, des différences dans la réponse des individus dispersants et philopatriques aux variations environnementales pourraient permettre la coexistence de plusieurs stratégies au sein d'une population. D'un autre côté, les différences observées pourraient principalement venir de différences de qualité individuelle. Dans cette thèse, nous avons étudié la réponse d'individus dispersants et philopatriques aux contraintes énergétiques, en termes d'équilibre oxydo-réducteur, de succès reproducteur et de comportement parental. Nous nous sommes notamment demandés si la gestion de l'équilibre oxydo-réducteur pourrait compenser leur différence de succès reproducteur face à une contrainte énergétique (stratégies alternatives) ou si la reproduction et l'équilibre oxydo-réducteur étaient modifiés dans le même sens par les contraintes énergétiques (différences de qualité individuelle). Afin de dissocier l'effet direct de l'environnement de différences fixées de phénotype, les contraintes énergétiques ont été expérimentalement (i) augmentées par une réduction de la surface alaire ou (ii) diminuées par une supplémentation en nourriture. Ces expériences ont été menées durant la reproduction pour contrôler l'effet de la sélection d'habitat et durant trois années afin de pouvoir étudier les variations longitudinales.

Dans une première étude, nous avons exploré les corrélations entre le taux métabolique au champ (en conditions d'activité normale), un marqueur du statut pro-oxydant (métabolites réactifs de l'oxygène ou ROMs) et un marqueur de capacité antioxydante (OXY), afin de déterminer l'impact relatif des différences entre individus et de variations environnementales sur ces marqueurs et leurs relations. Les défenses antioxydantes étaient corrélées positivement aux ROMs et cette relation étaient plus forte lorsque les contraintes énergétiques étaient élevées : lorsque les coûts de vol étaient augmentés ou lorsque la saison de reproduction était particulièrement mauvaise, en 2014 par

rapport à 2012 et 2013. La supplémentation en nourriture en 2014 n'a cependant pas diminué la corrélation entre marqueurs, peut-être parce qu'elle était insuffisante pour contrebalancer les mauvaises conditions environnementales (basses températures et faible abondance de nourriture). Malgré de faibles tailles d'échantillon, les résultats suggèrent aussi des corrélations positives entre l'activité métaboliques et les deux marqueurs de statut oxydo-réducteur chez les femelles où la surface de vol a été réduite. Comme les défenses antioxydantes, mais pas les ROMS, étaient répétables entre années, les corrélations plus fortes sous contraintes énergétiques impliquent que l'activité métabolique pourrait être contrainte par la capacité d'un individu à acquérir des antioxydants. Cette étude confirme l'importance des variations individuelles et environnementales dans la gestion de l'équilibre oxydo-réducteur.

Nous avons ensuite étudié les différences de réponses de femelles dispersantes ou philopatriques à une augmentation de l'activité métabolique stimulée expérimentalement par une réduction de la surface alaire (Chapitre IV). Les ROMs étaient plus élevés chez les femelles philopatriques handicapées que chez les contrôles, alors qu'aucun effet du handicap n'a été observé chez les dispersantes. Comme les femelles philopatriques avaient aussi une capacité antioxydante plus élevée indépendamment du handicap, une hypothèse est que les philopatriques pourrait supporter des niveaux de pro-oxydants plus importants. Cependant, comme les femelles philopatriques n'avaient pas un succès reproducteur plus élevé que les dispersantes en condition de handicap, il est aussi possible que les philopatriques soient moins capables de gérer une augmentation des contraintes énergétiques et aient maintenu leur succès reproducteur au prix d'un stress oxydatif.

Les femelles dispersantes et philopatriques géraient aussi différemment leur équilibre oxydo-réducteur en fonction des variations naturelles de la qualité d'habitat, qui est positivement corrélée à la densité de couples reproducteurs dans cette population. Les ROMs augmentaient dans les plus faibles densités d'habitat chez les femelles philopatriques, mais restaient basses qu'elle que soit la densité chez les dispersantes. Ces différences de marqueurs étaient accompagnées d'un succès reproducteur plus faible des dispersantes, particulièrement à faible densité. Par conséquent, la gestion différente des processus oxydo-

réducteurs et de l'investissement reproducteur ne permettait pas aux individus dispersants de parvenir au même succès reproducteur que les individus philopatriques. Cependant, comme les individus philopatriques étaient exposés à plus de pro-oxydants dans les habitats de faible densité, ils pourraient souffrir de plus faibles perspectives de survie et de reproduction qui compenseraient leur succès reproducteur plus élevé l'année de l'étude.

L'effet direct de la qualité d'habitat sur les différences entre dispersants et philopatriques a été confirmée par une expérience de supplémentation en nourriture (Chapitre V). La supplémentation a en effet supprimé l'interaction entre dispersion et densité sur la masse corporelle et le succès reproducteur des mâles observée dans les nids contrôle. Cette interaction n'est donc pas seulement dues à des décisions de dispersions différentes d'individus de faible ou bonne qualité face à la qualité d'habitat, mais sont directement influencées par la qualité d'habitat post-dispersion. La supplémentation n'a eu cependant aucun effet sur la différence de réponse des ROMs à la densité entre dispersants et philopatriques.

La supplémentation en nourriture a aussi modifié les différences de comportement de défense du nid entre individus dispersants et philopatriques. Les dispersants défendaient moins intensivement leur nid que les philopatriques dans les nids contrôles, mais augmentaient leur défense pour égaler celle des philopatriques dans les nids supplémentés. La supplémentation n'a cependant pas modifié l'interaction entre dispersion et densité sur la défense du nid, la défense augmentant avec la densité chez les dispersants uniquement.

Les résultats des chapitres IV et V sont en partie contradictoires. En 2012 et 2013 (Chapitre IV), les individus philopatriques subissent plus d'attaques pro-oxydantes aux faibles densités mais maintiennent leur succès reproducteur, alors qu'en 2014 ce sont les dispersants qui subissent plus d'attaques pro-oxydantes et une perte de masse aux faibles densités. La masse des adultes était aussi sensible aux variations de densité en 2014 mais pas en 2012 et 2013. Ces différences pourraient en partie trouver leur origine dans les variations très importante de conditions environnementales entre années. Du fait des conditions météorologiques, les nids contrôles ont eu un succès reproducteur beaucoup plus faible que les années précédentes. Des études suivies sur plusieurs années seraient nécessaires pour

s'assurer que les variations temporelles de l'environnement peuvent modifier si radicalement l'effet de la densité selon le statut de dispersion.

Enfin, la longueur des télomères, des séquences répétées qui protègent l'extrémité des chromosomes, répondait aussi différemment à la densité selon le statut de dispersion (Chapitre VI) : les individus dispersants avaient des télomères plus longs que les philopatriques dans les habitats de faible densité mais pas dans les habitats denses. Ceci suggère soit que les individus dispersants se sont installé dans les habitats différents selon la longueur de leurs télomères, soit qu'ils souffraient de dommages oxydatifs plus importants dans les habitats de faible densité. Cependant, nous avons trouvé une corrélation négative entre la longueur des télomères et le taux de retour. D'autre part, un autre prédicteur du taux de retour, la proportion d'hémoglobine glyquée, ne différait pas en fonction du statut de dispersion ou de la densité. Des analyses de capture-marquage-recapture seraient nécessaires pour confirmer le lien négatif contre-intuitif entre longueur des télomères et survie dans cette population.

Pour résumer, l'équilibre oxydo-réducteur des gobemouches à collier en reproduction est influencé par des interactions complexes entre facteurs intrinsèques (statut de dispersion) et extrinsèques (densité de couples reproducteurs, année, traitement expérimental). La capacité antioxydante est influencée à la fois par des différences permanentes entre individus et par la disponibilité en nourriture, alors que les pro-oxydants présentent de grandes variations intra-individu. Les variations environnementales et les contraintes énergétiques modulent aussi les différences de succès reproducteur et de comportement parental liées au statut de dispersion. Nos résultats confirment que les oiseaux dispersants et philopatriques diffèrent dans leur gestion de l'équilibre oxydo-réducteur lorsqu'il est en compétition avec l'investissement reproducteur. Ces différences pourraient avoir des conséquences à long terme sur la valeur sélective et compenser les différences de succès reproducteur entre individus dispersants et philopatriques dans les habitats de faible qualité. Ce travail souligne que les traits associés à la dispersion sont souvent déterminés par des normes de réaction à l'environnement et non des différences fixées entre individus, et améliore notre compréhension des syndromes de dispersion.

It would be instructive to know not only by what physiological mechanisms a just apportionment is made between the nutrient devoted to gonads and that devoted to the rest of the parental organism, but also what circumstances in the life history and environment would render profitable the diversion of a greater or lesser share of the available resources towards reproduction.

Ronald A. Fisher

REMERCIEMENTS

Un projet de recherche aura rarement aussi bien illustré son propre sujet : dans cette entreprise, j'aurai voyagé aux quatre coins de l'Europe (avec un barycentre tout de même décalé vers le nord). Si l'on peut discuter les coûts de cette « dispersion », elle aura, c'est certain, eu au moins un bénéfice, en étant l'occasion de nombreuses rencontres. Beaucoup resteront des amis et des collaborateurs.

Pour commencer par la dernière rencontre de cette thèse, je tiens à remercier les membres de mon jury, Jean Clobert, Philippe Christe, Emmanuel Desouhant et Bruno Faivre, d'avoir accepté d'évaluer mon travail. I would also like to thank Anna Qvarnström for agreeing to review this work.

Je remercie également mes directeurs de thèse, Pierre Bize, Blandine Doligez et Alexandre Roulin. Merci à Blandine pour avoir initié ce projet avec moi et pour m'avoir fait confiance dans sa conduite. Sa rigueur et son sens du détail auront été précieux dans la préparation des protocoles et l'interprétation des données. Merci d'avoir mis tant de couleurs dans mes manuscrits et de m'avoir remis les pieds sur terre lorsque je faisais de l'équilibrisme sur des spéculations ! Je remercie conjointement Pierre et Alexandre pour m'avoir ouvert les portes du DEE et m'avoir fait découvrir les vins suisses. Merci à Pierre pour son investissement dans ce projet et son soutien sans faille, ses commentaires éclairés, sa curiosité qui aura fait prendre des directions parfois inattendues mais passionnantes à ce travail, et pour les raclettes en toute saison ! Un grand merci enfin pour m'avoir donné l'opportunité de réaliser cette dernière année de thèse en Ecosse. Merci à Alexandre d'avoir donné l'impulsion nécessaire pour lancer ce projet sur une nouvelle voie plus fructueuse.

Un tel projet ne se fait pas sans collaborations et je remercie François de m'avoir accueilli au DEPE. Un grand merci à Stéphane, Alexandre et Isabelle pour m'avoir fait découvrir le monde merveilleux de l'eau doublement marquée et pour leur contribution précieuse à la préparation des protocoles et l'analyse des échantillons. Merci également à Mathilde pour son aide au laboratoire. Plus largement, merci aux chercheurs, doctorants et stagiaires du DEPE, en particulier Anne-Mathilde, Antoine, Floriane, Laure et Sophie, pour leur accueil à chacun de mes passages. Merci également à Adélaïde, Benoît et Françoise qui ont fourni une partie non négligeable du travail de laboratoire à Lausanne et à Hannes et Kathryn pour leur aide dans la mise au point et la réalisation des qPCR. Sans les citer tous, un grand merci à tous les stagiaires qui ont contribué à l'analyse des données comportementales.

Les saisons à Gotland auront rythmé ma thèse, toutes différentes mais toutes intenses. Un énorme merci à tous les assistants de terrain, stagiaires ou volontaires, pour leur investissement dans la récolte des données mais aussi les moments partagés durant ces trois mois de vie en communauté. Une mention particulière à Audrey et Joëlle pour leur bonne humeur à toute épreuve. Merci à Arthur, l'ornithopathe, (et à Camille) pour nous avoir invité à la « chasse » à l 'élan. Thanks to Kevin, Juho, Barbara and the « Swedish teams» for the barbecues and aquavit, the *midsommar* nights and an unforgettable « frying party». Thanks to Szymek for the orchids hotspots watch.

Le terrain, et à vrai dire ces quatre années, n'auraient pas été tout à fait les mêmes sans des co-thésards de choc, qui auront été bien plus que des collègues. Je remercie Laure et Marion pour leur énergie et leur bon sens sur le terrain, pour les sorties sur Gotland et à New-York, et les discussions animées qui lâchent la pression quand les soupapes atteignent le niveau de sécurité. Merci à Anaïs pour sa gentillesse et pour son accueil à Montpellier. Je remercie Grégory pour son aide sur le terrain, pour les beignets de sureau, et pour les soirées jeux à

Lyon comme à Chaumont. Merci enfin à la jeunesse du LBBE pour les moments passés ensemble lors de mes passages irréguliers.

Merci à la fine équipe aberdonienne du Dr Bize, dont lui-même, Barbara, Camille, Luiza et Quentin pour les soirées au pub et les barbecues avec vue sur les dauphins. Thank you especially to Barbara and Mateus for the nice weekends and for hosting us last spring.

J'envoie un merci à tous les amis d'ailleurs, pour tous les moments partagés en dépit de la distance. Merci aux descendants de EBE 2011 pour les discussions plus ou moins constructives sur la recherche en général et l'écologie en particulier, et en particulier à Pierre sans qui je n'aurai jamais commencé ce projet. Je remercie aussi les « naturalistes parisiens » qui me rappellent qu'il n'y a pas que les oiseaux dans la vie, il y aussi les p'tites fleurs (et les libellules, et les papillons, et un raton-laveur).

Bien sûr, merci à Bertrand d'avoir été à mes côtés, en pensée sinon toujours en personne, durant ces quatre années, pour essuyer les coups durs et fêter les succès. Cette thèse aura véritablement été un travail d'équipe, tant sur le terrain qu'à la maison. Merci pour ton amour et merci pour le thé !

Tous le reste de ma famille mérite aussi un grand merci pour l'énergie et la confiance qui me viennent d'eux, parce qu'ils auront toujours cru en moi sans mettre leurs espoirs en moi, ces espoirs souvent si lourds à porter. Sur un plan plus terre-à-terre, un merci particulier à tous les membres de ma famille qui ont un jour ou l'autre pris sur eux les tâches du quotidien pour que je puisse me consacrer à mon travail, et en particulier Anne, Isabelle, Jean-Paul et Geneviève.

Merci enfin à ceux qui sont partis durant ces quatre année, et qui ne seront plus là pour me rappeler, toujours avec une grand subtilité, que la reproduction reste une composante essentielle de la valeur sélective.

Illustrations par Bertrand Couillens (pp. 39, 161), Alice Goossens (p. 67), Olivia Mercier (p. 135) et Charlotte Récapet (pp. 1, 31, 95, 185).

CONTENTS

Chapter I – General introduction

1 DISPERSAL: LIFE-HISTORY CONSTRAINT OR LIFE-HISTORY STRATEGY?

1.1 Defining dispersal and the processes involved

Dispersal can be broadly defined as "any movement of individuals or propagules with potential consequences for gene flow across space" with "no restriction on the ploidy of the dispersing stage" (Ronce 2007). This wide definition includes such diverse processes as pollen dispersal, seed, spore, larvae or young dispersal, adults' movements between, and sometimes within (e.g. oviposition-site selection), breeding events, and the movements of parts of an individual, such as some plants' stolons. This definition is interesting in that it adopts an evolutionary framework by focusing on spatial gene flow and excludes movements without any direct impact on population genetics, such as foraging trips or seasonal and diurnal migrations [1]. Restricting the definition of dispersal to gene flow does however not fully convey the important consequences of dispersal on local population dynamics, for example by a change in competition pressures (Hanski 2001). Moreover, at the individual level, such distinction between dispersal and other type of movement might not always be relevant: the processes involved in information acquisition and movement are often the same for different types of movements and can occur simultaneously (Burgess *et al.* 2015).

In vertebrates with internal fecundation, the main distinction is usually made between natal dispersal and breeding dispersal. Natal dispersal takes place before the first breeding attempt, whereas breeding dispersal relates to movements between successive breeding attempts (Greenwood & Harvey 1982). Extra-pair mating and brood parasitism might provide other mechanisms for dispersal within a breeding attempt, although their relevance to spatial gene flow has been less studied. For example, intra-specific brood parasitism might actually increase with relativeness (Waldeck *et al.* 2008). Throughout this

[1] The terminology for movements is still ambiguous and debated in the ecology literature, as terms are often inconsistent among particular sub-fields for historical reasons. Migration can be used to refer to dispersal (e.g. in population genetics and the metapopulation literature) as well as regular movements among a fixed set of locations (e.g. seasonal and diurnal migrations but also nomadism), whereas dispersal can also refer to the population level result of individuals moving apart and is then opposed to aggregation (Dingle & Drake 2007).

thesis, we will thus refer to dispersal as a movement from the natal site to the first breeding site (natal dispersal) or between two successive breeding sites (breeding dispersal; Clobert, De Fraipont & Danchin 2008).

Dispersal processes can usually be split into three phases: (a) emigration when an individual decides to leave its natal or current breeding site, (b) transfer between the departure and arrival sites, and (c) immigration when the individual decides to settle in a new breeding site (Matthysen 2012). Decisions during these three phases can be sequential and thus independent from each other, or they can occur simultaneously when multiple habitats can be compared. In particular, an individual might initiate prospecting movements to explore potential breeding sites before emigration (Reed *et al.* 1999; Pärt & Doligez 2003; Debeffe *et al.* 2013). Depending on the dispersing form, the transfer phase can be active, with the individual controlling its movement, or passive, when the direction or speed of the movement is out of the organism control. Some organisms, especially insects or aquatic animals, combine these two dispersing modes (Burgess *et al.* 2015). For example, spiders often alternate between passive long-distance dispersal through "ballooning" and active moves on shorter distances (Bonte *et al.* 2009). However, even in species that can have a very good control of their movements, external factors can cause "non-intentional" dispersal. It is particularly true in aerial and aquatic organisms that can be displaced by strong winds and currents (Grinnell 1922; Cain, Damman & Muir 1998).

1.2 Why move at all: ultimate factors promoting a costly behaviour

Dispersal entails costs to the individual (Bonte *et al.* 2012). First, locomotion in itself requires time and energy, which could be invested in other functions. In some species, long-distance movements require particular locomotor structures, such as wings, that represent developmental costs. For example, macropterous insects often have delayed development or reproduction compared to wingless morphs of the same species (Zera & Denno 1997). The transfer phase, as any movement, is risky. It often occurs through unsuitable and/or unknown habitat (Wiens 2001; Haynes *et al.* 2007) and moving makes animals more conspicuous to predators (Yoder, Marschall & Swanson 2004). Avoiding such risks by using

safer corridors increases dispersal distance (Adriaensen *et al.* 2003) and is more sensitive to disruption (Epps *et al.* 2007). When movement is entirely passive or when information acquisition before and during transfer is low, there is a high risk to land in an unsuitable habitat (Cheptou *et al.* 2008). Some additional costs might arise from differences between the departure and settling sites. The dispersing individual will have a lower knowledge of its new environment (Pärt 1995), a lower social familiarity and a lower genetic relatedness with its neighbours. It will therefore have less cooperation opportunities and might experience a stronger intra-specific competition (Ekman, Bylin & Tegelström 2000; Dickinson *et al.* 2014). Moreover, it might lose many benefits of local adaptation unless they are maintained by habitat selection (Peterson, Hilborn & Hauser 2014).

The persistence of dispersal despite these costs implies some fitness benefits of dispersing. At the individual level, dispersal is an adaptation to temporal and spatial variation in the environment (Lurz, Garson & Wauters 1997; Gandon & Michalakis 2001), especially in ephemeral habitats (Ronce 2007) and range margins (Kokko & Lopez-Sepulcre 2006; Hughes, Dytham & Hill 2007). The quality of a habitat is influenced by its physical and chemical characteristics (temperature, salinity, natural or synthetic xenobiotic compounds), its resources (food, water, light or breeding sites availability) and its parasites and predators community, but also by its social environment (density of interspecific and conspecific competitors or collaborators) and by its genetic environment (Clobert *et al.* 2008). The spatial structure of variation is not always the same for these different components and might be more or less predictable. For example, the spatial structure of genetic relatedness with other individuals is often easily predictable, as it usually decreases with increasing distance from the natal site (Pusey 1987). Theoretical models have extensively shown that inbreeding depression and spatio-temporal variation in habitat quality can promote dispersal behaviour (reviewed in Starrfelt & Kokko 2012).

At the gene level, kin selection can also explain the evolution of dispersal in a stable environment, even with high dispersal costs: if there is local competition for resources, the dispersal of some individuals can enhance the fitness of related philopatric individuals and therefore the inclusive fitness of the dispersing individuals (Hamilton & May 1977). More

recent models have attempted to evaluate the relative contribution of environmental fluctuations, population density and kin competition on dispersal evolution and have shown that kin selection could play a large role in real metapopulations with a high cost of dispersal and therefore strong genetic structure (Poethke, Pfenning & Hovestadt 2007).

1.3 Sources of variation in dispersal decisions: proximal factors promoting dispersal

1.3.1 Gathering information on environmental factors

The proximate costs and benefits of dispersal are strongly linked to the ultimate factors that promote its evolution or restrict it: environmental quality, population density and kin relationships, but also ease of locomotion between suitable habitats. Optimizing dispersal decisions thus requires assessing environmental parameters before or during dispersal and thus gathering information from various sources. Individuals might get direct information from their habitat, such as parasites abundance (Brown & Bomberger Brown 1992), or use their own internal condition and reproductive success as indirect cues of habitat quality (Ims & Hjermann 2001), which more accurately measures the interaction between an individual phenotype and its habitat's characteristics. The sources of information can be extended in time and space by using information from other individuals (Ims & Hjermann 2001). Social information, such as the density or reproductive success of conspecifics, can be used to assess habitat quality through wider spatial scales or before the first reproduction of natal dispersers (Greenwood, Harvey & Perrins 1979; Doligez *et al.* 1999; Doligez, Danchin & Clobert 2002; Doligez *et al.* 2004). Maternal effects can fine-tune the dispersal decision to environmental clues anterior to their offspring birth (Tschirren, Fitze & Richner 2007; Bitume *et al.* 2014; Bestion *et al.* 2014; Duckworth, Belloni & Anderson 2015). Environmental characteristics are however not the only determinants of dispersal costs and benefits.

1.3.2 Phenotype-dependent dispersal

The potential costs and benefits of dispersal are often relative to an individual. Young or low quality individuals with low competitive abilities, but also aggressive or asocial individuals might perform badly in or be competitively excluded from high density habitats, even if other habitat characteristics are favourable (Duckworth 2008) and will settle accordingly in lower density, but often lower non-social quality, habitats (Doligez *et al.* 2004; Cote & Clobert 2007; Hénaux, Bregnballe & Lebreton 2007). Successful breeders, as well as young from large or successful families, might be more affected by kin competition and inbreeding (Kisdi 2004). Then, some phenotypes might also be more successful in the transfer phase, for example if they have a more efficient locomotion or larger nutrients stores. Accordingly, dispersing individuals can show morphological adaptations to the transfer phase such as larger wings or fat store (O'Riain, Jarvis & Faulkes 1996; Breuker, Brakefield & Gibbs 2007). They can also be more aggressive (Duckworth & Badyaev 2007), display more exploratory behaviour (Dingemanse *et al.* 2003; Krackow 2003; Hoset *et al.* 2010; Quinn *et al.* 2011; Debeffe *et al.* 2013, 2014) and have higher hormones or antibodies level (O'Riain *et al.* 1996; Snoeijs *et al.* 2004), all traits that might be advantageous for the exploration of a new habitat and in competitive encounters (Duckworth & Badyaev 2007; Fogarty, Cote & Sih 2011). In some cases, particular structures are absolutely required for dispersal and are specific to these individuals or species that disperse. It is now recognized that individual dispersal tendency covaries with other behavioural and physiological traits and that such differences can modulate the success of dispersal strategies and eventually the whole metapopulation dynamics (Bowler & Benton 2005; Hanski & Saccheri 2006; Clobert *et al.* 2009; Fogarty *et al.* 2011; Ronce & Clobert 2012).

In a (meta)population where multiple causes of dispersal act simultaneously, the proximate triggers of dispersal can differ between individuals, according to their phenotype. A well documented example is the difference in dispersal causes between males and females. The territorial sex is usually more sensitive to competition, and will thus emigrate more at high density or when of low body condition (Doligez *et al.* 1999; Hardouin *et al.* 2012). Females can specifically avoid areas of male-biased sex-ratio were sexual harassment will be stronger (Legrand *et al.* 2015). Social information is also used differently according to

competitive abilities in common lizards *Zootoca vivipara*, suggesting that some individuals seek to maximise non-social habitat quality whereas others minimise competition (Vercken, Sinervo & Clobert 2012). Phenotype-by-environment interactions are thus crucial in determining the observed dispersal patterns at the individual and population levels.

The optimization of dispersal decisions according to environmental conditions and individual phenotype seems to require a high behavioural flexibility. In contradiction, dispersal propensity has been found to be heritable in insects and birds species (reviewed in Roff & Fairbairn 2001 and Zera & Brisson 2012; Hansson, Bensch & Hasselquist 2003; Pasinelli, Schiegg & Walters 2004; Doligez *et al.* 2012, but see Wheelwright & Mauck 1998). Various phenotypic and behavioural traits strongly associated with dispersal are also heritable in plants, insects and, with more limited evidence, birds and mammals (Roff & Fairbairn 2001; Zera & Brisson 2012). Eventually, genetic polymorphism has been associated with dispersal in the fruit fly *Drosophila melanogaster* (Edelsparre *et al.* 2014) and in the Glanville fritillary *Melitaea cinxia (Hanski 2012).* Theoretical explanations of the maintenance of heritable variation in dispersal propensity rely on phenotype-dependent mechanisms of dispersal. An allele for body condition or inbreeding-dependent dispersal can be selected for because dispersal is an opportunity to find a better genetic background or to decrease inbreeding (Gueijman *et al.* 2013). Alternatively, if some traits are more beneficial to dispersers than to philopatric individuals, correlational selection on dispersal and these traits will eventually lead to their genetic integration and thus fix a genetic basis of dispersal propensity (Duckworth 2012).

1.4 Dispersal syndromes: integrated life-history strategies or by-products of constraints on dispersal?

The previous section illustrated through empirical evidence the idea that dispersal is associated with a suite of phenotypic traits that define a "dispersal syndrome" and could explain the maintenance of heritable dispersal behaviour if they enhance the success of dispersal. However, not all phenotypic traits associated with dispersal have a direct positive effect on the fitness of dispersers, and the observed correlations between dispersal and life-

history traits are much more inconsistent between, and sometimes within, species than the correlations with other phenotypic traits (Bélichon, Clobert & Massot 1996; Doligez & Pärt 2008; Ronce & Clobert 2012). Because different selective pressures can act on dispersal, different non-mutually exclusive hypotheses can be formulated on the evolutionary origin of the observed differences of life history traits between dispersing and non-dispersing individuals (Stevens *et al.* 2014). Their co-existence in natural population might explain the diversity of the patterns of associations between dispersal and life-history traits, and might also hinder the detection of these associations.

1.4.1 Indirect correlation generated by differences in individual quality

On the one hand, dispersers might be constrained to disperse because of their low ability to compete (Murray 1967; Waser 1985). Emigrants would then have a lower condition and/or reproductive success before dispersal, especially in high quality habitat, and immigrants might have a lower condition and/or reproductive success after dispersal. Accordingly, individuals with a smaller body size and lower competitive abilities sometimes disperse more than larger and more competitive individuals (McCauley 2010; Solmsen, Johannesen & Schradin 2011; Tarwater 2012). In this case, we expect the proportion of emigrants to be higher in plots with higher competition. Recent theoretical work has however shown that a direct negative correlation between dispersal and individual quality could evolve because dispersal is more beneficial to genes associated with deleterious alleles even in the absence of environmental variation: through dispersal, they can recombine with better genetic backgrounds or genetic backgrounds with different deleterious mutations, thus masking their effect when they are recessive (Gueijman *et al.* 2013). Inbred individuals will thus be more prone to disperse (Shafer *et al.* 2011). Although low quality dispersers might increase their fitness relative to their habitat of origin, they are still expected to have a lower fitness than philopatric individuals.

On the other hand, dispersers might be higher quality individuals that can sustain the costs of dispersal, which generate opposite predictions. Natal dispersal behaviour is often consistent with this hypothesis, with dominant or higher quality individuals dispersing

more, further or earlier, and then achieving higher fitness (Nilsson 1989; Dufty Jr & Belthoff 2001; Barbraud, Johnson & Bertault 2003; Bonte 2013; Camacho, Canal & Potti 2013; Scandolara *et al.* 2014; Bitume *et al.* 2014). This is particularly true when dispersal is involved in avoiding inbreeding and/or kin competition, because young from successful broods might be expected to both be of higher quality and have more siblings, and thus have a higher risk of inbreeding or kin competition. In that case, dispersing individuals will have a higher fitness than philopatric ones, despite potential costs of dispersal.

Of course, the two previous hypotheses can occur simultaneously within the same population, with low quality individuals constrained to disperse by their low competitive ability, whereas high quality dispersers take the opportunity to settle in a plot of higher quality thanks to their high competitive ability. For natal dispersal, this is an expression of the "silver spoon" effect (Stamps 2006). In competition is higher in high quality habitats, disperser from low quality to high quality habitats would have a lower condition (and reproductive success for breeding dispersal) before dispersal and a lower fitness than dispersers from high to low quality habitats. For example, great tits dispersing to high quality areas have a larger body mass than those dispersing to low quality areas (Garant *et al.* 2005). The relative importance of both mechanisms depend on the nature of environmental variability and on the costs of dispersal (Bonte & De La Peña 2009; Kisdi, Utz & Gyllenberg 2012).

1.4.2 Indirect correlation generated by other differences in individual phenotypes

More generally, dispersal patterns might be the result of adaptive decisions ("matching habitat choice") from individuals varying in their ecological optima (Edelaar, Siepielski & Clobert 2008; Ravigné, Dieckmann & Olivieri 2009). Dispersal is then but one side of individuals' differences in their response to the environment. In this case, knowledge of individuals' underlying preferences, or their phenotypic correlates, will be necessary to correctly interpret the observed patterns of departure and settlement (Edelaar *et al.* 2008). Emigrants from and immigrants to a population should then show opposite differences in their fitness response to environmental characteristics. However, dispersing individuals are

often considered a homogeneous group, without knowledge or distinction of their environmental origin. Moreover, differences in life-history traits between dispersing and philopatric individuals have rarely been assessed in interaction with environmental variables. Detailed studies of emigration and immigration decisions adjusted to different ecological optima between individuals thus remain scarce. Individuals can show adaptive habitat choice based on non-social environment: common lizards dispersing from warm habitats preferred lower temperature before dispersal and had lower post-dispersal survival in warm habitats than in cooler ones (Bestion, Clobert & Cote 2015). A correlative study in the barn owl *Tyto alba* showed that both habitat preference and reproductive success in forested vs. more open habitats were both linked to plumage colouration in females (Dreiss *et al.* 2012). Another example is the interaction of aggressiveness/asociality and breeding density on dispersal decision, although its generality remains to be tested (Wey *et al.* 2015): emigration increases and immigration and fitness prospects decrease with breeding density in aggressive/ asocial individuals, whereas non-aggressive/social individuals might benefit from cooperation and thus show opposite relationships (Myers & Krebs 1971; Cote & Clobert 2007). Although social behaviour was not assessed, two-spotted spider mite *Tetranychus urticae* dispersing from high to low density populations increase their fitness (Bonte *et al.* 2014). In the only study that tested the life-history traits responses to environmental variation in dispersers from different habitats (Bestion *et al.* 2015), only individuals dispersing from warm habitats showed differences in survival according to temperature: the opposite reaction norms in terms of dispersal were not paralleled by strictly opposite reaction norms in terms of life-history traits. As lifetime reproductive success was not measured, we cannot exclude differences in fitness due to differences in reproductive success. Testing the occurrence of "matching habitat choice" in dispersal decisions will thus require further experimental tests combining manipulations of the environment or the phenotype with accurate measures of dispersal and fitness (Edelaar *et al.* 2008).

1.4.3 Functional correlation between dispersal and other phenotypic traits

Finally, dispersal might be functionally linked to other physiological and behavioural traits. In that case, emigration and immigration decisions are the expression of the same trait (dispersal propensity), thus emigrants and immigrants should not differ from each other but should differ from residents. Because dispersal involves costly movements during the transfer phase, as well as exploratory and competitive processes during the settlement phase, one hypothesis is that some traits reduce the cost of dispersal *per se* or benefit dispersers more than residents (Clobert *et al.* 2009). As detailed before, dispersing individuals can show morphological, physiological and behavioural adaptations to the transfer and settlement phases. Some traits can be more beneficial (or less detrimental) to dispersers than to philopatric individuals because environment automatically differ according to dispersal. For example, in the western bluebird, philopatric males benefit from low levels of aggression by being close to kin (Aguillon & Duckworth 2015) and are less aggressive than immigrants to and emigrants from the same area (Duckworth & Kruuk 2009). These results are also coherent with the "social cohesion" hypothesis that predicts lower dispersal in more social individuals, independently of conspecifics density, because they benefit from established social relationships, especially with kin (Clutton-Brock & Lukas 2012). A consistent negative association between sociability and dispersal has been found in mammals (Ims 1990; Blumstein, Wey & Tang 2009; Armitage *et al.* 2011) and fishes (Schürch & Heg 2010; Cote *et al.* 2010).

Natural selection is then expected to select for the functional and/or genetic integration of dispersal propensity with traits that reduces the cost of dispersal or are beneficial to dispersers (Duckworth 2012). Empirical evidence remain scarce, because of the methodological limitations when testing for genetic correlations in wild populations: low power because of low sample size and incomplete pedigrees, imperfect knowledge of the pedigree due to extra-pair reproduction (Kruuk, Charmantier & Garant 2014; Teplitsky, Robinson & Merilä 2014). Despite these difficulties, dispersal distance or ability were found to be genetically correlated to exploratory behaviour in great tits *Parus major* (Korsten *et al.* 2013), to aggressiveness in Western blue birds *Sialia mexicana* (Duckworth & Kruuk 2009) and to boldness in alpine swift *Apus melba* (Grégory Daniel, personal communication). At the

genomic level, a single nucleotide polymorphism is strongly associated with both dispersal rate and flight metabolic rate in the Glanville fritillary *Melitaea cinxia* (Hanski 2012).

Although the traits themselves are beneficial to dispersers, they might have a negative impact on other traits, especially when they use resources that could be allocated to other functions. Evolutionary trade-offs could thus constrain the associations between dispersal and other life-history traits. For example, although aggressive dispersive western blue bird have a much higher reproductive success in the low-density habitats they colonise than in high-density habitats, they still experience a decrease in reproductive success with increasing aggressiveness (Duckworth 2008).

1.4.4 The difficulties of estimating fitness components according to dispersal

Distinguishing between these hypotheses can however be challenging. It requires assessing the fitness costs and benefits of each strategy, and notably, whether dispersal is associated with a decrease or increase in fitness at the within-individual level, as well as whether dispersers eventually achieve a lower or higher fitness than non-dispersers. First, isolated life-history traits might not correlate with fitness, because dispersers and non-dispersers may achieve similar fitness through different life-history strategies (Julliard, Perret & Blondel 1996; Lemel *et al.* 1997; Marr, Keller & Arcese 2002). Second, fitness estimates are sensitive to differences in recapture probability between dispersers and non-dispersers if dispersal is repeatable (in species where multiple dispersal events can occur), as well as between their offspring if there is a correlation between parents and offspring dispersal propensity (Doligez & Pärt 2008). Traits that predict survival independently of dispersal (i.e. that would predict survival similarly for dispersing and non-dispersing individuals) would thus be helpful to investigate potential trade-offs associated with dispersal. Finally, it is also necessary to control for relevant environmental variables and/or to distinguish between pre-dispersal differences (between emigrants and residents) and post-dispersal differences (between immigrants and residents), because of the confounding effects of spatially variable habitat quality on dispersal propensity and on other life-history traits. However, estimating within-individual variation according to environment, or pre-dispersal

differences between-individuals, in reproductive success will only be possible if dispersal can occur between breeding seasons, and is not restricted to natal dispersal.

1.5 Flexibility in dispersal syndromes: the importance of environmental conditions

1.5.1 Environment modulates the association between dispersal and phenotypic traits.

Studies of phenotypic differences between dispersing and philopatric individuals often assume that these differences are fixed in time and across environmental contexts. Although this has proved right in some cases (Ims 1990; Meylan *et al.* 2009), these differences, especially in behavioural traits, can be exhibited transiently during or close to the dispersal event (Hoset *et al.* 2010) and/or be dependent on environmental conditions including social context (Clobert *et al.* 2009; Ronce & Clobert 2012). In the case of individual difference in quality linked to dispersal, the costs of dispersal are often negatively linked to individual condition, and indirectly to the quality of the habitat of origin, whereas the benefits of dispersal are usually decrease with the increasing quality of the habitat of origin relative to other habitats that could be reached through dispersal. In particular, individual with higher dispersal ability are only expected to disperse when they can benefit from dispersal. The winged dispersing morphs in insects will decrease dispersal if the conditions become favourable (Shaw 1970, cited in Ims & Hjermann 2001). Increase of emigration propensity with body mass is only observed in low quality habitats in great tits (Verhulst, Perrins & Riddington 1997). In the spider *Erigone dentipalpis*, food deprivation of the offspring influences the mode of dispersal and reduces the increase in long-distance dispersal induced by food deprivation of the mother (Mestre & Bonte 2012). More generally, any change in the costs of dispersal might modify the correlation between dispersal and other traits. For example, mosquito fish balance the benefits of dispersing for asocial individuals with the risks of dispersing under high predation (Cote *et al.* 2013), thus generating an interaction between social behaviour and perceived predation risk on dispersal propensity.

1.5.2 Environment could also modulate the life-history correlates of dispersal.

Despite this impact of environmental variation on dispersal-dependent phenotypes and the numerous studies that investigated differences in life-history traits according to dispersal (reviewed in Doligez & Pärt 2008), the influence of environmental variation in shaping life-history differences between dispersing and non-dispersing individuals has often been overlooked. In all the hypotheses detailed above, dispersers are expected to differ from philopatric individuals in their response to habitat characteristics. In that case, the magnitude and even the direction of the observed differences between dispersers and non-dispersers will depend on the habitat and the stage of dispersal considered. For example, differences in individual quality can result in fitness differences in unfavourable habitats, where resources are limiting, but not in favourable habitats. The fitness consequences of boldness can be modulated by predation intensity: survival of female bighorn sheep *Ovis canadensis* was positively correlated with boldness in years of high predation, but not when there was no or little predation (Réale & Festa-Bianchet 2003). In particular, observations under laboratory conditions might not reflect actual differences in the wild (Zera & Denno 1997), as trade-offs between traits might also only be observed under challenging conditions, when resources are limiting (Beaulieu *et al.* 2015). Failure to take into account the relevant environmental variables, or to sample different types of environments, will hinder the ability to detect such dispersal-related differences (Ducatez *et al.* 2014).

In addition to habitat preferences directly linked to their ecological optimum, individuals might exhibit a preference for their natal habitat type when exploring or dispersing (Davis & Stamps 2004). The habitat of origin can also influence exploratory behaviour, and consequently settlement patterns: American red squirrels born at habitat edges and thus exposed to a wider variety of habitat types are more prone to explore and thus disperse further distances (Haughland & Larsen 2004). Although these mechanisms are often adaptive and associated with higher fitness in the preferred habitat, they can also mask other adaptive preferences, or even be maladaptive (Gilroy & Sutherland 2007; Chalfoun & Schmidt 2012). In these all cases, habitat preferences can restrict the expression of multiple

traits and generate correlations between traits directly modulated by the environment (Dubois & Giraldeau 2014).

Understanding the maintenance of individual differences in life-history traits thus requires examining the response of dispersers and non-dispersers to relevant environmental variation. Overall, disentangling the various hypotheses about dispersal-related differences in life-history traits requires (i) a better understanding of the mechanisms underlying potential trade-offs between dispersal and other life-history traits, (ii) defining estimates of individual condition that could predict future survival and reproductive success of dispersers, independently of their recapture probability, and (iii) taking into account the effect of the environment on life-history differences between dispersers and non-dispersers.

2 METABOLISM AND OXIDATIVE BALANCE AS MECHANISMS UNDERLYING THE TRADE-OFF BETWEEN DISPERSAL AND OTHER LIFE-HISTORY TRAITS

Dispersal involves direct energetic costs, such as the costs of movement during transfer and exploration (Bonte *et al.* 2012). As detailed above (§ 1.3.2), dispersal is also correlated with other energetically demanding traits and behaviours, such as increased hormone and antibodies levels, increased mobility and exploration or increased aggressiveness toward competitors. Selection may thus favour a higher metabolic scope in dispersers so that dispersers could mobilise additional energy for movements *per se* and traits facilitating movements to new environments. Consistently, dispersal is positively associated with peak metabolic rate in females of the Glanville fritillary butterfly (Niitepõld *et al.* 2011). Energy management is thus expected to be central to the within-individual trade-offs and between-individual correlations between dispersal and other life-history traits. Energy management can be separated in three steps in heterotrophic organisms: energy acquisition by the consumption of energy rich nutrients, energy transformation through respiration and fermentation to generate energy forms that are usable by the cell, and finally energy allocation between different functions. Although life-history trade-offs have often been explained at the level of energy allocation, the costs associated with energy

transformation could also generate trade-offs between life-history traits expressed at different points in time, such as dispersal and subsequent reproductive success and survival.

2.1 Energy metabolism is associated with oxidative costs

2.1.1 Aerobic respiration is a source of reactive oxygen species.

From bacteria to multicellular organisms, energy transformation through respiration is an ubiquitous process (Schäfer, Purschke & Schmidt 1996). Electrons are transferred from donors (reducers), resulting from the breakdown of nutrients (carbohydrates, lipids and proteins), to a range of cofactors fixed to the mitochondrial or bacterial inner membrane (Nicholls & Ferguson 2003). Electrons are then transferred between these cofactors and eventually to an electron acceptor (oxidant). In aerobic respiration, dioxygen is the final electron acceptor. The energy released during these electron transfers allows the translocation of protons across the inner membrane to generate an electrochemical gradient that is used to produce adenosine triphosphate (ATP), the short-term energy currency in the cell (Mitchell 1961).

When both dioxygen and adenosine diphosphate (ADP), which is required for ATP production, are not limited, the electrochemical gradient remains low, and dioxygen is mostly reduced into water at the end of the electron transport chain (Murphy 2009). However, if the proton electrochemical gradient increases, some dioxygen is reduced by only one electron into superoxide ($O_2^{-}\bullet$) at various points in the transport chain (Korshunov, Skulachev & Starkov 1997; Turrens 2003). The ion superoxide is a powerful and instable oxidant and a precursor for a variety of pro-oxidants called reactive oxygen species, or ROS. Other endogenous mechanisms, notably in catabolic reactions (beta-oxidation of lipids) and in innate immune response (phagocytosis, inflammation), also produce reactive oxygen species. Additionally, they can be generated by environmental factors, such as UV and ionizing radiations, metallic ions (iron, copper) and heavy metals, some pesticides (e.g. paraquat) and other pollutants (Limón-Pacheco & Gonsebatt 2009). The relative importance of mitochondrial ROS production as opposed to other sources remains poorly known

(Murphy 2009), but the mitochondria is the main site of endogenous production under non-pathological conditions (Sanz, Pamplona & Barja 2006).

2.1.2 Reactive oxygen species should be regulated for optimal cellular function.

Reactive oxygen species can damage lipids, proteins and DNA. First, they can directly oxidise a function of the macromolecule, resulting for example in hydroperoxides in polyunsaturated fatty acids, 8-hydroxydesoxyguanosine from the deoxyguanosine base of DNA, or carbonyl radicals on some amino-acid side chains in proteins (Cadenas & Davies 2000; Mateos & Bravo 2007). Second, pro-oxidant can break polymeric chains, generating single- and double-strand breaks in DNA or protein cleavage (Beckman & Ames 1998). Following breakage, DNA and polypeptides can react to form inter-strand, intra-strand or DNA-protein cross-links (Mateos & Bravo 2007). Finally, some oxidised molecules, especially lipids, remain highly reactive. As they are more long-lived than ROS and can cross membrane and cytosolic material, they can extend the damages far from the original site (Hulbert *et al.* 2007). All these modifications result in impaired fluidity of lipidic membranes, loss of functional and structural efficiency of proteins, and accumulation of mutations and deletions in nuclear and especially mitochondrial DNA (Beckman & Ames 1998; Raha & Robinson 2000; Hulbert *et al.* 2007).

Despite their detrimental effects, ROS are also fundamental to many cellular function and signalling pathways. They are involved in the signalling of cell proliferation, differentiation, metabolic adaptation to hypoxia, and reproduction (Metcalfe & Alonso-Alvarez 2010). Moreover, there are both actors and signals in immunity, programmed cell death (apoptosis) and autophagy (Sena & Chandel 2012). For example, humans with defective NADPH-oxidase, an enzyme that reduces dioxygen into superoxide, suffer from recurrent and persistent infections (Holland 2010). The inactivation of an uncoupling protein in mice strongly increased both ROS production by isolated macrophages and resistance to a lethal infection (Arsenijevic *et al.* 2000). Although evidence of the importance of ROS for normal biological functions in wild population is still scarce, white-browed sparrow weavers *Plocepasser mahali* with a high baseline superoxide dismutase activity also have a lower

immune response (Cram *et al.* 2015a). Efficient immune and signalling functions of ROS require a good balance and timing of ROS activity: if they remain active too long, they can promote chronic inflammation (Sorci & Faivre 2009). It is therefore widely agreed nowadays that the redox state of the cell is a homeostatic balance finely tuned to avoid excessive damages while allowing cellular communication and immune processes (Finkel & Holbrook 2000).

2.1.3 Antioxidant defences limit ROS production and scavenge pro-oxidants.

Various mechanisms are involved in the control of ROS production and activity. The first line of defence is to directly reduce ROS production. The production of superoxide in the mitochondria can be prevented by a direct decrease in the proton gradient, due to uncoupling proteins (UCPs) that allow protons to flow through the inner membrane. These proteins, first identified for their role in thermogenesis in brown adipose tissue, are also involved in the regulation of ROS production and energy flow in mammals (Brand 2000; Sluse 2012). Whether they are implicated in regulating ROS production in birds remains controversial (Criscuolo *et al.* 2005; Bicudo *et al.* 2010 p. 91; Stier, Massemin & Criscuolo 2014c; Stier *et al.* 2014b). As a second line of defences, ROS and pro-oxidants can be reduced into less damaging molecules. Antioxidant scavenger reactions can transform superoxide into less oxidizing compounds (Valko *et al.* 2007). Superoxide is first transformed into hydrogen peroxide (H_2O_2) by the enzyme superoxide dismutases (SOD). Hydrogen peroxide is still a strong oxidant, and various enzymes such as catalase, glutathione peroxidases and peroxiredoxins can then catalyse the reduction of hydrogen peroxide into water (and dioxygen in the former case). Beside these enzymatic defences, non-enzymatic antioxidants (vitamin E, vitamin C, carotenoids, bilirubin, uric acid) can reduce various molecules not targeted by enzymes, and notably quench the reaction chains of lipid peroxidation (Halliwell & Gutteridge 2007). They often work synergistically but can interfere when targeting similar molecules (Niki 2010). Finally, oxidative damages can also be prevented or repaired through interventions directly targeting the biomolecules that are most sensitive to oxidation. Organisms can limit oxidative damages with macromolecules that are more resistant to

oxidation, such as saturated fatty acids instead of polyunsaturated fatty acids (Hulbert *et al.* 2007). When the damage is done, repair mechanisms can still restore functional molecules: oxidised proteins can be recognised and degraded in the proteasome (Cadenas & Davies 2000), whereas abnormal DNA nucleotides can be excised and replaced (Hoeijmakers 2001). The energetic costs of antioxidant protection might thus vary widely depending on the mechanisms involved: whereas dietary non-enzymatic antioxidants (vitamin E, vitamin C, carotenoids) and some enzymes (glutathione peroxidises) might be limited by nutrients availability (Isaksson, Sheldon & Uller 2011), some enzymatic antioxidant protections, such as catalase or superoxide dismutase might be almost costless (Speakman & Garratt 2014).

The oxidative state is therefore a balance between ROS production and control mechanisms (Figure 1). When the latter are not sufficient to equilibrate the former, oxidative damages accumulate with detrimental effects on the organism, a situation called oxidative stress (Finkel & Holbrook 2000). Given the variety of cellular processes involved in ROS production or controlled by ROS signalling, the need to control redox homeostasis might be a central mechanism of life-history trade-offs (Metcalfe & Alonso-Alvarez 2010; Monaghan & Costantini 2014).

2.2 The role of oxidative balance in life-history trade-offs

Because of the energetic requirement of dispersal, dispersal-related traits, and other life-history traits, disruption of the oxidative balance is a good candidate mechanism for potential trade-offs between dispersal and other life-history traits. Indeed, investment in life-history traits has been found to influence the oxidative balance, which can in turn constrain future expression of life-history traits. We will here focus on the links between oxidative balance, reproduction and survival.

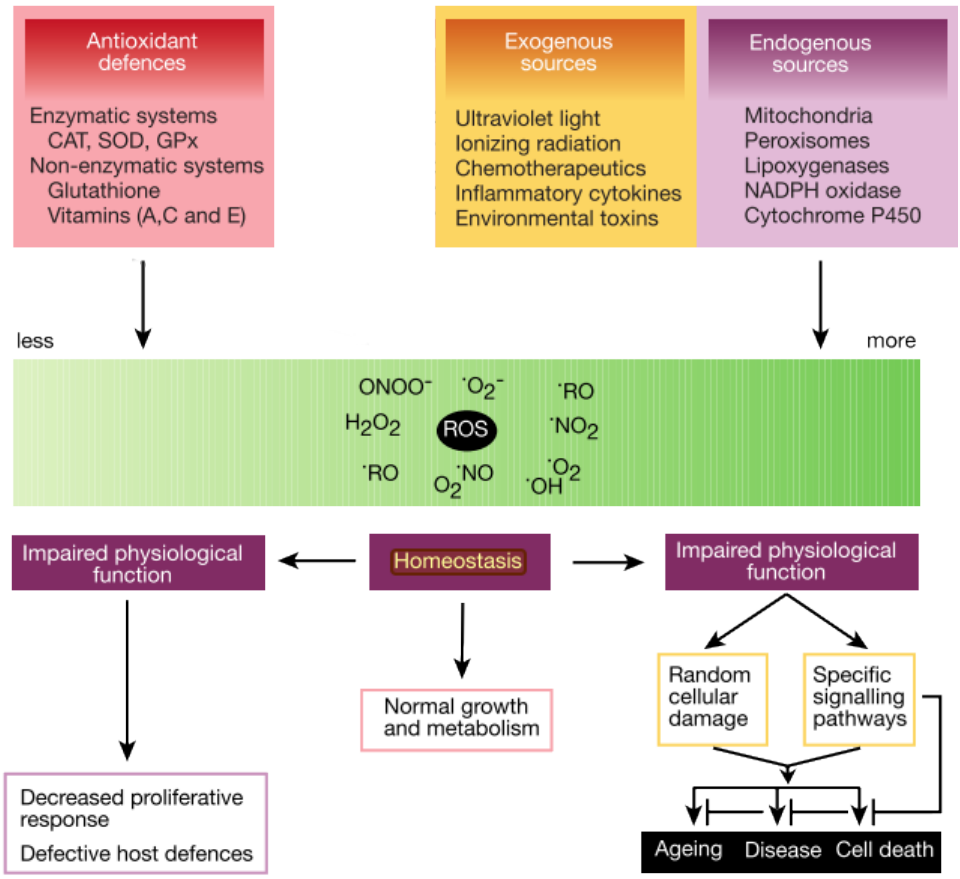


Figure 1: The sources of and responses to reactive oxygen species (adapted from Finkel 2000)

2.2.1 Effects of reproduction on oxidative stress.

Although intensively studied, the effect of reproduction on oxidative balance remains elusive (Stier *et al.* 2012; Metcalfe & Monaghan 2013; Speakman & Garratt 2014; Blount *et al.* 2015). Comparison of breeding and non-breeding individuals in the field often found higher oxidative damages in breeders or an increase over the breeding season, but laboratory studies on rodents and recently on birds highlighted the lack of consistency between markers and tissues and often found a decrease in oxidative damages in breeding individuals (Speakman & Garratt 2014; Costantini *et al.* 2014b; Costantini, Casasole & Eens 2014a; López-Arrabé *et al.* 2014; Sharick *et al.* 2015). The differences between breeding and non-breeding individuals could also be environment-dependent: females of the *Bicyclus anynana* butterfly

increased antioxidant capacity but had similar oxidative damage after mating compared to virgin females at high temperatures but not at moderate ones (Beaulieu *et al.* 2015).

Early studies of natural or experimental variation in egg production or brood/litter size in birds, mammals and insects have often found a negative effect of reproductive effort on resistance to oxidative stress and antioxidant enzymes, but did not assess oxidative damages (Harshman & Zera 2007; Monaghan, Metcalfe & Torres 2009; Christe *et al.* 2012). When examined, the effects of reproductive effort on oxidative damages could be positive but were most often non-significant, and sometimes negative (Speakman & Garratt 2014; Wegmann, Voegeli & Richner 2015a). Even when the manipulation had an energetic cost, it did not systematically translate into a change in oxidative balance (Aloise King, Garratt & Brooks 2013). Results were not always consistent for a given species, depending on the experimental design and the tissues considered (Speakman & Garratt 2014). Significant correlative increases in oxidative damages were also found with other markers of reproductive investment, such as male-male competition or higher investment in daughters than in sons in rhesus macaque *Macaca mulatta* (Georgiev *et al.* 2015).

Given the inconsistency between studies, the appropriate protocol to test for a trade-off between reproductive effort and oxidative balance is still been discussed. If individuals adjust the number of their offspring to their capacity to rear them (Lack 1954), reproductive effort could be limited by factors independent of offspring number or even food availability and manipulation of brood/litter size or food availability might not alter reproductive investment (Speakman & Król 2010). However, enlarging brood size does often increase absolute reproductive success (van der Werf 1992) and manipulations of different components of reproductive effort showed that, in birds, the cost of egg-laying could be more limiting than that of nestlings rearing and that individuals were thus not at an energetic ceiling during the latter (Visser & Lessells 2001). As many of the studies cited above reported an increase in antioxidants or energy expenditure but not in oxidative damages, individuals seems to be able to increase antioxidant protection to maintain their oxidative balance (Blount *et al.* 2015; Zimmer & Spencer 2015). In particular, breeding females might maintain a low oxidative stress at all costs because of the potential deleterious

effects on offspring, a process called oxidative shielding (Blount *et al.* 2015). Such differences in the management of oxidative stress might partly explain the inconsistency between species: short-lived species are expected to sacrifice oxidative balance for early reproduction, whereas longer-liver species might invest more in antioxidant defences and suffer low damages (Rey *et al.* 2015). Experimental manipulations of other energetic costs, such as immunity, thermoregulation or foraging, were thus suggested to test for a potential trade-off between reproduction and oxidative maintenance. However such experimental handicap increased antioxidant capacity but not oxidative damages in breeding Adélie penguins *Pygoscelis adeliae* (Beaulieu *et al.* 2011) and had no effect on parents' physiology in great tits (Wegmann, Voegeli & Richner 2015b).

Although the results overall point toward oxidative costs of accelerated growth and reproduction, the exact nature of these cost (increased oxidative damage or increased antioxidant production/acquisition) and their relevance to life-history trade-offs in wild populations might be highly dependent on the species studied and the environment where it is studied. Experimental manipulations of reproductive costs and food availability might help understand the constraints (or their absence) on oxidative balance.

2.2.2 Oxidative stress negatively impact future reproduction and survival.

Because some antioxidants, and especially carotenoids, are involved in sexual signals, the link between antioxidants, oxidative damage and colouration have been extensively studied and showed a negative impact of oxidative stress on sexual signals in many species (Catoni, Peters & Martin Schaefer 2008; López-Arrabé *et al.* 2014; Esteban *et al.* 2015). Effects of the oxidative balance on other components of reproductive success have been less studied. Resistance to oxidative stress was positively correlated with reproductive performance in alpine swifts (Bize *et al.* 2008). Oxidative damages were negatively related to subsequent litter size in mice (Stier *et al.* 2012) and to reproductive effort in male Florida scrub jay *Aphelocoma coerulescens* (Heiss & Schoech 2012) but no effects were found in the wandering albatross (Costantini *et al.* 2015). These correlative studies could not tell apart the direct effect of oxidative stress from differences in quality between individuals, but experimental data

have confirmed the role of oxidative balance in constraining reproduction. The negative effect on reproduction of oxidative pollutants is well documented in humans, rodent, and non-model species (Fry 1995; Agarwal, Saleh & Bedaiwy 2003; Peterson *et al.* 2010). There is strong evidence that antioxidant supplementation increases sperm quality and thus male fertility (Catoni *et al.* 2008; Costantini *et al.* 2010). Sperm have high metabolic rate and their membrane is rich in polyunsaturated fatty acids, which makes them particularly vulnerable to oxidation (Blount, Moller & Houston 2001). The effect of supplementation experiments on female fecundity seems less universal, but no negative effect was found so far (Catoni *et al.* 2008; Costantini *et al.* 2010).

The deleterious effects of oxidative stress during chronic inflammation or ischaemia-reperfusion have been well described in humans, model animals and hibernating mammals (Costantini *et al.* 2010; Chouchani *et al.* 2014), as well as the deleterious effects of oxidative pollutants (Limón-Pacheco & Gonsebatt 2009; Yang & Omaye 2009; Lichtfouse, Schwarzbauer & Robert 2013). A few studies so far have addressed the effect of oxidative stress on survival and lifespan outside these extreme conditions. Resistance to oxidative stress or antioxidant capacity was positively correlated with survival in birds (Alonso-Alvarez *et al.* 2006; Bize *et al.* 2008; Saino *et al.* 2011; Geiger *et al.* 2012; Losdat *et al.* 2013) and with reproductive lifespan in zebra finches (Kim *et al.* 2010). Oxidative damages were also negatively associated with survival in king penguin chicks (Geiger *et al.* 2012; but see Stier *et al.* 2014d) and in European shags *Phalacrocorax aristotelis* (Herborn *et al.* 2015), but no correlation was found between oxidative damages and survival in the wandering albatross (Costantini *et al.* 2015). Consistently, mitochondrial uncoupling, which decreases the rate of ROS production for a given metabolic rate, was found to increase lifespan in mice (Speakman *et al.* 2004). However, experimental supplementations in antioxidants produced contradictory results. Antioxidant supplementation reduced the effects of age-related diseases, such as degenerative brain diseases or arthritis, in humans and laboratory rodents (Catoni *et al.* 2008; Banks, Speakman & Selman 2010; Pallauf *et al.* 2013). The effects on lifespan of antioxidant supplementation or manipulation of antioxidant genes in a wide range of taxa are however mixed: longevity was mostly unaffected, but it could also increase

or decrease depending on the antioxidant molecule and the species considered (Sanz *et al.* 2006; Hulbert *et al.* 2007; Catoni *et al.* 2008; Selman *et al.* 2013; Pallauf *et al.* 2013). Vitamin supplementation was suggested to disrupt the natural increase of antioxidant defences under moderate oxidative stress, which should increase lifespan through a hormetic[2] process (Ristow & Zarse 2010).

These limited, and often contradicting, results suggest that oxidative balance might be influenced by and in turn influence the expression of life-history traits in a species-specific way. The potential of the oxidative balance to mechanistically link dispersal with other life history traits and help us predict survival and reproductive outcomes of dispersal should be investigated.

2.3 Telomere length as an integrator of oxidative state?

Telomeres are tandem-repeated simple sequences located at the ends of chromosomes (Moyzis *et al.* 1988), and highly conserved between Eukaryotes (Meyne, Ratliff & Moyzis 1989). Their primary role is to protect the extremities of the DNA sequence against (i) the fusion that readily occurs between chromosome breaks, and (ii) the degradation due to the end-replication problem, i.e. the fact that DNA polymerase does not replicate the very end of the 3′ DNA strand (Chan & Blackburn 2004). Compromised telomeres represent a high risk for the genome integrity and eventually the cell function, and telomere dysfunction directly elicits programmed cell-death as a protective mechanism (Sahin *et al.* 2011). At the whole organism level, short telomeres are associated with various degenerative disorders, progeria syndromes, and increased mortality in humans (Cawthon *et al.* 2003; Decker *et al.* 2009; Ehrlenbach *et al.* 2009; Armanios & Blackburn 2012; Boonekamp *et al.* 2013). Longer telomeres and slower telomere shortening are also positively linked to longevity or survival within various species in the wild and in the laboratory (Table 1) as well as between species (Haussmann *et al.* 2003). Experimental increase of telomere length, mainly through genetic manipulations, consistently increased lifespan (Joeng *et al.* 2004; Vera *et al.* 2012). Although

[2] Hormesis describe a bi-phasic stress response, where "a low dose of a stressful stimulus activates an adaptive response that increases the resistance of the cell or organism to a moderate to severe level of stress" (Calabrese *et al.* 2007).

longer telomeres can also be associated with higher annual or lifetime reproductive success in some species, the opposite relationship is also found (Table 1).

Telomere shortening occurs naturally with age in many organisms as the enzyme telomerase, responsible for telomere synthesis, is down-regulated in most adult somatic tissues (Taylor & Delany 2000; Forsyth, Wright & Shay 2002). Furthermore a wide range of stressors and energetic challenges, such as accelerated growth or siblings competition during development, reproductive effort, immune challenges or social stressors (isolation or crowding), can accelerate telomere shortening (Table 2). Because of the deleterious effects of short telomeres on cell function, and their association with lower health and survival outcomes, telomeres could be one of the mechanisms linking stress exposure to lower survival and longevity.

Oxidative stress could be a mechanism integrating the effects of various stressors on telomere length (Houben *et al.* 2008). Oxidative stress has been shown to increase telomere shortening in cell cultures (von Zglinicki 2002; Richter & von Zglinicki 2007), whereas mitochondrial uncoupling slows down telomere shortening (Passos *et al.* 2007). Stressors that had a negative effect on telomere length or dynamics also increased oxidative damages and two studies found a negative relation between telomere length and oxidative damages (Table 2). Telomere length has therefore been proposed to be a good candidate marker of the cumulative effects of oxidative stress over time and their impact on future survival and reproductive prospects.

Table 1: Effect of natural variation in telomere length on fitness components in non-human animals.

Species	L/F	Measure	Age/Stage	Surv.	Longevity	Annual RS	Lifetime RS	Référence
tree swallow *Tachycineta bicolor*	F	Length	1 y (first breeding)	+	+			Haussmann *et al.* 2005
sand martin *Riparia riparia*	F	Length	variable		+			Pauliny *et al.* 2006
dunlin *Calidris alpina*	F	Length	variable		0		0 (F) + (M)	Pauliny *et al.* 2006
alpine swift *Apus melba*	F	Length Shortening	variable	+ +				Bize *et al.* 2009
jackdaws *Corvus monedula*	F	Shortening	variable	+				Salomons *et al.* 2009
water python *Liasis fuscus*	F	Length	adults hatchlings	- 0				Ujvari & Madsen 2009
southern giant petrel *Macronectes giganteus*	F	Length	variable	+				Foote *et al.* 2010
sand lizard *Lacerta agilis*	F	Length			+ (F) 0 (M)		+ (F) 0 (M)	Olsson *et al.* 2011
zebra finch *Taeniopygia guttata*	L	Length Shortening	25 d 1 y 3, 4, 6, 7 y 25d to 1y		+ (+) 0 0			Heidinger *et al.* 2012
king penguin *Aptenodytes patagonicus*	F	Length	~2.5 m (before fasting)	+				Geiger *et al.* 2012
leatherback turtle *Dermochelys coriacea*	F	Length	variable			+		Plot *et al.* 2012
house mouse *Mus musculus musculus*	L	Length Shortening	4, 8, 12, 25 m 4 to 25 m		0 +			Vera *et al.* 2012
American redstart *Setophaga ruticilla*	F	Shortening	variable	+				Angelier *et al.* 2013
Seychelles warbler *Acrocephalus sechellensis*	F	Length Shortening	variable	+	0 +			Barrett *et al.* 2013
common tern *Sterna hirundo*	F	Length				-		Bauch, Becker & Verhulst 2013
barn swallows *Hirundo rustica*	F	Shortening	nestling		0			Caprioli *et al.* 2013
African grey parrot *Psittacus erithacus erithacus*	(F)	Length	variable	0				Aydinonat *et al.* 2014
common tern *Sterna hirundo*	F	Length		+				Bauch *et al.* 2014
jackdaw *Corvus monedula*	F	Length Shortening	nestlings	0 +		0		Boonekamp *et al.* 2014
zebra finch *Taeniopygia guttata*	L	Length	adults	0				Reichert *et al.* 2014
great reed warbler *Acrocephalus arundinaceus*	F	Length	nestlings		+ (U) 0 (I)			Asghar *et al.* 2015

L/F: Laboratory or field study. The effects could differ according to sex (F=females, M=males) and lifetime infection status (U = uninfected, I = infected).

Table 2: Studies of the negative effect of various stressors and challenges on telomere length.

Type of stressor / challenge	Species	Negative effects on telomeres	Marker of OS	Effect on OS	r^2	Référence
Compensatory growth	rat *Rattus norvegicus*	Length (kidney)				Jennings *et al.* 1999
	king penguin *Aptenodytes patagonicus*	Length	Damages AO	+ (+)	0.40 n.s.	Geiger *et al.* 2012
Early-life competition	European starling *Sturnus vulgaris*	Shortening				Nettle *et al.* 2013, 2015
	jackdaw *Corvus monedula*	Shortening				Boonekamp *et al.* 2014
	zebra finch *Taeniopygia guttata*	Length	OS AO	+ +	+	Reichert *et al.* 2015
Reproduction	zebra finch *Taeniopygia guttata*	Length Shortening				Heidinger *et al.* 2012
		Length	Damages AO	0 0	n.s. n.s.	Reichert *et al.* 2014
Low habitat quality	American redstart *Setophaga ruticilla*	Shortening				Angelier *et al.* 2013
	thick-billed murre *Uria lomvia*	Length				Young *et al.* 2013
Immune challenge	house mouse *Mus musculus musculus*	Shortening				Ilmonen, Kotrschal & Penn 2008
	great reed warbler *Acrocephalus arundinaceus*	Shortening				Asghar *et al.* 2015
Psychological and social stress	human *Homo sapiens*	Telomerase Length	OS	+		Epel *et al.* 2004
		Shortening				Puterman *et al.* 2015
	house mouse *Mus musculus musculus*	Shortening				Kotrschal, Ilmonen & Penn 2007
	African grey parrot *Psittacus erithacus erithacus*	Length				Aydinonat *et al.* 2014
	spotted hyena *Crocuta crocuta*	Length				Lewin *et al.* 2015
Corticosterone treatment	black-legged kittiwakes *Rissa tridactyla*	Shortening				Schultner *et al.* 2014
Prenatal maternal stress or corticosterone in the egg	human *Homo sapiens*	Length				Entringer *et al.* 2011
	domestic chicken *Gallus domesticus*	Short telomeres	Damages AO	+ (-)		Haussmann *et al.* 2012
Combination (immune + social stress)	Eurasian blackbird *Turdus merula*	Length Shortening	Damages AO	+ 0		Hau *et al.* 2015

Length = telomere length, Shortening = telomere shortening, Telomerase = telomerase activity, Short telomeres = proportion of short telomeres. Oxidative stress was measured, the effect of the stressor was given, as well as the correlation between telomere length or dynamics and oxidative markers, or the sign of the relationship when the correlation coefficient was not given in the study. Damages: oxidative damage to biomolecules. AO: antioxidant capacity, concentration of antioxidants and/or activity of antioxidant enzymes. OS: damages corrected for antioxidant defences.

3 AIMS OF THE THESIS

The main question guiding this work will be to investigate whether dispersal behaviour is related to a different management of oxidative balance and reproduction under varying environmental conditions. Dispersing individuals might have a selective advantage over non-dispersing (hereafter called philopatric; see chapter IV for the measure and definition of philopatry and dispersal in our study system) individuals in some environmental conditions or compensate their lower reproductive success in constrained conditions through lower oxidative stress, and thus future survival and breeding success. Most of the results presented were obtained on individuals breeding between 2012 and 2014, but for the last study (Chapter VI) that used data on individuals breeding in 2011 for which we had sufficient data on subsequent return rate and reproductive success.

As a preliminary step (Chapter III), I sought to clarify the correlations between two components of the oxidative balance in the study population, independently of the dispersal status: intermediate oxidative damages, measured through the concentration of reactive oxygen metabolites, and antioxidant defences, through a test of non-enzymatic antioxidant capacity. As I focussed on markers measured in only one tissue (the plasma), it was necessary to describe how they related to each other and to energy expenditure in the study population, because measures might differ among tissues (Veskoukis *et al.* 2009; Speakman & Garratt 2014). The role of energetic constraints in modulating these correlations was investigated through two experimental procedures during breeding, one increasing energy expenditure through a manipulation of wing load, another increasing food availability by providing additional food.

A first experiment (Chapter IV) aimed at testing the hypothesis that dispersing and philopatric individuals manage oxidative costs differently during reproduction and at describing the reproductive consequences of such differential management. I examined the differences in energy expenditure, oxidative damages, antioxidant capacity and reproductive success between dispersing and philopatric birds. To test whether these differences depend on the level of energetic demand, I experimentally manipulated this demand by increasing

flight costs (through reducing wing area) during reproduction. Energetic demand, as well as energy and antioxidant availability, might also vary between habitats. The responses of dispersing and philopatric individuals to habitat quality were thus investigated correlatively, using breeding density as a marker of habitat quality in this population (Doligez *et al.* 2004).

To complement this approach, a second experiment (Chapter V) tested whether habitat quality directly modulated the observed differences according to dispersal. Given the multicausal nature of dispersal, many differences between dispersing and philopatric individuals could indeed stem from a bias in settlement according to individual quality. This is why assessing a direct effect of habitat requires an experimental manipulation of habitat quality. Many features of habitat quality, such as food productivity, but also competition, predation and cooperation, ultimately impact food availability, which in turn has a strong impact on breeding success. I experimentally relieved the environmental constraints by providing additional food during the nestling feeding period and investigated whether it modified the oxidative and reproductive response of dispersing and philopatric individuals to naturally varying habitat quality, measured through breeding density (Chapter V, Paper 1). Differences in personality traits according to dispersal have often been reported and could underlie differences in oxidative balance and reproductive success, but the role of environment in mediating those remains unclear. The effect of the food supplementation was therefore studied on a behavioural traits linked to parental care, nest defence toward a predator (Chapter V, Paper 2).

Finally, the longer-term consequences of the differences in response to the environment between dispersing and philopatric individuals were studied (Chapter VI). Given the potential bias in the probability of recapture between dispersing and philopatric individuals, three potential predictors of future survival and reproduction were identified: an index of body condition based on body mass, the mean telomere length and the level of glycated haemoglobin as a proxy of glucose homeostasis. Differences in these markers according to dispersal and breeding density were investigated, then the predictive value of these markers for return rate and future reproductive success was quantified.

This thesis is organised in seven chapters. The first (introduction), second (methods) and seventh (discussion and conclusion) parts are unpublished material. The third part is not currently formatted for publication and might yield one to two publications in peer-reviewed journals. The fourth and fifth parts correspond to respectively one and two papers submitted or in preparation for publication. The sixth part as presented here is not formatted for publication. However, a paper is currently in preparation, adapted from the results on glycated haemoglobin, whereas a second paper on telomere length is planned.

Chapter II – Some methodological considerations

1 THE COLLARED FLYCATCHER ON THE ISLAND OF GOTLAND, A MODEL IN THE STUDY OF DISPERSAL

The collared flycatcher *Ficedula albicollis* is a small insectivorous passerine bird, breeding in Central Europe and wintering in sub-Saharan Africa. The breeding populations of the collared flycatcher have been studied intensively since the first ecological studies of the species in central Europe (Tinner & G 1951; Löhrl 1957; Hajek 1968; Balat 1971; Głowaciński 1973). They naturally breed in tree cavities and are very prone to use nest boxes, which made them very popular models in population ecology.

The Swedish populations of Gotland and Öland are at the northern limit of the species breeding range and spatially isolated from other populations (Figure 2). Their existence is dependent on the presence of some of the biggest deciduous woodland areas in Sweden (Diekmann 1994), and especially of the Öja-Fide forest on Gotland. However, on Gotland, the landscape remains extremely fragmented, with areas unsuitable for breeding separating the suitable breeding patches (Figure 3). Collared flycatchers preferably breed in woodland, gardens and forested pastures (or *änge*) with a majority of deciduous trees and no dense shrub cover, but can also use coniferous woodland (Adamík & Bureš 2007; Kralj *et al.* 2009). The non-breeding areas include non-planted pastures and cropped land but also alvars, particular limestone formations with a thin soil and sparse grassland vegetation.

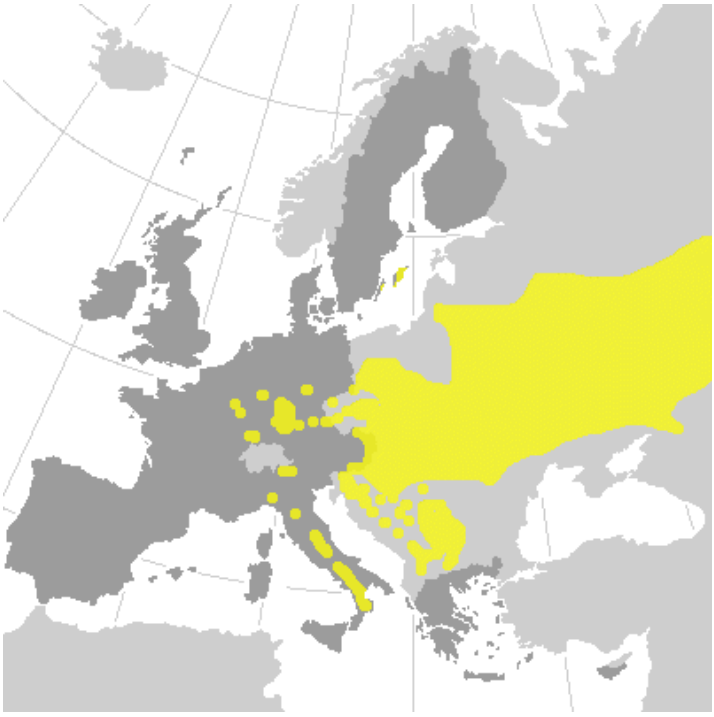


Figure 2: Breeding distribution of the collared flycatcher in Europe, in yellow (European commission © 2015)

The University of Uppsala has been monitoring the Gotland population in artificial nest boxes since 1980, and natal and breeding dispersal have been extensively studied since 1985, first as a potential source of error when evaluating recruitment (Gustafsson 1985), then as the very object of study (Pärt & Gustafsson 1989; Pärt 1990). Thanks to the fragmented structure of this population, dispersal can be defined as a change of breeding plot between birth and the first breeding event (natal dispersal) or between two consecutive breeding events (breeding dispersal; Doligez, Gustafsson & Pärt 2009). Natal dispersal is high and slightly sex biased toward females with 78.4% of female recruits and 72.3% of males recruits ringed as nestlings being dispersers (N = 2713 females and 2283 males caught between 1981 and 2011; $X^2_1 = 24.58$, $P < 0.0001$). There is also a considerable degree of strongly female-biased breeding dispersal with 24.3% of females and 4.8% of males being dispersers in their second or latter breeding attempt (N= 5279 females and 3928 males caught between 1981 and 2011; $X^2_1 = 640.56$, $P < 0.0001$). Sex-biased dispersal might contribute to inbreeding avoidance given the high inbreeding depression in this population (Kruuk, Sheldon & Merilä 2002).

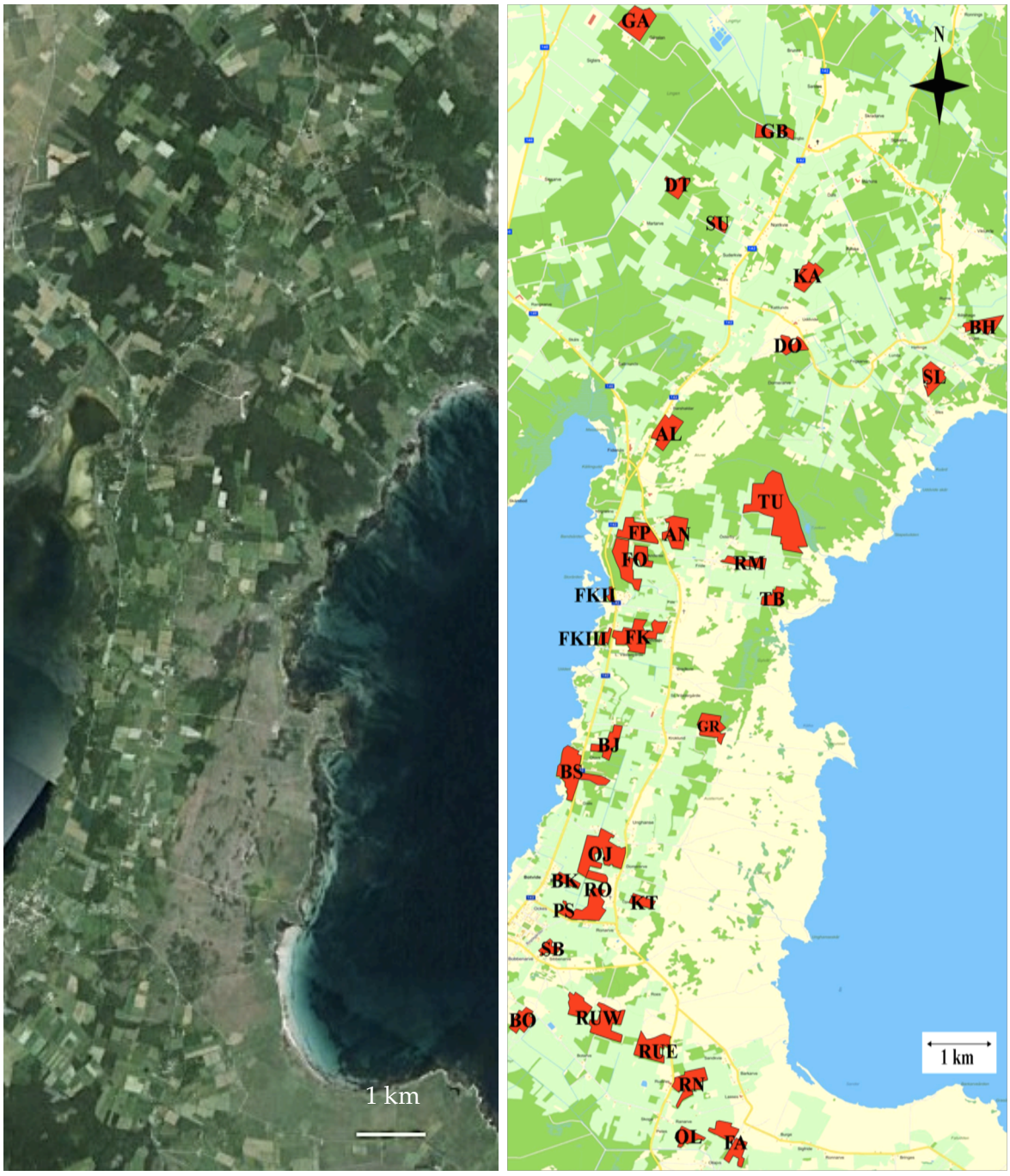


Figure 3: Satellite photograph (Google earth © 2015 TerraMetrics) and land cover map of the study site on Gotland Island, Sweden. Dark green: woodland and other planted areas; Light green: non-planted pastures and cropped farmland; Light yellow: dry meadows and alvars; Brown: buildings.

Dispersal in this population follow a model of balanced dispersal, without clear "source" and "sink" populations (Doncaster *et al.* 1997). Dispersal decisions are influenced by habitat characteristics, assessed through own experience (Doligez *et al.* 1999) as well as public information (Doligez *et al.* 1999, 2002, 2004), and their interaction with individual condition or abilities (Doligez *et al.* 1999, 2004). On the contrary, size, body mass, and body condition are not associated with natal or breeding dispersal behaviour when considered in isolation (Pärt & Gustafsson 1989; Pärt 1990). Because nest box fidelity is very low, occupation of the previous nest box by another bird did not strongly influence dispersal (Pärt & Gustafsson 1989), whereas boxes previously occupied by a conspecifics were preferentially chosen (Kivelä *et al.* 2014). Despite these sources of intra-individual variability, dispersal was heritable, with heritability estimates obtained using animal models of 0.39 for natal dispersal and 0.21 for the combination of natal and breeding dispersal (Doligez *et al.* 2012).

Natal philopatry was associated with higher local survival and mating success in males and with higher reproductive success in females (Pärt 1990, 1991, 1994). However, a recent study has shown that natal dispersers only experienced a cost in term of lifetime reproductive success under naturally or experimentally constrained conditions, notably an experimental increase in brood size (Germain 2014). The effect of breeding dispersal on subsequent fitness was restricted to females and depended on current reproductive success, with unsuccessful females increasing their future fitness through an increased number of offspring, whereas successful females decreased their future fitness through decreased survival (Pärt & Gustafsson 1989).

2 MEASURING OXIDATIVE STATE AND TELOMERE LENGTH IN WILD ANIMALS

The variety of processes involved in maintaining the redox homeostasis makes it difficult to measure it through a simple trait. The effects of various pro- and anti-oxidants are not only linked to their redox potential measured in standardized conditions, but also depend on their location in the cell, on the configuration of their active site (for enzymes),

and on the other molecules around (Cadenas & Davies 2000; Niki 2010). The current approach is therefore to measure a range of markers varying at different temporal and spatial scales, from mitochondrial parameters to more integrated measures on whole tissues, and combining measures of pro-oxidant and anti-oxidant concentrations, with markers of oxidative damages to macromolecules (Monaghan *et al.* 2009; Lowe 2014). As the protocols of the studies presented here involved repeated sampling within the breeding season, blood samples for oxidative stress markers were limited to 40μL in most cases, yielding approximately 20μL of plasma. Given this limiting amount, we described the oxidative balance by focussing on two measures: a marker of oxidative damage, the concentration of reactive oxygen metabolites, and a marker of antioxidant capacity in the plasma. These assays have been successfully used in collared flycatchers (Markó *et al.* 2011).

Plasma hydroperoxides (R-OOH) are relatively stable products (metabolites) of the oxidation of lipids by highly instable reactive oxygen species. They represent an intermediate step of oxidative damages, as they can still be reduced by antioxidants. The d-ROM test (Diacron international) measure them indirectly by adding acetate buffer (pH = 4.8) to release iron (chelated in transport proteins) that then react with hydroperoxides to produce peroxy (R-OO•) and alkoxy (R-O•) radicals through Fenton's reaction. These radicals' reaction with a chromogenic alkylamine (N,N-diethyl-*para*-phenilendiamine) produces a pink-coloured anion in proportion to the concentration of hydroperoxides in the sample (Alberti *et al.* 2000). The values of the d-ROM test were well correlated with total peroxides measured through a peroxide-peroxidase reaction (PoxAct assay) in patients with various medical conditions (and associated high peroxides concentrations) but not in healthy subjects with lower peroxides concentrations (Lindschinger *et al.* 2004). It has been shown that measures of plasma peroxidation levels, such as the measure obtained with the d-ROM test or measures of malondialdehyde, might be strongly linked to plasma lipids concentration (Pérez-Rodríguez *et al.* 2015). However, using a portable test-strip reader to measure triglycerides levels, we found no correlation with the results of the d-ROM test (Chapter III, Supplementary Information S1).

Plasma antioxidant capacity was measured through the OXY test (Diacron international), which estimates the capacity of plasma to oppose the massive oxidation action of the hypochlorous acid HClO by quantifying the pink-coloured anion produced by the reaction of the remaining HClO with N,N-diethyl-*para*-phenilendiamine. This measure integrates the activity of several non-enzymatic antioxidants, which could not be measured individually due to the low amount of plasma available. Contrary to other assays, this measure of total antioxidant capacity is not sensitive to uric acid (Costantini 2011).

Plasma antioxidant capacity tends to decrease and ROM concentrations to decrease with handling stress (Haussmann *et al.* 2012). Differences in these plasma parameters following handling could therefore reflect different stress responses rather than different basal oxidative states.

Tissues available for small wild animals are often restricted to blood samples, whereas oxidative stress levels can vary among different tissues (Veskoukis *et al.* 2009) and the variation of biomarkers in blood might not correlate with their variation in other tissues (Speakman & Garratt 2014). These limitations should be kept in mind when interpreting plasmatic markers of oxidative balance.

Chapter III – Repeatability and correlation of markers of the oxidative balance in a free-living passerine bird

The regulation of pro-oxidants and antioxidants processes in the organism results in a homeostatic oxidative balance. Imbalance in the favour of pro-oxidants, or oxidative stress, can indeed have a deleterious effects on cell function and thus on the whole organism health and survival. Because pro-oxidants are mainly produced through aerobic metabolism and can increase following energy investment in major life-history traits such as reproduction, oxidative balance as been proposed as a mediator of life-history trade-offs. Variation in the oxidative balance will directly depend on the variability of pro-oxidant and antioxidant mechanisms, but little is known about their repeatability, their within-individual variation and their correlation across different life-stages and conditions. We manipulated energetic constraints through increased flight costs via a wing load manipulation in 2012 and 2013 and through a food supplementation in 2014 in collared flycatchers. We then tested whether the effect of the experimental treatments and the relative importance of within- and between-individual variation on two markers of the oxidative balance measured in the plasma: reactive oxygen metabolites (ROM), a marker of pro-oxidant status, and non-enzymatic antioxidant defences (OXY test). We also investigated whether the experimental treatments modified the correlation between these markers. Antioxidant defences, but not ROM, were repeatable between years. Antioxidants increased under the food supplementation treatment, but did not vary between breeding stages, whereas ROM increased during reproduction in females and were higher in females than in males. Antioxidant defences and ROM concentration were positively correlated, and more strongly so when wing load was increased. The effects of energetic demands on oxidative balance were thus probably mediated through pro-oxidant mechanisms, whereas antioxidant capacity was determined by permanent individual characteristics and food availability.

Authors : Charlotte Récapet, Alexandre Zahariev, Stéphane Blanc, Mathilde Arrivé, François Criscuolo, Blandine Doligez, and Pierre Bize

Keywords: energetic constraint, daily energy expenditure, doubly-labelled water, reactive oxygen metabolites, antioxidant defences, oxidative stress, *Ficedula albicollis*

1. Introduction

Reactive oxygen species (ROS) produced during aerobic respiration can have deleterious effects by oxidising biomolecules and thereby disrupting cell function. They were thus soon proposed as a potential constraints on life-history trade-offs: the free radical theory of aging postulated that the accumulation of oxidative damages with age leads to cell degeneration and death, then eventually to whole organism dysfunction and senescence (Harman 1956). Genomic and transcriptomic studies of lifespan and aging in laboratory models then confirmed the importance of the respiratory chain and antioxidant enzymes in the aging process (Beckman & Ames 1998; Balaban, Nemoto & Finkel 2005; Ricklefs 2008). Studies across many taxa have also found a positive link between energy expenditure, notably during reproduction, and oxidative stress (Adelman, Saul & Ames 1988; Fletcher *et al.* 2013), or between oxidative stress and future survival and reproductive outcomes (Tolmasoff, Ono & Cutler 1980; Beckman & Ames 1998; Bize *et al.* 2008), thus establishing oxidative stress as a credible mechanism underlying life-history trade-offs (Monaghan *et al.* 2009; Metcalfe & Alonso-Alvarez 2010).

To understand the potential role of oxidative balance in life-history trade-offs, it is however crucial to understand the variability of pro- and antioxidants in realistic ecological settings. Variation in pro-oxidants production can often be related to variations in metabolism, but the generality of this relationship is far from clear. First, a higher metabolic and respiratory rate might not be associated with a higher free radicals production in many cases (Glazier 2015; Salin *et al.* 2015b). In fact, uncoupling proteins (UCPs) and changes in the mitochondrial inner membrane structure can modulate the relationship between energy consumption and free radicals production (Brand 2000; Stier *et al.* 2014a) or future survival and reproduction (Speakman *et al.* 2004). The rapid mobilisation of oxygen when respiration rate is high might also explain this somewhat contradictory relationship (Bonawitz *et al.* 2007; Salin *et al.* 2015a). Second, a higher free radicals production does not automatically translates into higher oxidative damages. In fact, the antioxidant capacity can be modulated to counteract the deleterious effects of free radicals, thus resulting in a redox balance. It has

been proposed that energetic constraints, if antioxidant defences are energetically costly, or a deficit of dietary antioxidants might result in a shift of the redox balance toward pro-oxidants. However, it is not clear whether antioxidant protection, especially through antioxidant enzymes, represents such a high cost (Speakman & Król 2010; Isaksson *et al.* 2011; Gems & Partridge 2013). Finally, there is no *a priori* expectation on how measures of antioxidant defences should relate to measures of ROS production and oxidative damages (Costantini & Verhulst 2009). If circulating antioxidants are used up to protect against additional reactive oxygen species, they should covary negatively with metabolic rate, ROS production or oxidative damages. Conversely, compensation by an increase in antioxidant in response to ROS production or oxidative damages could led to a positive, or an absence of, correlation. Markers of antioxidant capacity should thus be compared with at least a marker of damages to fully describe the oxidative balance.

The interpretation of oxidative stress measures in ecological studies therefore requires a better understanding of their variability in the wild and the actual relationships between markers. Moreover, relationships between traits can differ greatly when measured at the within-individual level, where life-history trade-offs can be expressed, or at the between-individuals level, where differences in individual quality or permanent environment are expected to play a large role (Stearns 1989; Wilson & Nussey 2009). We therefore measured field metabolic rate (FMR) and two plasmatic markers of oxidative state (reactive oxygen metabolites through the d-ROM test and non-enzymatic antioxidant capacity through the OXY test) in adults of a small passerine bird, the collared flycatcher *Ficedula hypoleuca*, during consecutive breeding seasons. These two markers, frequently used in wild bird populations, were sensitive to manipulations of energy expenditure or mitochondrial ROS production (Stier *et al.* 2014c; Vaugoyeau *et al.* 2015) and have been linked to dietary antioxidant intake (Beaulieu & Schaefer 2014), to reproductive effort (Beaulieu *et al.* 2011; Markó *et al.* 2011; Reichert *et al.* 2014; Wegmann *et al.* 2015a) and to fitness outcomes (Geiger *et al.* 2012; Herborn *et al.* 2015). Although measures of oxidative balance are often variable across tissues (Veskoukis *et al.* 2009), we focussed on plasma because it could be repeatedly sampled within and between years and because we expect

quick variation of oxidative balance in the blood in response to energetic constraints (Nikolaidis *et al.* 2008). We first tested the repeatability of oxidative state markers within the same season, as well as between seasons, and then assessed the correlation among physiological variables, and between physiological and behavioural variables, at the between- and within-individual levels.

To explain the inconsistent results across many studies to date, it has been proposed that antioxidant protection might be constrained by resource availability and could not adjust to an increased free radicals production. To test that hypothesis, in 2012 and 2013 we manipulated breeding females' wing load to increase their energy expenditure, and in 2014 we food supplemented breeding males and females to alleviate their energetic constraints. We expected the correlations between markers of oxidative state to be stronger in handicapped females compared to control ones and in parents from control broods compared to food supplemented ones.

2. Material and methods

2.1. Study population

The study was conducted during the springs 2012 to 2014 in a natural population of collared flycatchers breeding on the island of Gotland, Sweden (57°07'N, 18°20'E). This hole-nesting passerine bird readily accepts to breed in artificial nest boxes, which were erected in the nine forest plots used for the study (between 13 and 78 nest boxes per plot distributed homogeneously). Nests were visited regularly to estimate laying date, incubation date and hatching date. Females were first caught on average (± S.D.) 7.2 ± 1.2 days after the start of incubation, then males and females were caught when the nestlings were 9.0 ± 2.1 days old. Birds were ringed if not previously ringed and blood sampled (see below). The data and samples were collected under permission from the Ringing Centre of the Museum in Stockholm (bird catching, measuring and ringing) and the Ethical Committee for Experiments on Animals in Sweden (experimental manipulation of flight feathers and blood sampling).

2.2. Experimental manipulations of energetic constraints

In 2012 and 2013, we increased energetic constraints on females during the end of incubation and nestling feeding by cutting the two innermost primaries of each wing at their base when catching them in incubation, to mimic feather loss at the onset of moult (Moreno *et al.* 1999; Sanz, Kranenbarg & Tinbergen 2000; Ardia & Clotfelter 2007). Upon capture during incubation, females were alternatively assigned to the manipulated or the control group (same handling conditions but no feathers cut). The manipulation was successful at increasing energy expenditure (Chapter IV).

In 2014, we relieved energetic constraints on both parents during the nestlings feeding period by providing additional food (Chapter V). When nestlings were two days old, transparent plastic containers were attached to the front side of the nest box, below the nest hole. For supplemented nests, 30g live maggots were placed in the containers once a day until nestlings were 12 days old (included), i.e. over 10 days in total. Each experimental nest therefore received a total of 360g maggots over the whole nestling phase. Control nests received no food, but were visited daily to control for disturbance linked with human presence. Nests were assigned either to the control or supplemented group alternatively in space, so as to distribute treatments homogenously in space both within and between study plots.

2.3. Measure of field metabolic rate

Field metabolic rate was measured using the two-point doubly-labelled water method (Lifson & McClintock 1966). Upon capture during the nestling feeding phase, females were injected intraperitoneally with 30μL of a premixed solution composed of 0.6005g of 94% $H_2{}^{18}O$, 0.1514g of 99.99% D_2O and 2.8000g of 9‰ NaCl in 2012, and 1.2010g of 94% $H_2{}^{18}O$, 0.3028g of 99.99% D_2O and 2.0481g of 9‰ NaCl in 2013. These doses were calculated to obtain an *in vivo* enrichment of about 68 ‰ and 496 ‰ in 2012, and 135 ‰ and 992 ‰ in 2013, for ^{18}O and deuterium respectively.

After injection, females were kept in a cloth bag during 45 to 60 min so that the isotopes equilibrate with body water (Tinbergen & Dietz 1994; Moreno *et al.* 1999). A 50μL blood sample was then taken and females were released. Females were caught again 48h42min ± 41min after the injection and a second 50μL blood sample was taken to estimate isotope elimination. To limit the amount of blood taken from each female, 12 non-experimental females in 2012 and 20 in 2013 were sampled to estimate the background level of isotope enrichment for a given year (mean ± S.D.: δD = -41,9 ± 5,6 ‰ and δ^{18}O = -1,7 ± 0,6 ‰ in 2012; δD = - 41,7 ± 6.0 ‰ and δ^{18}O = -2.6 ± 0,6 ‰ in 2013). Blood samples were collected in heparinised glass capillaries and immediately flame-sealed.

After fieldwork, samples were cryo-distillated for about 10 min under a vacuum system. Each sample was measured four times and, for each measurement, 0.1μL distillate was injected into an elemental analyser with thermal conversion (TC/EA) connected to a continuous-flow isotope ratio mass spectrometer (IRMS DELTA V PLUS, Thermo Scientific, Waltham, MA, USA). Each measure was first corrected for drift and memory effect, then normalized to the VSMOW2/SLAP2 international scale.

CO2 production (mol/day) was calculated according to Lifson modified by Speakman (single pool model; Speakman 1997; Speakman & Król 2005) and converted to field metabolic rate (kJ/day) using Weir's equation (de V. Weir 1949), assuming a food quotient of 0.8 for these insectivorous birds (Williams 1987). Due to technical problems, reliable measures were obtained on 38 females only out of 138 injected females (61 in 2012 and 77 in 2013), all sampled in 2013.

2.4. Measures of markers of the oxidative balance

To measure blood markers of oxidative state, a 40μL blood sample was taken from the brachial vein into heparin-coated Microvettes (Sarstedt, Nümbrecht, Germany). Blood samples were maintained at 5°C in the field before being centrifuged in the evening to separate plasma from red blood cells. Plasma and red blood cells were then stored at -80°C.

Two oxidative state markers were measured: reactive oxygen metabolites (ROM) concentration and plasma antioxidant capacity. Plasma concentration of ROM was measured

using the d-ROMs test (MC0001 kit, Diacron International, Grosseto, Italy): 4μL of plasma were mixed with 198μL acidic buffer and 2μL chromogenic substrate (N,N-diethylparaphenilendiamine) and left to incubate for 75mn at 37°C, before measuring OD at 550nm. Haemolysed samples with a light orange or pink to bright red colouration were excluded visually (N = 104). To control for the natural opacity of some samples, 5 samples with $OD_{800nm} > 0.100$ were excluded from the analysis. The inter-assay CV was 18.9% on 46 duplicates and the intra-assay CV 12.7% on 20 duplicates. As this assay mainly measures lipid peroxides, it may be influenced by the concentration of triglycerides in the plasma (Pérez-Rodríguez *et al.* 2015). However, preliminary analyses on a subset of our samples provide no support for such association in our study species, and thus we did not control for the concentration of triglycerides in our analyses (Supplementary Information S1). Plasma antioxidant capacity was measured by the capacity of plasma to oppose the oxidative action of the hypochlorous acid HClO (OXY adsorbent test, MC434 kit, Diacron International, Grosseto, Italy). This assay is less sensitive to variations in uric acid concentration in the plasma than other methods (Costantini 2011). Each plasma sample was diluted at 1/100 in ultra-pure water. 5μL diluted sample were incubated 10mn at 37°C with 200μL HClO solution. 2μL chromogenic substrate (N,N-diethylparaphenilendiamine) were then added and OD at 550nm was measured to quantify HClO excess. The inter-assay CV was 10.1% on 33 duplicates and the intra-assay CV 6.5% on 45 duplicates.

2.5. Statistical analyses of repeatability

To assess the correlation between the physiological markers measured on different years but at the same breeding stage in a given individual, we modelled ROM concentration and antioxidant capacity in (i) females sampled in incubation (ROM concentration: 214 observations of 202 individuals; antioxidant capacity: 251 observations of 234 individuals), and (ii) males and females sampled during chicks feeding (ROM concentration: 662 observations of 535 individuals; antioxidant capacity: 838 observations of 652 individuals) through separate linear mixed-effects models with individual identity and assay as random effects, and a fixed effect of year. The interaction between sex and manipulations was added

in the models describing the variables during chicks feeding. Repeatability was calculated as the ratio of between-individual random variance on residual variance. Its significance was tested using a loglikelihood-ratio test on the between-individual random effect.

To assess the correlation between the oxidative state markers measured at different breeding stages within the same year in a given individual, we modelled ROM concentration and antioxidant capacity through linear mixed-effects models with individual identity, individual identity within a year, and assay as random effects, and year and the interaction of breeding stage and manipulation as fixed effects, on females sampled during incubation and/or chicks feeding (ROM concentration: 535 observations of 310 individuals; antioxidant capacity: 681 observations of 359 individuals). Repeatability was calculated as the ratio of the individual within year random variance on the sum of the individual and residual variances. Its significance was tested using a loglikelihood-ratio test on the individual within year random effect. Including body mass as a covariate in any of the models above did not change the variance estimates, and there was no significant effect of body mass, thus the models reported do not include body mass.

The parameters of the univariate models for the repeatability analyses were estimated by restricted maximum likelihood (REML) using the *lmer* function in R (Bates *et al.* 2014). The significance of the fixed effect was tested by F-tests with Satterthwaite estimation for the denominator degree of freedom, using the function *anova* from the *lmerTest* library (Kuznetsova, Brockhoff & Christensen 2013).

2.6. Statistical analyses of the relationships between traits

Studying the relationship between two non-controlled variables is a classic situation in ecology. Although this is easily done using orthogonal regression and correlation methods in very simple cases, methods taking into account potential interactions with other variables and complex variance structures are not well implemented. As we were interested in the effect of the experimental treatment on the relationships between markers, we needed a method to test the differences in slope according to the treatment. Moreover, given that the random error for ROM concentration and antioxidant capacity were structured with

individual and assay random terms, these should be modelled when assessing the correlation between markers, to avoid spurious correlations to arise from pseudoreplication or methodological biases. Indeed, samples were usually analysed on the same day for ROM concentration and antioxidant capacity to avoid freeze-thaw cycles. We thus chose to use two statistical methods in parallel to describe the relationships between physiological markers. First, we modelled one variable as a function of another in a univariate mixed-effects regression framework to study the effect of experimental treatment, year and sex on the slope of the relationship. In that case, only the random structure of the response variable could be modelled. In a second step, we modelled the two variables as responses in a multivariate mixed-effects regression framework, which allowed us to estimate the correlation coefficients at the individual and residual levels, while correcting for the random structure of both variables. Although the correlation coefficients could be estimated separately for the different experimental groups, potential differences in slope could not be formally tested for. We present and discuss the results from both methods together as they complement each other.

In both cases, the response variables were standardized to mean zero and variance one within each year, treatment and sex to account for potential differences in mean and variance between these groups.

To test for differences in the slopes of the relationship between ROM concentration and antioxidant capacity according to experimental treatment, year and sex, ROM concentration was modelled in a linear mixed effects model as a function of antioxidant capacity, its two-way interaction with year, with sex and with experimental treatment, and its three-way interaction with year and sex and with year and experimental treatment. The simple effects of year, sex and experimental treatment were not modelled. Individual identity and the assay for the measure of ROM concentration were included as random effects. Both ROM concentration and antioxidant capacity were then modelled as a function of field metabolic rate and its interaction with the experimental treatment for females in 2013, with the assay for the measure of respectively ROM concentration or antioxidant capacity as random effect. Fixed effects were selected by stepwise elimination, starting with interactions.

Selection criteria were the p-values of type-III F-tests for LMM, with denominator degrees of freedom calculated using Satterthwaite's approximation (R package 'lmerTest', function *anova*, Kuznetsova, Brockhoff & Christensen 2013). No selection was performed on random effects, which were thus kept in all final models. The homoscedasticity and normality of residuals were checked graphically.

To investigate the correlation between ROM concentration and antioxidant capacity, the two variables were modelled in a bivariate mixed-effects model with a random effect of the assay for antioxidant capacity and ROM concentration, as well as a common random effect of individual identity when pooling multiple years. These analyses were conducted for all nests together, then separately for control nests (all years), females that were handicapped (2012-2013), males whose female was handicapped (2012-2013), and food supplemented nests (2014 only). The covariance was estimated at the between- and within-individual levels when studying multiple years together. The total phenotypic variance-covariance matrix was computed as the sum of the between- and within-individual variance-covariance matrices. Only the phenotypic covariance is reported when there were less than 25 individuals with multiple measures. The correlation between field metabolic rate and respectively ROM concentration and antioxidant capacity were studied separately in bivariate mixed-effects models with a random effect of the assay. Only the phenotypic covariance was estimated.

The bivariate models' parameters were estimated in a Bayesian framework that allowed us to fit different random effects for each response variable and to estimate their covariance at the between- and within-individual levels. The priors for the fixed effects estimates were set to a multinomial distribution with expected values of 0 and a diagonal variance-covariance matrix with a low strength of belief (10^{10}). The priors were set to inverse-Wishart distributions with the variances set to $1/n_i$ where n_i was the number of variance components estimated for the parameter i, null covariances, and a degree of belief equal to the dimension of the variance-covariance matrix for the parameter. Pilot studies showed that the value used for the covariances were quite informative on the posterior distribution of the individual and residual correlations, but not on the phenotypic correlation. The analyses were performed with Markov chain Monte Carlo sampling using the *MCMCglmm* function

in R (Hadfield 2010), with 110000 iterations, a burn-in period of 10000, and a thinning interval of 50, to obtain autocorrelation values lower than 0.10 and an effective sample size higher than 1400 for all variables. The reported results are the mode of the posterior distribution as point estimate and the Highest Posterior Density as 95% credibility interval.

3. Results

3.1. Inter-annual repeatability of OXY and ROM

In females measured during incubation in different years, the repeatability of antioxidant capacity was high (r = 0.630, X^2_1 = 3.88, P = 0.049), whereas the repeatability of ROM concentration was very low and not significant (r = 0.033, X^2_1 = 0.05, P = 0.83).

In males and females measured during feeding in different years, the repeatability of antioxidant capacity was low but significant (r = 0.154, X^2_1 = 4.46, P = 0.03), whereas that of ROM concentration was not significant (r = 0.064, X^2_1 = 0.47, P = 0.50). There was no effect of the manipulations or their interaction with sex on antioxidant capacity (manipulations: $F_{2,812}$ = 1.55, P = 0.21; manipulations x sex = $F_{2,796}$ = 0.08, P = 0.92) or ROM concentration (manipulations: $F_{2,650}$ = 0.41, P = 0.67; manipulations x sex = $F_{2,640}$ = 0.17, P = 0.84). Antioxidant capacity was independent of sex ($F_{1,646}$ = 0.39, P = 0.54). ROM concentration was lower in males than in females (-0.095 ± 0.045, $F_{1,512}$ = 12.34, P = 0.0005).

3.2. Intra-annual repeatability of OXY and ROM

The repeatabilities of ROM concentration and antioxidant capacity measured at incubation and feeding of the same breeding event were both null and non-significant (r = 0.000, X^2_1< 0.01, P = 1.00). The food manipulation had a positive effect on antioxidant capacity between the incubation and breeding stage (interaction breeding stage x manipulations: 19.3 ± 6.9, $F_{2,428}$ = 3.34, P = 0.04, Figure 4). There was no effect of the manipulations, alone ($F_{2,388}$ = 1.08, P = 0.34) or in interaction with the breeding stage on ROM concentration ($F_{2,341}$ = 0.14, P = 0.87). ROM concentration was higher during nestling feeding compared to the incubation stage (+0.17 ± 0.06, $F_{1,100}$= 13.75, P = 0.0003, Figure 4).

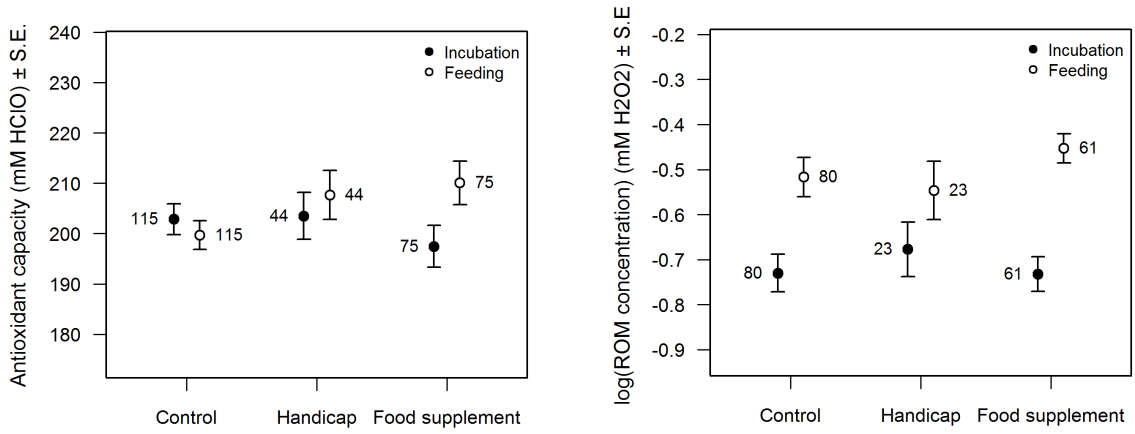


Figure 4: Antioxidant capacity and log-transformed ROM concentration by experimental treatment and breeding stage. Values were corrected for the year effect.

3.3. Correlations between physiological markers

Overall, there was a positive correlation between ROM concentration and antioxidant capacity at the phenotypic level (Table 3). The effect of antioxidant capacity on ROM concentration however differed according to the sex and the experimental group (interaction: $F_{2,629} = 3.03$, $P = 0.049$). More specifically, the effect of antioxidant capacity was independent of sex in control ($F_{1,291} = 1.99$, $P = 0.160$) and food supplemented nests ($F_{1,121} = 0.00$, $P = 0.995$), but was sex-dependent in nests were the females were handicapped ($F_{1,202} = 4.13$, $P = 0.044$): ROM concentration tended to increase with antioxidant capacity in handicapped females (0.20 ± 0.10, $F_{1,88} = 3.65$, $P = 0.060$) but not in males whose females had been handicapped (-0.01 ± 0.09, $F_{1,95} = 0.01$, $P = 0.933$). Although not significant, the relationship was also positive when pooling both sexes in control nests (0.08 ± 0.06, $F_{1,301} = 2.09$, $P = 0.149$) and food supplemented nests (0.17 ± 0.09, $F_{1,126} = 3.49$, $P = 0.064$). Consistently, there was a positive correlation in food-supplemented nests, and the estimates in control nests and in handicapped females were positive although not significantly so (Table 3). The differences between the experimental groups did not stem from differences between years, as there was no significant interaction between antioxidant capacity and year ($F_{2,633} = 1.05$, $P = 0.352$) or antioxidant capacity, year and sex ($F_{2,626} = 0.00$, $P = 0.999$). However, it should be noted that the positive correlation found in supplemented nests (in 2014) was not much stronger than

that in control nests in the same year, whereas the values were much lower in 2013 and especially 2012 (Table 3). Despite the increase in the strength of the correlation from 2012 to 2014 in control nests (Table 3) and the slight increase in the estimates of the slope (0.06 ± 0.10, $F_{1,67} = 0.38$, P = 0.541 in 2012; 0.07 ± 0.10, $F_{1,95} = 0.66$, P = 0.420 in 2013; 0.14 ± 0.10, $F_{1,109} = 1.85$, P = 0.176 in 2014), the interaction of year by antioxidant capacity was not significant either in control nests only ($F_{2,294} = 0.05$, P = 0.956).

When modelling ROM concentration as a function of field metabolic rate, the interaction between field metabolic rate and wing load manipulation group ($F_{1,20} = 1.70$, P = 0.207) and the effect of field metabolic rate ($F_{1,19} = 0.62$, P = 0.543) were not significant. Similarly, the effect of field metabolic rate on antioxidant capacity was not influenced by the wing load manipulation ($F_{1,28} = 0.39$, P = 0.538) and was non-significant ($F_{1,29} = 1.47$, P = 0.154). Consistently, the correlation coefficients were all positive but non-significantly so (Table 4). The coefficients' estimates were higher in handicapped than in control females for both relationships.

Table 3: Mode and 95% credibility interval of the posterior distribution of the coefficients of correlation between ROM concentration and antioxidant capacity, for different experimental groups, sex and year. The number of individuals is given in parentheses below the sample sizes when the samples covered multiple years, and thus some individuals were sampled more than once.

Treatment	**Sex**	**Years**	**N**	**Individual**	**Residual**	**Total (phenotypic)**
All	F + M	2012-2014	646 (527)	0.289 [-0.245 – 0.643]	-0.076 [-0.038 – 0.193]	**0.102 [0.034 – 0.188]**
Control	F + M	2012-2014	307 (274)	0.029 [-0.561 – 0.538]	0.137 [-0.061 – 0.312]	0.101 [-0.020 – 0.202]
Wing load handicap	F	2012-2013	91 (87)	-	-	0.181 [-0.012 – 0.400]
Wing load handicap	M	2012-2013	120 (112)	-	-	0.022 [-0.167 – 0.210]
Food supplementation	F + M	2014	128	-	-	**0.171 [0.021 – 0.350]**
Control	F + M	2012	72	-	-	0.015 [-0.175 – 0.272]
Control	F + M	2013	124	-	-	0.075 [-0.099 – 0.260]
Control	F + M	2014	111	-	-	0.155 [-0.044 – 0.334]

Table 4: Mode and 95% credibility interval of the posterior distribution of the coefficients of correlation between field metabolic rate and antioxidant capacity and ROM, respectively, for both experimental groups, control, or handicapped females in 2013.

Treatment	Sex	Years	N	FMR and OXY	N	FMR and ROMs
All	F	2013	34	0.260 [-0.120 – 0.522]	23	0.232 [-0.255 – 0.532]
Control	F	2013	16	0.107 [-0.440 – 0.560]	12	0.153 [-0.453 – 0.650]
Handicapped	F	2013	18	0.427 [-0.053 – 0.722]	11	0.412 [-0.233 – 0.793]

Additional analyses showed that the variations in field metabolic rate were partly explained by parental behaviours, but these were not correlated with oxidative balance (Supplementary Information S2).

4. Discussion

In this study, we aimed at describing the sources of variation and covariation between two plasmatic markers of the oxidative balance, and at testing the role of energy requirements in modulating their covariation. We also explored directly their correlation with energy metabolism, measured through the doubly-labelled water method. Individual identity and food availability were important determinants of antioxidant capacity, but not ROM concentration. All three physiological traits covaried or tended to covary positively. The correlations between traits were stronger in handicapped females and tended to be stronger in 2014 compared to the previous years, but relieving energetic constraints through food supplementation did not lessen the correlation.

Antioxidant capacity at a given breeding stage was repeatable between years, but not when comparing values measured during incubation and nestling feeding in the same year. The repeatability was higher in incubation (r = 0.630, P = 0.049) than during nestling feeding (r = 0.154, P = 0.03), maybe because uncontrolled environmental factors had a stronger effect during nestling feeding. It thus seems that the between-year variations in antioxidant capacity are partly determined by individual characteristics, such as the ability to find higher quality food rich in antioxidants, to acquire a better territory or to produce more enzymatic antioxidants and spare the dietary antioxidant pools. Similarly, within-individual

repeatability among breeding seasons was found in barn swallows *Hirundo rustica* (r = 0.487, P < 0.001, Saino *et al.* 2011), Seychelles warblers *Acrocephalus sechellensis* (*r* = 0.122, P = 0.043, van de Crommenacker *et al.* 2011) and in European shags *Phalacrocorax aristotelis*, although repeatability varied with age (2-9 years old: *r* = 0.20, P = 0.36; 10 to 22 years old: *r* = 0.33, P = 0.020; Herborn *et al.* 2015). The individual characteristics involved are yet not the same or do not act in the same way at different breeding stages, with an absence of correlation between breeding stages (*r* = 0.000, P = 1.00). Such absence of repeatability in antioxidant defences was reported in non-breeding captive greenfinches *Carduelis chloris* (*r* = 0.21, P = 0.29, Sepp *et al.* 2012). Conversely, antioxidant capacity was repeatable over a month in captive non-breeding European kestrels *Falco tinnunculus* (r = 0.30, P < 0.001, Costantini *et al.* 2007), within breeding season in barn swallows, although the exact breeding stage at which birds were repeatedly sampled was unknown (r = 0.50, P < 0.001, Saino *et al.* 2011), and between pre-mating and post-weaning measures in mice (r = 0.178, P < 0.034, Stier *et al.* 2012). The absence of correlation within a given year in our study was associated with increases in antioxidant capacity in food-supplemented females between incubation and nestling feeding and could thus be partly explained by individual variation in the response to food supplementation. Likewise, when experimentally manipulating energy expenditure or oxidative balance in birds, measures of antioxidant capacity were only repeatable in control birds (*r* = 0.71, P = 0.018 in control birds, P > 0.1 in birds treated with paraquat, Meitern *et al.* 2013) or were not correlated across different treatments (mean *r* ± s.e.m. = 0.124 ± 0.111, Beamonte-Barrientos & Verhulst 2013). The role of energy constraints on the oxidative balance is highlighted by the positive effect of food supplementation on antioxidant capacity. Similar experiments also impacted the oxidative balance, although not always the antioxidant component: food supplementation decreased ROM concentration but had no effect on antioxidant capacity in breeding female great tits (Giordano *et al.* 2015). Using different markers of oxidative stress, Fletcher *et al.* (2013) found a higher antioxidant capacity and lower oxidative damages in American red squirrel *Tamiasciurus hudsonicus*.

Variations in ROM concentration were not repeatable either between- (incubation: *r* = 0.033, P = 0.83; nestlings feeding: *r* = 0.064, P = 0.50) or within-year (*r* = 0.000, P = 1.00) and

were thus mainly determined by external factors or physiological changes, apart from a difference between males and females. This is in contrast with studies of ROM that found individual consistency even under different experimental treatments (Stier *et al.* 2012; Beamonte-Barrientos & Verhulst 2013; Herborn *et al.* 2015; but see van de Crommenacker *et al.* 2011). Individual consistency in ROM measurements can however vary with age (Herborn *et al.* 2015). ROM concentrations were strongly influenced by the changes in physiology between incubation and nestling feeding in females, with a strong increase between stages, but were not influenced by the experimental manipulations of energetic constraints.

Males during nestlings feeding and incubating females had lower ROM concentration than females feeding nestling, whereas no effect of sex and breeding stage was found on antioxidant capacity. In mice, ROM concentration increased in breeding females, but no in non-breeding ones, whereas no effect of the breeding status was found on antioxidant capacity (Stier *et al.* 2012). This effect can be conditional on other constraints experienced by the individual: ROM levels increased during nestlings feeding in female Seychelles warblers naturally infected with malaria but not in uninfected females (van de Crommenacker et al. 2012). However, antioxidant capacity significantly decreased over the breeding season in that species. The increase between breeding stages in females might reflect the costs of reproduction or the fact that oxidative damages are adaptively reduced in females during egg-laying to avoid negative effects on offspring, a process called oxidative shielding (Giordano *et al.* 2015; Blount *et al.* 2015). Testing whether reproductive costs are higher in females than in males, or whether the observed differences are only due to lower baseline oxidative damages in males, would require sampling males before hatching, which is difficult in that species.

Antioxidant capacity was positively correlated with ROM concentration when considering all individuals irrespective of their experimental treatment (mode [95% CI] = 0.102 [0.034 – 0.188]). This correlation seemed to lie at the between-individual level rather than be a within-individual trade-off, although our power to separate these two sources of variation was limited. This is consistent with a role of individual quality in this correlation, with some individuals being able to mount more efficient antioxidant defences. Despite the

low sample size (N = 18 and 11 respectively), our results also suggested a positive but non-significant correlation between metabolic rate and both antioxidant capacity and oxidative damages in handicapped females. We cannot conclude to an absence of correlation or a positive correlation between metabolism and oxidative balance, but a negative correlation is unlikely. Further investigations are needed to confirm this relationship and test whether it translates to the within-individual level. It would however be consistent with a further study of mitochondrial function on the same population in 2014, where we found that ATP production during nestlings feeding was positively correlated with total reactive species (including both reactive oxygen species and reactive nitrogen species, derived from the oxidation of dinitrogen) in the mitochondria and with ROM concentration in the plasma (Annexe I). An absence of relationship, at least when energetic constraints remain low, would suggest that a higher metabolic rate does not systematically translate into higher oxidative damages because relatively cheap defence mechanisms can be mobilised, such as uncoupling proteins (Stier *et al.* 2014a) or enzymatic antioxidants (Isaksson *et al.* 2011).

The positive relationships we observed are inconsistent with the hypothesis that circulating antioxidants are used up to protect against the reactive oxygen species produced through increased metabolism. In that case, antioxidant defences should correlate negatively with metabolic rate and oxidative damages or be modified in opposite ways by individual and environmental factors (Fletcher *et al.* 2013; Yang *et al.* 2013; Hanssen *et al.* 2013; López-Arrabé *et al.* 2014, 2015). Positive correlations between metabolic rate and antioxidant capacity were however found in some birds (Cohen, Hau & Wikelski 2008), as well as positive correlations or variation in the same direction between oxidative damages and antioxidant capacity (van de Crommenacker *et al.* 2011; Stier *et al.* 2012; Isaksson 2013; Xu *et al.* 2014; Beaulieu *et al.* 2015; Vaugoyeau *et al.* 2015). Antioxidant protection could thus adaptively build up to face increased exposure to pro-oxidants in periods of higher energy demands, especially in breeding females in which oxidative stress could be particularly harmful for offspring (Blount *et al.* 2015). In our population also, antioxidant capacity is increased in response to increased oxidation, and possibly in response to increased metabolic rate. This positive correlation in the plasma could be due to an increase in antioxidant

defences to partially counter higher oxidative damages in some individuals. Contrary to other by-products of dietary lipids oxidation, hydroperoxides are degraded in the stomach (Kanazawa & Ashida 1998) and have a low uptake by intestinal cells (Maestre *et al.* 2013). Their contribution to plasma ROM concentration is most likely low.

The relationship between ROM concentration and antioxidant capacity was sensitive to variation in the constraints experienced by the individuals. First, it was stronger in handicapped females in 2012-2013, which experienced stronger energetic constraints (0.181 [-0.012 – 0.400]). The role of food availability did not appear very strong, as there was no noticeable difference between the correlation in control nests (0.155 [-0.044 – 0.334]) and in food-supplemented nests (0.171 [0.021 – 0.350]) in 2014. It is however possible that the treatment had not been long enough at the time of sampling to produce a difference. Second, the strength and slope of the relationship increased from 2012 to 2014, although not significantly so. This hints at a role of environmental variables in modulating the correlation between antioxidant defences and oxidative damages. Indeed, particularly sunny and dry meteorological conditions in 2012 and particularly rainy and cold conditions in 2014 resulted in strong differences in mortality rate among nestlings and thus in reproductive performances (mean fledging success in control nests ± S.D. (N) = 4.7 ± 2.6 (89) in 2012, 3.0 ± 2.4 (99) in 2013, and 1.5 ± 2.0 (82) in 2014). The relationship between oxidative damages and antioxidant capacity might thus be stronger when environmental conditions are harsher, although this cannot be tested correlatively with only three successive years. Overall, despite the low values of the correlations measured, these were more salient when individuals were energetically constrained experimentally or naturally. Similarly, the estimates of the correlation coefficient between field metabolic rate and the markers of oxidative balance were higher in handicapped vs. control females, although non-significantly so. The relationship between metabolism and oxidative balance, as well as among different components of the oxidative balance, were not fixed but modulated by individual condition and environmental quality.

Manipulating food availability or activity levels in laboratory experiments might allow a better understanding of the actual relationships between metabolism and oxidative

balance. Alternatively, studies from wild populations might enlighten them, if enough relevant variation in individual and environmental conditions can be measured or if these can be experimentally manipulated. Several limitations will however hinder the measure of relevant biomarkers of oxidative balance in free-ranging populations. The oxidative processes, and thus markers, often vary between tissues (Veskoukis *et al.* 2009; Speakman & Garratt 2014; Blount *et al.* 2015). As oxidised fatty acids are mostly recycled within the cells and not eliminated in the general circulation (Hulbert *et al.* 2007), additional measures in metabolically active tissues, such as muscles, liver or brain, would have been necessary to thoroughly evaluate whether ROM concentration in the plasma also reflect processes in organs in our study. As blood is often the only available tissue when working on wild populations, designing adequate studies in captive population might give a deeper insight in these relationships. The timing of sampling in the wild is also often constrained by the biology of the species and by field constraints. Some markers can vary transiently in response to stressors or antioxidant consumption (Cohen *et al.* 2008; Beaulieu & Schaefer 2014), and different biomarkers can have different timing of response to such interventions (Nikolaidis *et al.* 2008; Niki 2010), therefore limiting the inferences that can be drawn from one-time measures. More detailed studies of the time-course of pro- and antioxidant biomarkers in different species would help their interpretation. Finally, our study shows that relationships between markers of oxidative state can be relatively weak and large sample sizes will be required under even the harsher conditions within the natural range.

Overall, we demonstrate that individuals were consistent between years in antioxidant capacity, but not ROM levels, and that the relationships between these two markers are conditional on the energetic constraints experienced by each individual: correlations between traits are stronger when wing load was slightly increased experimentally or when environmental conditions were naturally poorer. This probably comes from a tighter adjustment of antioxidant defences to free radicals production when conditions were constrained, either by increasing non-enzymatic antioxidant acquisition or enzymatic antioxidant production, or by decreasing the production of free radicals to match the available antioxidants. As non-enzymatic antioxidant defences were repeatable between

years, an individual quality, and in particular its ability to acquire high-quality territories or to find antioxidant-rich food, might play an important role in its ability to respond to oxidative stress. Understanding which are the factors modulating the correlations between markers of oxidative balance could eventually help explain the variation in these correlations among studies, species and populations.

Acknowledgements.

Many thanks to Jukka Forsman and the landowners on Gotland for access to the field sites, to Lars Gustafsson for logistics on the field and to three successive teams of field assistants for their precious help on the field. This work was supported by the French Ministry of Research (PhD fellowship to CR), the CNRS (PICS grant to BD), the Région Rhône-Alpes (CMIRA Explora'doc to CR), the Rectors' Conference of the Swiss Universities (mobility grant to CR), the Fédération de Recherche 41 BioEnvironnement et Santé (training grant to CR), the Fondation pour l'Université de Lausanne (grant to CR) and the Journal of Experimental Biology (travel grant to CR).

Supplementary Information S1: Correlation between markers of oxidative balance and nutritional state in the plasma.

In 2014, the concentrations of triglycerides, glucose and lactate were measured in whole blood immediately after blood taking using a portable test-strips reader designed for point-of-care measures in humans (Accutrend, Roche Diagnostics), whereas antioxidant capacity and ROM concentrations were measured through the OXY and d-ROMs tests, respectively. The quantity of blood deposited on the test-strip was 10μL for triglycerides and glucose and 15μL for lactate. ROM concentrations were not significantly correlated with triglycerides (Spearman's rank correlation test: ρ = -0.01, N = 76, S = 74136, P = 0.91), lactate concentrations (ρ = 0.18, N = 19, S = 937, P = 0.47), or glucose concentration (ρ = -0.37, N = 20, S = 1817, P = 0.11). There was no correlation between the total antioxidant capacity of the plasma and triglycerides (ρ = -0.16, N = 93, S = 155677, P = 0.12), lactate (ρ = 0.30, N = 21, S = 1072, P = 0.18) or glucose concentrations (ρ = -0.09, N = 24, S = 2510, P = 0.67).

Supplementary Information S2: Correlation between the physiological markers and breeding behaviour.

To investigate the importance of behavioural differences in determining energy expenditure and oxidative balance, we measured two components of reproductive effort, nestling feeding and nest defence against a predator, in 2013 and 2014 and studied their correlation with ROM concentration, antioxidant capacity and field metabolic rate.

1. Measure of feeding rate and nest defence score

When nestlings were five-days old, parental feeding rate was recorded during one hour by a camouflaged camera located at least six meters from the nest box. At the beginning of the recording period, the experimenter went to the nest and emptied the feeders if necessary (in food supplemented nests in 2014). Feeding rate was then calculated for each parent as the number of feeding events per hour and per nestling starting from the first feeding event until the end of the recording, to account for the latency before returning to the nest that could be influenced by the birds' response to the disturbance when setting the camera. Four nests for which the feeders had not been properly emptied before the test were excluded and 16 birds that only came to feed less than 20min before the end of the test were excluded, as their feeding rate could not be known with confidence. Individuals that did not come to feed during the entire test were excluded, as it was not possible to know whether they were prospecting elsewhere or were too disturbed when setting the camera.

Nest defence was measured when chicks were 13-days old by placing a stuffed red squirrel on the entrance of the nest box, mimicking a nest predator attack on nestlings. To avoid premature fledging, nest box entrance was closed during the test. The stuffed squirrel was left for no longer than 5min from the arrival of the second parent and no longer than 15 min from the observer's arrival. If no adult was seen, it was removed after 10min. An observer hidden under a camouflage net and sitting at least 10 meters from the nest box recorded the behaviour of the pair. Because some parents arrived less than 5 min before the end of the test, only behavioural responses during the 4.5min following an individual first sighting were available for all individuals and used in the analyses. One individual that arrived only 16s before the end of the test was considered absent during the test. Following

an exploratory multivariate analysis of the behavioural data (cf. chapter V), a nest defence score was computed based on the behaviours that best described the intensity of the response: the time spent within 2 meters of the box, the number of moves, and the presence/absence of direct attacks to the dummy (Table S2.1).

Table S2.1: Construction of the nest defence score. The cut-off values for the two quantitative variables are the tertiles in the whole population. Individuals that were not seen during the test were assigned a score of 0. If individuals attacked the dummy, the score was increased by 1. Thus the final score varied between 0 and 6.

Not seen: 0		Time spent within 2m of the nest box		
		$t \leq 4.0\%$	$4.0\% < t \leq 66.7\%$	$t > 66.7\%$
Activity = number of moves	$a \leq 7$	1	2	3
	$7 < a \leq 20$	2	3	4
	$a < 20$	3	4	5

2. Statistical analyses

The statistical analyses followed a two-steps procedure similar to that used for studying the relationships between the markers of oxidative balance. The effect of each behavioural traits on each marker of oxidative balance was studied in linear mixed models, with the physiological trait as a response variable, the behavioural trait and its interactions with year, with sex, with experimental treatment, with sex and year, and with sex and experimental treatment as fixed effects and the assay and individual identity as random effects. Field metabolic rate was modelled as a function of each behavioural variable and its interaction with the experimental treatment in a linear model (no random effects).

Each behavioural trait was then modelled in a trivariate linear mixed model, together with ROM concentration and antioxidant capacity. The random effects of the test date and observer for behavioural variables, the random effects of the assay for antioxidant capacity and ROM concentration, as well as a common random effect of individual identity when pooling multiple years were modelled. These analyses were conducted for all nests together (2013-2014), then separately for control nests (2013-2014), females that were handicapped (2013), males whose female was handicapped (2013), and food supplemented nests (2014).

The correlation between field metabolic rate and each behavioural variable were studied separately in bivariate mixed-effects models with random effects of the day and observer. Only the phenotypic covariance was estimated.

3. Results

When modelling ROM concentration and antioxidant capacity as a function of feeding rate or nest defence behaviour, and their interaction with year, sex, and experimental treatment, none of these effects were significant (Table S2.2). Consistently, there was no robust correlation between the oxidative balance markers and feeding rate or the nest defence score in the whole dataset or any of the subsets considered (Tables S2.3 and S2.4).

When modelling field metabolic rate as a function of feeding rate, the interaction between feeding rate and experimental group ($F_{1,25} = 0.77$, $P = 0.39$) and the effect of feeding rate ($F_{1,26} = 2.59$, $P = 0.12$) were not significant and no robust correlations were found between these two variables (Table S2.5). The field metabolic rate increased with the nest defence score (0.34 ± 0.14, $F_{1,33} = 5.92$, $P = 0.02$), independently of the wing load manipulation ($F_{1,32} = 0.09$, $P = 0.76$). Consistently, the field metabolic rate and nest defence score were significantly positively correlated when pooling control and handicapped females, and the estimates were similar, although non significant, in both groups separately (Table S2.5).

Table S2.2 Effect of feeding rate on the markers of oxidative balance and marginal effects of the interactions with sex, year and experimental treatment. The sample size and number of different individuals are given beside the response variable.

Response variable	Effect	Estimate	F	Num. d.f.	Denom. d.f.	P
ROM concentration (N = 314, N_{ind} = 287)						
	Feeding rate	-0.00 ± 0.05	0.00	1	306	0.969
	Feeding rate:sex		0.54	1	303	0.462
	Feeding rate:year		0.15	1	307	0.695
	Feeding rate:treatment		0.33	2	302	0.719
	Feeding rate:sex:year		0.79	1	301	0.455
	Feeding rate:sex:treatment		0.39	2	300	0.680
Antioxidant capacity (N = 379, N_{ind} = 332)						
	Feeding rate	0.01 ± 0.05	0.06	1	380	0.813
	Feeding rate:sex		3.50	1	379	0.062
	Feeding rate:year		0.00	1	377	0.960
	Feeding rate:treatment		0.52	2	376	0.597
	Feeding rate:sex:year		0.99	1	378	0.319
	Feeding rate:sex:treatment		0.53	2	375	0.590
ROM concentration (N = 379, N_{ind} = 332)						
	Nest defence	0.02 ± 0.05	0.21	1	369	0.646
	Nest defence:sex		-0.56	1	371	0.574
	Nest defence:year		0.36	1	361	0.547
	Nest defence:treatment		0.00	2	369	0.997
	Nest defence:sex:year		1.46	1	365	0.227
	Nest defence:sex:treatment		2.62	2	364	0.074
Antioxidant capacity (N = 469, N_{ind} = 403)						
	Nest defence	0.02 ± 0.04	0.19	1	452	0.664
	Nest defence:sex		0.87	1	449	0.353
	Nest defence:year		0.36	1	402	0.549
	Nest defence:treatment		0.47	2	423	0.624
	Nest defence:sex:year		0.21	1	404	0.649
	Nest defence:sex:treatment		1.04	2	417	0.355

Table S2.3: Mode and 95% credibility interval of the posterior distribution of the coefficients of correlation between feeding rate and the markers of oxidative balance, for different experimental groups, sex and year. The number of individuals is given into parentheses below the sample size.

Treatment	Sex	Years	N	Feeding rate and ROMs	Feeding rate and TAC
All (individual)	F + M	2013-2014	314 (287)	0.182 [-0.364 – 0.625]	-0.386 [-0.655 – 0.433]
All (residual)				-0.100 [-0.336 – 0.184]	0.047 [-0.256 – 0.337]
All (phenotypic)				0.010 [-0.111 – 0.107]	-0.044 [-0.166 – 0.061]
Control	F + M	2013-2014	158 (149)	-0.057 [-0.215 – 0.106]	-0.103 [-0.268 – 0.054]
Wing load handicap	F	2013	24	0.305 [-0.138 – 0.647]	-0.321 [-0.674 – 0.178]
Wing load handicap	M	2013	30	-0.073 [-0.455 – 0.338]	0.085 [-0.313 – 0.456]
Food supplementation	F + M	2014	102	0.019 [-0.160 – 0.225]	0.061 [-0.132 – 0.248]

Table S2.4: Mode and 95% credibility interval of the posterior distribution of the coefficients of correlation between nest defence and the markers of oxidative balance, for different experimental groups, sex and year. The number of individual is given into parentheses below the sample size.

Treatment	Sex	Years	N	Nest defence and ROMs	Nest defence and TAC
All (individual)	F + M	2013-2014	379 (332)	-0.073 [-0.432 – 0.526]	0.203 [-0.365 – 0.555]
All (residual)				0.033 [-0.140 – 0.195]	-0.043 [-0.266 – 0.179]
All (phenotypic)				0.020 [-0.084 – 0.124]	0.022 [-0.081 – 0.122]
Control	F + M	2013-2014	172 (160)	0.047 [-0.093 – 0.207]	-0.053 [-0.198 – 0.119]
Wing load handicap	F	2013	38	-0.211 [-0.543 – 0.112]	0.037 [-0.349 – 0.335]
Wing load handicap	M	2013	53	0.183 [-0.150 – 0.428]	-0.109 [-0.374 – 0.212]
Food supplementation	F + M	2014	116	0.045 [-0.145 – 0.238]	**0.174 [0.016 – 0.379]***

*Excluding the outlying lowest value of antioxidant capacity in food supplemented individuals gave a non-significant and much lower correlation with the nest defence score: 0.084 [-0.114 – 0.267].

Table S2.5 Mode and 95% credibility interval of the posterior distribution of the coefficients of correlation between feeding rate or nest defence score and field metabolic rate, for both groups and control and handicapped females separately in 2013.

Treatment	Sex	Years	N	Feeding rate and FMR	N	Nest defence and FMR
All	F	2013	30	-0.377 [-0.680 – 0.020]	35	**0.417 [0.073 – 0.657]**
Control	F	2013	15	**-0.593 [-0.860 – -0.055]***	17	0.311 [-0.262 – 0.669]
Handicapped	F	2013	15	-0.037 [-0.589 – 0.473]	18	0.489 [-0.023 – 0.790]

*Excluding the outlying highest value of feeding rate gave a non-significant correlation with the field metabolic rate: -0.361 [-0.751 – 0.300].

4. Discussion

Studies in various bird species have shown a positive relation between metabolic rate and feeding rate or activity levels (Bryant & Tatner 1991; Nilsson 2002). Field metabolic rate indeed increased with the intensity of nest defence, but it also decreased with feeding rate in control nests. Field metabolic rate integrate both resting metabolic rate and the costs of the additional activities measured through behavioural tests. Yet resting metabolic rate can correlate with personality traits, and especially social dominance, aggressiveness and boldness (Biro & Stamps 2010). Further work should investigate whether the counterintuitive relationship between feeding rate and metabolic rate might actually reflect a positive correlation between boldness and metabolic rate, as suggested by the correlation between nest defence toward a predator and metabolic rate. Given the low sample size and the lack of robustness of this last result, further data would anyway be required before concluding to a negative relationship in this population.

Chapter IV – Responses of dispersing and philopatric individuals to experimentally increased energetic constraints

PAPER – DIFFERENCES IN THE OXIDATIVE BALANCE OF DISPERSING AND NON-DISPERSING INDIVIDUALS: AN EXPERIMENTAL APPROACH IN A PASSERINE BIRD

Charlotte Récapet, Alexandre Zahariev, Stéphane Blanc, Mathilde Arrivé, François Criscuolo, Pierre Bize, and Blandine Doligez

Submitted to *Functional Ecology*

Keywords: dispersal, energetic constraint, breeding density, daily energy expenditure, doubly-labelled water, reactive oxygen metabolites, antioxidant defences, oxidative stress, reproductive output, *Ficedula albicollis*

Dispersal is considered an energetically demanding behaviour that may entail costs through increased exposure to oxidative stress. Indeed, higher energy expenditure can lead to the production of highly reactive oxidative molecules that are deleterious to the organism if left uncontrolled. Thus dispersing and philopatric individuals may differ in their management of energy production and oxidative balance, especially under energetically demanding situations. To explore such differences, we experimentally increased flight costs via a wing load manipulation in female collared flycatchers (*Ficedula albicollis*) breeding in a patchy population and measured the effects of the manipulation on field metabolic rate, plasmatic markers of oxidative state and reproductive success of dispersing and philopatric females. The wing load manipulation successfully increased female energy expenditure independently of their dispersal status. Its impact on the oxidative balance however differed according to dispersal status: the concentration of reactive oxygen metabolites (ROM), a marker of pro-oxidant status, was higher in the manipulated than the control group in philopatric females only. Independently of the manipulation, plasma antioxidant capacity differed according to dispersal status: philopatric females showed higher antioxidant capacity than dispersing ones. Nestlings raised by philopatric females also had a higher fledging success. Interestingly, differences between dispersing and philopatric individuals also depended on habitat quality, as measured by local breeding density. Reactive oxygen metabolites showed opposite trends with increasing density between philopatric and dispersing females, and nestling body mass increased with increasing density in dispersing females only. Our results suggest that dispersing individuals maintain a stable oxidative state when facing challenging conditions, through a reduction of their reproductive effort. Conversely, philopatric individuals can adjust their effort to current conditions to maintain their reproductive output, possibly by taking advantage of a better knowledge of their environment to acquire higher quality resources. These differences may impact life-history strategies depending on dispersal.

1. Introduction

Dispersal, defined as a movement between the birth site and the first breeding site (natal dispersal) or between two successive breeding sites (breeding dispersal; Greenwood & Harvey 1982), has important ecological and evolutionary consequences both at the individual and the population level (Clobert *et al.* 2001; Matthysen 2012). Dispersal allows individuals to escape adverse conditions and thereby enhance their fitness. It is also a key driver of gene flow and metapopulation dynamics (Hanski 2001; Whitlock 2001).

Individual dispersal propensity often covaries with other behavioural, morphological and physiological traits (Bowler & Benton 2005; Hanski & Saccheri 2006; Clobert *et al.* 2009). These associations are thought to have evolved because they reduce the costs of movement and/or of settlement in the new habitat (Ronce 2007). Accordingly, dispersing individuals can show adaptations to movement, such as larger wings or fat store (O'Riain *et al.* 1996; Breuker *et al.* 2007). They also show adaptations to competitive encounters, such as higher aggressiveness (Duckworth & Badyaev 2007), and to exploration of a new habitat plot, such as higher exploratory behaviour (Dingemanse *et al.* 2003; Korsten *et al.* 2013; Debeffe *et al.* 2014), lower xenophobia (O'Riain *et al.* 1996) or higher immune response (O'Riain *et al.* 1996; Snoeijs *et al.* 2004).

Although associations with phenotypic traits may enhance dispersal success, they may also entail costs in terms of reproductive success or survival prospects, especially when resources are scarce (Hanski 2012). Indeed, most of the phenotypic traits associated with dispersal (e.g. high aggressiveness, exploration, immunity, or metabolic rate; Clobert *et al.* 2009) are likely to be energetically demanding (Debeffe *et al.* 2014). Due to such energetic constraints, dispersing and philopatric individuals may evolve different life-history strategies, with different relative investment in maintenance and reproduction (Dytham & Travis 2006; Cotto, Kubisch & Ronce 2014). Although metabolic requirements could play an important role in shaping these strategies (Hanski, Saastamoinen & Ovaskainen 2006), the physiological constraints that underlie life-history variation in relation to dispersal remain unclear.

Among the metabolic processes that could be involved in shaping such life-history variation, the regulation of the oxidative balance is expected to play a particularly important role. Energy production through aerobic metabolism leads to the production of highly unstable oxidative components, called reactive oxygen species or ROS (Finkel & Holbrook 2000; Balaban *et al.* 2005). ROS can damage the structure of biological macromolecules through oxidation and thereby disturb from cell to whole organism functioning, i.e. impose an oxidative stress (Halliwell & Gutteridge 2007; Costantini *et al.* 2010), but they are also messengers in central cell signalling pathways such as cell death signals (Kamata & Hirata 1999; Finkel 2003). If a higher metabolic rate is selected in dispersing individuals compared to philopatric ones to face increased energetic requirements, dispersers could thus be exposed to a higher production of ROS that could lead to more oxidative damage and reduced life expectancy. Oxidative damages can be prevented through antioxidant defences including inducible enzymes (such as the superoxide dismutase; Balaban *et al.* 2005) or molecules acquired through the diet (such as vitamin E; Halliwell & Gutteridge 2007). Therefore, dispersing individuals may also regulate a higher production of ROS via an increased investment in antioxidant defences, either internally produced or externally acquired. Thereby they could maintain high survival prospects possibly at the expense of other functions, in particular reproduction. Interestingly, both oxidative state and antioxidant defences have been linked to exploratory behaviour (Herborn *et al.* 2011; Arnold *et al.* 2015), a behavioural trait associated with dispersal (Korsten *et al.* 2013). Direct links however remain poorly investigated so far.

Here, we explored whether dispersing and philopatric individuals differ in metabolism and oxidative state during reproduction in a patchy population of a small passerine bird, the collared flycatcher *Ficedula albicollis*. We compared different markers of energy management and oxidative state (field metabolic rate, energy stores, oxidative damages and antioxidant capacity), as well as reproductive output, between individuals having or not dispersed between habitat plots. In addition, to test whether metabolic and oxidative differences between dispersing and philopatric individuals depend on the level of energetic demand, we experimentally manipulated this demand by increasing flight costs

(through reducing wing area) during reproduction. We focused on females because they can easily be manipulated during incubation in this species, allowing sufficient time for the manipulation to impact reproductive decisions during nestling rearing. If dispersing and philopatric females differ in their energy management or reproductive investment, they should respond differently to such an experimental handicap in terms of metabolism, oxidative balance and reproductive performance. Differences in these physiological parameters and/or reproductive output between dispersing and philopatric females could also arise from differences in habitat quality, either because dispersing and philopatric individuals respond differently to habitat quality or because they settle in habitat of different quality. Therefore, we also controlled for natural environmental variation in habitat quality, measured by the local breeding density of conspecifics, which positively relate to reproductive success in this population (Doligez et al. 2004). We predicted that differences in metabolism, oxidative balance or reproductive performance between dispersing and philopatric females could be manipulation- and/or environment-dependent. More specifically, we expected differences to be stronger in more constrained conditions (i.e. for manipulated females with higher flight cost and/or in low quality habitats), where dispersing females might either pay a higher cost through increased oxidative stress or decreased reproduction or show stronger defences against oxidative damage compared to philopatric females.

2. Material and methods

2.1. Study population and definition of dispersal

The study was conducted during the springs 2012 and 2013 on the island of Gotland, Sweden (57°07'N, 18°20'E). Collared flycatchers are hole-nesting passerine birds that readily accept to breed in the artificial nest boxes erected in the nine forest plots used for the study (between 13 and 78 nest boxes per plot distributed homogeneously). Nests were visited every third day to record laying date and clutch size. Close to hatching, nests were visited daily to record hatching date and number of hatched eggs. Nestlings were cross-fostered when two-

days old to measure post-hatching investment independently from pre-hatching effects (Supplementary Information S1). Females were first caught 5 to 12 days (on average 7.9 ± 0.9 (SD) days) after the start of incubation, then again when the nestlings were 5 to 16 days old (on average 8.8 ± 2.3 (SD) days). They were ringed if not previously ringed, weighed to the nearest 0.1g, aged (yearlings or older adults) based on plumage characteristics and their tarsus length was measured to the nearest 0.1mm by a single observer (C.R.). Nestlings were weighed and their tarsus length measured when 12 days old. After fledging, nests were checked for dead nestlings to record the final number of fledglings. The data and samples were collected under permission from the Ringing Centre of the Museum in Stockholm (bird catching, measuring and ringing) and the Ethical Committee for Experiments on Animals in Sweden (experimental manipulation of flight feathers and blood taking).

The study plots are separated mainly by habitat unsuitable for breeding in this species (fields and pastures). This spatially fragmented configuration allows defining dispersal as a change of breeding plot between birth and the first breeding event (natal dispersal) or between two consecutive breeding events (breeding dispersal; see Doligez *et al.* 1999 for a discussion of this binary definition of dispersal in this population). We considered in our analyses only previously ringed individuals, whose dispersal status is known (we excluded 143 unringed immigrant females out of 327 observations, i.e. 44.8%). Our final dataset included 97 females in 2012 and 87 females in 2013, among which 26 females were caught in both years.

2.2. Wing load manipulation

Female flight energy requirement was increased by cutting the two innermost primaries of each wing at their base to mimic feather loss at the onset of moult (Moreno *et al.* 1999; Sanz *et al.* 2000; Ardia & Clotfelter 2007). Upon capture during incubation, previously ringed females were alternatively assigned to the manipulated or the control group (same handling conditions but no feathers cut). Manipulated females did not differ from control ones in terms of main morphological and breeding characteristics (Supplementary Table S1).

2.3. Field metabolic rate and body composition

Field metabolic rate and body composition were measured using the two-point doubly-labelled water method (Lifson & McClintock 1966). Upon capture during the nestling feeding phase, females of known dispersal status were injected intraperitoneally with 30μL of a premixed solution composed of 0.6005g of 94% $H_2{}^{18}O$, 0.1514g of 99.99% D_2O and 2.8000g of 9‰ NaCl in 2012, and 1.2010g of 94% $H_2{}^{18}O$, 0.3028g of 99.99% D_2O and 2.0481g of 9‰ NaCl in 2013. These doses were calculated to obtain an *in vivo* enrichment of about 68 ‰ and 496 ‰ in 2012, and 135 ‰ and 992 ‰ in 2013, for ^{18}O and deuterium respectively (enrichment = $[R_{sample}/R_{standard} - 1]/1000$ with R being the ratio of heavy on light isotope).

After injection, females were kept in a cloth bag during 45 to 60 min so that the isotopes equilibrate with body water (Tinbergen & Dietz 1994; Moreno *et al.* 1999). After this period of time, a 50μL blood sample was taken and females were released. Females were caught again about 48h (on average 48h42min ± 41min) after the injection and a second 50μL blood sample was taken to estimate isotope elimination and thereby measure metabolic rate. To limit the amount of blood taken from each experimental female, 12 non-experimental females in 2012 and 20 in 2013 were sampled to estimate the background level of isotope enrichment for a given year (mean ± S.D.: δD = -41,9 ± 5,6 ‰ and $\delta^{18}O$ = -1,7 ± 0,6 ‰ in 2012; δD = - 41,7 ± 6.0 ‰ and $\delta^{18}O$ = -2.6 ± 0,6 ‰ in 2013). Blood samples were collected in heparinised glass capillaries and immediately flame-sealed.

After field work, samples were cryo-distillated for about 10 min under a vacuum system. Each sample was measured four times and, for each measurement, 0.1μL distillate was injected into an elemental analyser with thermal conversion (TC/EA) connected to a continuous-flow isotope ratio mass spectrometer (IRMS DELTA V PLUS, Thermo Scientific, Waltham, MA, USA). Each measure was first corrected for drift and memory effect, then normalized to the VSMOW2/SLAP2 international scale.

CO2 production (mol/day) was calculated according to Lifson modified by Speakman (single pool model; Speakman 1997; Speakman & Król 2005) and converted to field metabolic rate (kJ/day) using Weir's equation (de V. Weir 1949), assuming a food

quotient of 0.8 for these insectivorous birds (Williams 1987). Unfortunately, and despite doubling the enrichment in 2013 compared to 2012, we under-estimated the turnover rate. Consequently most final isotopic enrichments were close to background levels. Based on our analytical precision, only samples with final δD and $\delta^{18}O$ enrichments of respectively 20‰ and 5‰ above background levels should be kept in the calculations, which corresponded to 26 females only out of 117 injected females (61 in 2012 and 56 in 2013), all sampled in 2013. This sample of birds was not biased with respect to the combination of dispersal status and experimental group (Pearson's Chi-squared test: $X^2_3 = 1.25$, $P = 0.74$).

Body composition was calculated by hydrometry (Blanc *et al.* 2005) and was available for all 117 injected females, because it is measured before isotope elimination. Total body water was calculated from the 18-oxygen labelled water using a correction factor of 1.007 for exchange. Fat-free mass was derived from total body water and the average hydration coefficient (73.2%). Fat mass was calculated as the difference between total body mass and fat-free mass.

2.4. Oxidative state markers

To measure blood markers of oxidative state, a 40μL blood sample was taken from the brachial vein into heparin-coated Microvettes (Sarstedt, Nümbrecht, Germany) on females captured while feeding nestlings. Blood samples were maintained at 5°C in the field before being centrifuged in the evening to separate plasma from red blood cells. Plasma and red blood cells were then stored at -80°C.

Two oxidative state markers were measured: reactive oxygen metabolites (ROM) concentration and plasma antioxidant capacity. These markers have been related to reproductive output in different avian species (reviewed in Stier *et al.* 2012). Plasma concentration of ROM was measured using the d-ROMs test (MC0001 kit, Diacron International, Grosseto, Italy): 4μL of plasma were mixed with 198μL acidic buffer and 2μL chromogenic substrate (N,N-diethylparaphenilendiamine) and left to incubate for 75mn at 37°C, before measuring OD at 550nm. To control for the natural opacity of some samples, 15 samples with $OD_{800nm} > 0.100$ were excluded from the analysis. The mean intra-plate and

inter-plate CV were 20.9% on 9 samples and 22.4% on 35 samples, respectively. These high values were partly explained by the low absolute values for ROM concentration, an order of magnitude lower than measured in other bird species, and thus the larger relative measurement error (Stier *et al.* 2013; Wegmann *et al.* 2015b). ROM concentrations were however repeatable both in 2012 ($F_{19,20} = 2.80$, $P = 0.01$, $r = 0.474$) and 2013 ($F_{19,20} = 34.13$, $P < 0.001$, $r = 0.943$).

Plasma antioxidant capacity was measured by the capacity of plasma to oppose the oxidative action of the hypochlorous acid HClO (OXY adsorbent test, MC434 kit, Diacron International, Grosseto, Italy). Each plasma sample was diluted at 1/100 in ultra-pure water. 5μL diluted sample were incubated 10mn at 37°C with 200μL HClO solution. 2μL chromogenic substrate (N,N-diethylparaphenilendiamine) were then added and OD at 550nm was measured to quantify HClO excess. The mean intra-plate and inter-plate CV were 6.7% on 34 samples and 8.6% on 22 samples, respectively. Antioxidant capacity was repeatable both in 2012 ($F_{15,16} = 3.83$, $P = 0.006$, $r = 0.586$) and in 2013 ($F_{20,21} = 2.92$, $P = 0.009$, $r = 0.490$).

2.5. Statistical analyses

Factors affecting adult metabolism, oxidative state markers and body mass

We studied the effect of dispersal status and wing load manipulation on female body mass, body composition, field metabolic rate and oxidative state markers. We controlled for habitat quality at the plot scale by including as a covariate breeding density, measured as the proportion of available nest boxes occupied by flycatchers in a plot during the year considered. Plot density is positively correlated with plot fledging success in this population (Doligez *et al.* 2004) but unlike fledging success, it is unlikely to be affected by our wing load manipulation. A nest box was considered available to flycatchers when it contained no nest from another species, mainly great tit *Parus major* and blue tit *Cyanistes caeruleus*, up to five days after the earliest egg laying date for flycatchers in the same plot. Using local breeding success (i.e. average number of fledged young per nest in the plot) of control birds as an alternative measure of local habitat quality did not come to any significant link with

physiology and body mass, but drawing inferences from these results was hampered by different biases (detailed in Supplementary Information S2).

Because there were large differences in ROM and antioxidant capacity absolute values between 2012 and 2013 (Supplementary Table S2), probably due to differences in the experimental kits used in the laboratory, these values were centred and standardized within each year. Nevertheless, a year effect was included in the models to explore between-year differences in relevant biological processes.

In addition to dispersal status, wing load manipulation, plot density, nestling age on the day of parental sampling, brood size at hatching and year were included as fixed factors as well as all pairwise interactions between dispersal status, plot density and manipulation. For field metabolic rate, ROM concentration and plasma antioxidant capacity, adult body mass during nestling feeding was included as a covariate, and for body mass and body composition, tarsus length was included as a covariate. In addition, the time lapse between the expected sampling time (48h after equilibration) and the actual sampling time was also included as a covariate for field metabolic rate. To account for the non-independence of data for individuals measured in both years and for individuals breeding in the same plot, individual and plot were included as random effects in linear mixed models. The plate was also added as a random effect when modelling ROM concentration and antioxidant capacity. The effect of female age (yearling vs. older adult) and its interaction with dispersal status were included in preliminary analyses of adult metabolism, oxidative state markers and mass (as well as reproductive success) to account for potential differences between natal and breeding dispersal, which are under different selective pressures (Greenwood & Harvey 1982). Because they were retained in none of the final models, they are however not described in the results.

Factors affecting reproductive success

We investigated the effect of foster mothers' dispersal status on their nestlings' body mass when 12-days old, which is a predictor of future survival and recruitment (Lindén, Gustafsson & Pärt 1992), and their fledging success. Body mass was investigated using a

linear mixed model and fledging probability using a generalized linear mixed model with a logit link function and a binomial error distribution. Foster nest, nest of origin and plot were included as random effects. The dispersal status of the foster mother, its wing load manipulation and the foster plot density were included as fixed effects, as well as all pairwise interactions between dispersal status, manipulation and density. Year, female body mass during nestling feeding and brood size at hatching were included as covariates. For nestling body mass, weighing time was also included as a covariate.

In a second step, we directly tested the effect of female oxidative state on reproductive output. The foster mother antioxidant capacity and ROM concentration as well as their interaction with dispersal status, wing load manipulation, and plot density were included to the final models on nestling body mass and fledging success obtained in the first step.

Model selection

Fixed effects were selected by stepwise elimination, starting with interactions. Selection criteria were the p-values of type-III F-tests for LMM, with denominator degrees of freedom calculated using Satterthwaite's approximation (R package 'lmerTest', function *anova*, Kuznetsova, Brockhoff & Christensen 2013) and the p-values of type-III Wald chi-square tests for GLMM (R package 'car', function *Anova*, Fox & Weisberg 2011). No selection was performed on random effects, which were thus kept in all final models. The complete final models, as well as the partition of the random effect variances, are given in Supplementary Tables S3 and S4. The homoscedasticity and normality of residuals were checked graphically.

3. Results

3.1. Female metabolic rate, body mass and body mass composition

The field metabolic rate of females was not explained by their dispersal status, either alone ($F_{1,20}$ = 0.005, P = 0.94) or in interaction with the experimental wing load manipulation ($F_{1,17}$ = 0.02, P = 0.89). Nevertheless, field metabolic rate was higher in manipulated than

control females (estimate ± SE = 8.7 ± 3.5, $F_{1,19}$ = 6.10, P = 0.023) and decreased with brood size at hatching (-4.0 ± 1.6, $F_{1,20}$ = 6.54, P = 0.019). Female field metabolic rate was not significantly associated with plot density ($F_{1,3}$ = 1.07, P = 0.37), elimination time ($F_{1,22}$ = 0.17, P = 0.29), body mass ($F_{1,20}$ = 0.31, P = 0.58) or nestling age ($F_{1,22}$ = 1.37, P = 0.25).

Adult body mass did not differ according to dispersal status ($F_{1,165}$ = 2.43, P = 0.12), wing load manipulation ($F_{1,152}$ = 1.09, P = 0.30) or plot density ($F_{1,12}$ = 2.02, P = 0.18); all interactions between these variables were non-significant (all P > 0.11). Body mass however increased with tarsus length (0.48 ± 0.10, $F_{1,159}$ = 20.81, P < 0.0001), decreased with nestling age on the day of capture (-0.09 ± 0.02, $F_{1,131}$ = 19.11, P < 0.0001) and was lower in 2013 compared to 2012 (-0.17 ± 0.08, $F_{1,82}$ = 4.19, P = 0.04). Regarding the two components of body mass, neither fat-free nor fat mass differed between dispersing and philopatric females (fat-free mass: $F_{1,38}$ = 2.37, P = 0.13; fat mass: $F_{1,112}$ = 0.086, P = 0.77). Fat-free mass was however higher in manipulated females compared to control ones (0.18 ± 0.07, $F_{1,30}$ = 6.39, P = 0.017) and increased with plot density (1.10 ± 0.38, $F_{1,22}$ = 8.53, P = 0.008); all interactions between dispersal status, manipulation and plot density were non-significant (all P > 0.15). Fat-free mass also increased with tarsus length (0.60 ± 0.10, $F_{1,107}$ = 37.48, P < 0.0001), decreased with nestling age on the day of female capture (-0.05 ± 0.02, $F_{1,20}$ = 8.44, P = 0.009) and was lower in 2013 compared to 2012 (-0.56 ± 0.05, $F_{1,14}$ = 119.73, P < 0.0001). Fat mass did not differ between manipulated and control females ($F_{1,106}$ = 0.62, P = 0.43) and was not associated with plot density ($F_{1,17}$ = 0.30, P = 0.59) or tarsus length ($F_{1,80}$ = 0.21 , P = 0.65), but decreased with nestling age (-0.05 ± 0.02, $F_{1,109}$ = 9.13, P = 0.003) and was higher in 2013 compared to 2012 (0.49 ± 0.06, $F_{1,64}$ = 54.56, P < 0.0001). There was no effect of brood size at hatching on total body mass or its components (all P > 0.12).

3.2. Female oxidative state

The effect of wing load manipulation on ROM concentration depended on dispersal status (interaction dispersal status x manipulation: $F_{1,112}$ = 5.60, P = 0.02; Figure 5): among manipulated females, ROM concentration was higher in philopatric compared to dispersing females (0.40 ± 0.18, $F_{1,60}$ = 4.65, P = 0.035), while no difference was observed among control

females (-0.24 ± 0.19, $F_{1,56}$ = 1.48, P = 0.23). The effect of plot density on ROM concentration also depended on dispersal status (interaction dispersal status x plot density: $F_{1,116}$ = 5.28, P = 0.02; Figure 6), with inverse although non-significant relationships for dispersing and philopatric females: ROM concentration decreased with increasing plot density (but non-significantly so) among philopatric females (-0.95 ± 0.83, $F_{1,26}$ = 1.32, P = 0.26) whereas it increased (but non-significantly so) among dispersing females (0.62 ± 1.09, $F_{1,6}$ = 0.33, P = 0.59), leading to the overall significant interaction (Figure 6).

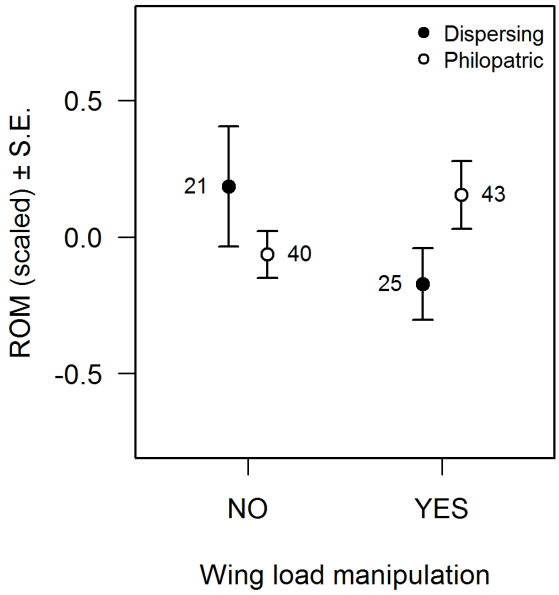


Figure 5: ROM concentration (scaled within each year) depending on wing load manipulation for dispersing and philopatric females.

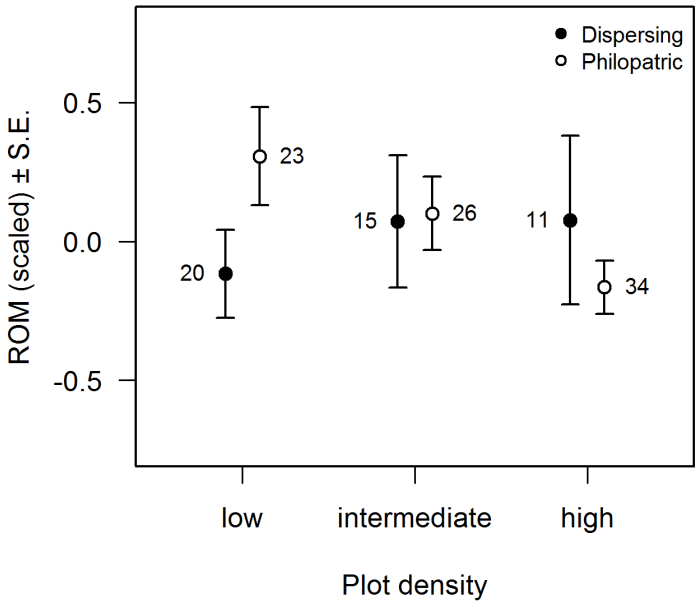


Figure 6: ROM concentration (scaled within each year) depending on plot density for dispersing and philopatric females. Plot density quantiles were used to define three density classes for the sake of illustration (low density: < 63.32% of nest boxes occupied, high density: ≥ 74.07%).

In addition, the effect of plot density on ROM concentration differed between manipulated and control females (interaction manipulation x plot density: $F_{1,116}$ = 6.01, P = 0.02), with again inverse although non-significant relationships for manipulated and control females: ROM concentration decreased with increasing plot density (but non-significantly so) among manipulated females (-1.39 ± 0.92, $F_{1,19}$ = 2.29, P = 0.15) whereas it increased (but non-significantly so) among control females (0.63 ± 0.82, $F_{1,56}$ = 0.60, P = 0.44), leading to the overall significant interaction. There was no effect of body mass ($F_{1,120}$ = 0.48, P = 0.49), brood size ($F_{1,119}$ = 0.01, P = 0.91) or nestling age ($F_{1,119}$ = 0.73, P = 0.39). Adding antioxidant capacity as a covariate yielded qualitatively similar results (not detailed here).

Antioxidant capacity was higher in philopatric females compared to dispersing ones (0.52 ± 0.18, $F_{1,145.95}$ = 8.19, P = 0.005). Wing load manipulation and plot density had no effect on antioxidant capacity, either alone (manipulation: $F_{1,146}$ = 0.01, P = 0.91; density: $F_{1,16}$ 2.36, P = 0.14) or in interaction with each other or with dispersal status (all P > 0.19). Finally, there was no effect of body mass ($F_{1,149}$ = 1.27, P = 0.26), brood size ($F_{1,154}$ = 0.55, P = 0.46) and nestling age ($F_{1,154}$ = 0.92, P = 0.34).

3.3. Nestling body mass and fledging success

The effect of plot density on 12 day-old nestling body mass differed between dispersing and philopatric foster mothers (interaction dispersal status x plot density: $F_{1,151}$ = 5.05, P = 0.026). Dispersing females reared nestlings with a lower body mass than philopatric females in low-density plots, but no difference was observed anymore when density increased (Figure 7). The wing load manipulation had no effect on nestling body mass, either alone or in interaction with the dispersal status of the mother or with plot density (all P > 0.18). Nestling body mass was also lower in 2013 compared to 2012 (-1.82 ± 0.23, $F_{1,164}$ = 64.28, P < 0.0001), decreased with increasing brood size (-0.31 ± 0.09, $F_{1,152}$ = 10.60, P = 0.001) and increased with the time at weighting (2.57 ± 0.86, $F_{1,156}$ = 8.99, P = 0.003). There was no effect of the body mass of the foster mother ($F_{1,134}$ = 1.55, P = 0.22) on nestlings' body mass.

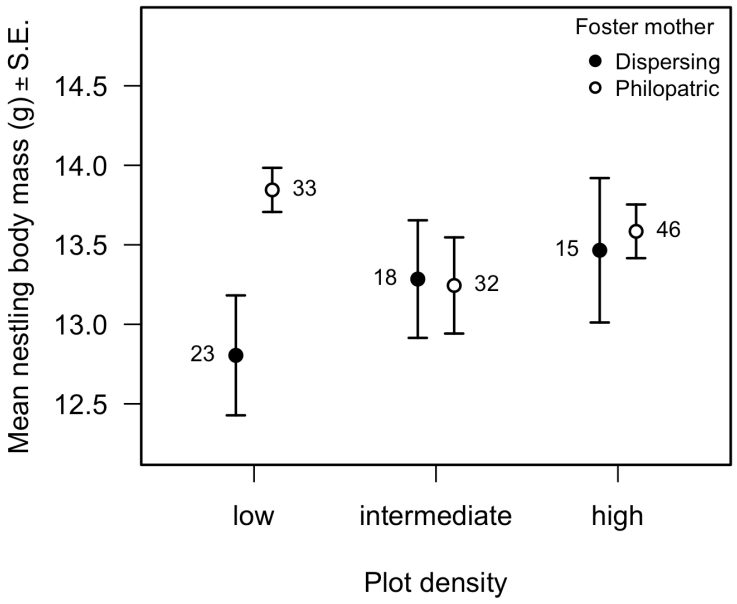


Figure 7: Mean nestling body mass at 12 days of age (corrected for year) depending on plot density for dispersing and philopatric foster mothers. Three density classes were defined for the sake of illustration (see Figure 6).

Fledging probability was higher when the foster mother was philopatric (odd-ratio [95% CI] = 5.74 [1.63 – 22.80], $X^2_1 = 7.11$, P = 0.008). Plot density and wing load manipulation had no effect on fledging probability, either alone or in interaction with each other and with the dispersal status of the mother (all P > 0.22). Fledging probability was lower in 2013 compared to 2012 (0.019 [0.004 – 0.071], $X^2_1 = 28.59$, P < 0.0001). There was no effect of the body mass of the foster mother ($X^2_1 = 0.15$, P = 0.70) or the initial brood size ($X^2_1 = 0.21$, P = 0.65) on fledging probability.

3.4. Link between oxidative state and reproductive output

Nestling body mass increased with the antioxidant capacity of the foster mother (+0.35 ± 0.13, $F_{1,92} = 7.34$, P = 0.008) but decreased with her ROM concentration (-0.42 ± 0.20, $F_{1,94} = 4.47$, P = 0.04). Nestling fledging probability was independent of the antioxidant capacity of the foster mother ($X^2_1 = 0.68$, P = 0.41) or her ROM concentration ($X^2_1 = 1.33$, P = 0.25). All these effects were independent of the dispersal status of the foster mother, plot density or wing load manipulation (all P > 0.07).

4. Discussion

To reduce energetic costs potentially associated with dispersal, dispersers may adopt specific metabolic strategies, with potential consequences on reproduction and survival. In this study, we investigated experimentally whether dispersing and philopatric individual differ in metabolic markers during reproduction depending on the energetic demand. Only plasma antioxidant capacity was higher in philopatric than dispersing females independently of the experimental increase in wing load and local breeding density. In response to the increase in wing load, metabolic rate increased in both dispersing and philopatric females, but ROM increased in philopatric females only. Similarly, only philopatric individuals showed higher ROM in low-density plots compared to high-density ones. Overall, nestlings raised by dispersing mothers had a lower fledging probability and body mass compared to philopatric mothers, especially in low-density plots. Our results

suggest that dispersing and philopatric individuals manage oxidative balance and reproductive investment differently under constrained energetic conditions.

Physiological differences between dispersing and philopatric individuals

Philopatric females showed higher plasma antioxidant capacity than dispersing ones. Plasma antioxidant capacity has been shown to be correlated with dietary non-enzymatic antioxidants (e.g. vitamins, carotenoids) in humans (Talegawkar *et al.* 2009; Wang *et al.* 2012b; a) and birds (Cohen & McGraw 2009). Moreover, the OXY-test used here to measure antioxidant capacity, through a reduction of the activity of the hypochlorous acid, mostly reflects the activity of these non-enzymatic antioxidants rather than enzymes targeting specific oxidants such as superoxide, hydrogen peroxide or lipid peroxide. Thus the difference between dispersing and philopatric females in antioxidant capacity supports the idea that philopatric individuals have higher familiarity with their habitat and thus may be more efficient at finding high quality resources (Pärt 1995). Alternatively, philopatric and dispersing individuals may differ in their antioxidant capacity prior to dispersal. Discriminating these alternatives would require sampling individuals before dispersal.

Some major enzymatic antioxidants, such as catalase and superoxide dismutase, are active in the plasma of birds (Kurhalyuk *et al.* 2009; Oropesa *et al.* 2013; Cram, Blount & Young 2015b). They could be alternative low-cost antioxidant mechanisms. Therefore, dispersing and philopatric individuals may mobilize different types of antioxidant defences. Quantifying multiple antioxidants would help determining whether different antioxidant mechanisms are correlated or on the contrary are traded against each other (Romero-Haro & Alonso-Alvarez 2014). It was however not possible here because of the small quantity of plasma available.

Other differences in markers of oxidative state between dispersing and philopatric individuals depended on external (breeding density) and/or internal (wing load manipulation) factors. This could result from the multicausal nature of dispersal and the resulting heterogeneity within the dispersing and philopatric groups (Cote & Clobert 2007; Cote *et al.* 2013; Baines, McCauley & Rowe 2014). Testing this hypothesis would require

identifying the motivation to disperse for each individual, which currently remains a challenge. The manipulation- or density-dependent differences between dispersing and philopatric individuals could also reflect different responses to environmental and physiological challenges.

Effect of the experimental manipulation of wing load

As expected, the wing load manipulation modified female energy budget, with a higher field metabolic rate and a higher fat free mass for manipulated compared to control females. The difference in fat free mass likely results from an increase in muscular mass, which has a critical influence on flight performance (Verspoor *et al.* 2007) and can increase following wing load manipulations (Lind & Jakobsson 2001). This would at least partly explain the absence of a decrease in body mass between control and manipulated females, a result previously observed in various passerine species (Winkler & Allen 1995; Swaddle & Witter 1997; Chai 1997; Hemborg & Lundberg 1998; Lind & Jakobsson 2001; Senar, Domenech & Uribe 2002; Carrascal & Polo 2006). Interestingly, female wing load manipulation affected neither the physiological parameters of their partners (Supplementary Table S5) nor the mass and fledging success of their nestlings. This suggests that manipulated females developed stronger flight muscles allowing them to maintain the same reproductive output as control females, without any noticeable compensation from their partner. Behavioural measures of reproductive investment, such as feeding rates, would help to confirm the absence of compensation by mates of manipulated females.

The increase of field metabolic rate in response to wing load manipulation is expected to come at an oxidative cost. The interaction between female dispersal status and manipulation on ROM concentrations suggests that this cost might differ between dispersing and philopatric females. Among philopatric females, manipulated females showed higher ROM concentrations than control ones, whereas there was no difference among dispersing females (Figure 5). This could suggest that philopatric females can sustain higher oxidative pressures thanks to their ability to mount more efficient antioxidant responses than dispersing ones. However, accounting for the level of antioxidant activity did not explain the

interaction. Therefore, either philopatric females mounted a more efficient enzymatic antioxidant response or dispersing females were able to mitigate the deleterious effect of increased metabolic rate, at least on the short-term, whereas philopatric females were not.

Habitat quality modulates differences between dispersing and philopatric individuals

We used the density of breeders in a plot as a measure of local habitat quality. We found increasing nestling body mass with increasing density. Thus, in general, individuals did not appear to undergo stronger competition in denser plots. On the contrary, denser plots appeared of higher quality in terms of reproduction and may thus be more attractive. This is in line with previous results in this population showing a positive correlation between local breeding density and success at the plot scale, and consequently higher immigration rate (Doligez *et al.* 2004).

Differences between dispersing and philopatric individuals depended on plot breeding density (Figure 6): although no significant effects were detected for dispersing and non-dispersing females separately, the overall interaction between dispersal status and plot density suggests that ROM concentrations of philopatric females decreased with increasing density, potentially reflecting a decreasing exposure to oxidants. Contrarily, dispersing individuals only showed slightly higher ROM concentrations with increasing breeding density, although, once again, the effect was not significant in dispersing females separately. Although the low sample size (N = 26) prevented us to explore an interaction between plot density and dispersal status on metabolic rate, our observations might be due to an increase in reproductive effort for philopatric females in less dense habitats. This allowed them to raise heavier nestlings compared to dispersing females in lower density plots, whereas no difference was observed in higher density plots. Again, such differences support the idea that philopatric individuals have a better access to resources, which may appear particularly important when resources are limited. Overall, the interactions observed between local breeding density, dispersal status and wing load manipulation on different measures of oxidative status and reproductive success suggest that habitat quality plays a key role in shaping oxidative costs during reproduction. However, these results remain correlative, and

we cannot exclude a confounding effect of individual quality, with high quality individuals settling in high quality habitats. An experimental manipulation of habitat quality, e.g. through food supplementation or parasite infestation, is needed to disentangle the role of habitat and individual quality on the management of oxidative costs.

Overall this study shows that dispersal-related differences in metabolic markers and reproductive success are often condition- or habitat-dependent. The results reveal no general associations between metabolic markers and dispersal, although dispersing and philopatric individuals manage oxidative costs differently depending on reproductive effort (wing load manipulation) and environmental conditions (breeding density). They suggest that dispersing individuals do not adjust reproductive effort even in challenging conditions, resulting in a lower reproductive output, contrary to philopatric individuals that may adjust their effort to the local conditions through physiological responses, possibly because of their better knowledge of the environment. Our study therefore calls for further work investigating the differential management of oxidative constraints between individuals, especially in the context of dispersal.

Acknowledgements

Many thanks to Jukka Forsman and the landowners on Gotland for access to the field sites, to Lars Gustafsson for logistics on the field and to Marine Kreder, Cédric Lionne, Antoine Gillet, Alice Goossens, Nicolas Guignard, Thomas Guyot, Audrey Le Pogam, Aurélie Pugnière, and Lise Sannipoli for their precious help on the field. This work was supported by the French Ministry of Research (PhD fellowship to CR), the CNRS (PICS grant to BD), the Région Rhône-Alpes (CMIRA Explora'doc to CR), the Rectors' Conference of the Swiss Universities (mobility grant to CR), the Fédération de Recherche 41 BioEnvironnement et Santé (training grant to CR), and the Journal of Experimental Biology (travel grant to CR).

Supplementary Information S1: Cross-fostering protocol

We aimed at measuring the post-hatching reproductive investment and outcome of females depending on their dispersal status, their wing load manipulation group and local habitat quality (measured by plot breeding density). Reproductive parameters can however also be influenced by genetic and pre-hatching parental effects. To control for such confounding effects, we cross-fostered two-day old nestlings and analysed the body mass and fledging success of nestlings depending on the characteristics of their foster mother. Nestlings of experimental females were cross-fostered within triplets of broods of same hatching date and similar average body mass (difference between broods in average body mass < 1g on day 2). Whenever possible, all nestlings were exchanged with nestlings from the two other broods in the triplet, so that an experimental female reared none of its own nestlings, but nestlings coming from two other broods. Partial cross-fostering were conducted when no or only one suitable other brood was available, or when brood sizes where too different. For practical reasons, most exchanges were performed within breeding plots. Nests where early mortality was high (i.e. 3 or more nestlings dead on day 2) were not cross-fostered.

Supplementary Information S2: Using local breeding success of control nests as an alternative measure of habitat quality

Local breeding success, i.e. the average number of fledged young per nest in a plot, could have been used as an alternative measure of local habitat quality (Doligez *et al.* 1999, 2002; Clobert *et al.* 2001). Here, this habitat quality index was not significantly related to our measures of adults' metabolism, oxidative balance, mass, and body composition (results not detailed here). This was not due to an effect of our wing load manipulation on reproductive success, because (i) local success was computed on control nests only and (ii) the wing load manipulation did not influence final measures of reproductive success (see text). The absence of relation between local breeding success and our adult measures may however be due to early failed nests (i.e. before or just after hatching), which were included in the computation of local breeding success and whose parents might be of lower quality than those included in this study. Excluding early failed nests would however have created another bias. Overall, the individuals studied here experienced very low nestling mortality before being sampled: only 83 out of 370 of the nestlings that did not fledge (22%) died before nine days of age, the average time when the parents were caught. A lot of the environmental variation shaping final local breeding success, and especially spatio-temporal variation in weather conditions and food availability, might not yet have impacted the adults at the time of sampling. Finally, because a large fraction of females were manipulated and their nests were thus excluded from the computation of local breeding success, we cannot exclude that low sample sizes prevented us from efficiently measuring local habitat quality using local breeding success. For all these reasons, we therefore did not use local success as a measure of habitat quality in this study.

Table S1: Comparison of the morphological and reproductive variables between wing load manipulation and dispersal status groups prior to the nestling feeding period, excluding females that were not caught again while feeding nestlings. Y:O = number yearlings:number older adults. Statistical tests are for the differences between experimental groups, without taking into account dispersal. They were never significant at the 5% level. The differences in body mass during incubation and tarsus length were tested with individual identity as a random effect to account for the presence of some individuals in both years.

Sex	Treatment group	Status	N	Y:O	Mass during incubation (g)	Tarsus length (mm)	Laying date from 1 May	Clutch size	Brood size at hatching
Females	Control	Philopatric	57	3:54	16.0 ± 0.8	19.8 ± 0.5	18.8 ± 3.3	6.5 ± 0.7	5.9 ± 1.2
		Dispersing	34	14:19	16.0 ± 0.9	19.8 ± 0.4	20.8 ± 5.4	6.3 ± 1.0	6.0 ± 1.1
	Manipulated	Philopatric	62	5:57	16.3 ± 0.7	19.8 ± 0.5	19.0 ± 3.9	6.5 ± 0.8	6.0 ± 1.1
		Dispersing	31	10:19	15.7 ± 1.1	19.7 ± 0.5	19.5 ± 3.6	6.4 ± 0.8	6.0 ± 1.1
	Difference between groups		$X^2_1 = 0.17$	$X^2_1 = 0.05$	$X^2_1 = 1.04$	$F_{1,82} = 0.0001$	$t_{177} = 0.58$	$t_{180} = -0.20$	$t_{179} = -0.33$
Males	Control	Philopatric	64	5:59	-	19.6 ± 0.6	19.1 ± 3.7	6.4 ± 0.9	5.9 ± 1.2
		Dispersing	20	11:9	-	19.8 ± 0.4	20.2 ± 4.6	6.6 ± 0.8	6.0 ± 1.2
	Manipulated	Philopatric	65	2:63	-	19.6 ± 0.5	19.0 ± 3.2	6.4 ± 0.8	6.1 ± 0.9
		Dispersing	26	9:16	-	19.7 ± 0.6	19.6 ± 2.9	6.2 ± 0.6	5.6 ± 1.2
	Difference between groups		$X^2_1 = 0.30$	$X^2_1 = 1.07$	-	$F_{1,43} = 0.10$	$t_{155} = 0.30$	$t_{156} = 0.80$	$t_{164} = -0.16$

Table S2: Comparison of physiological and morphological variables between the different wing load manipulation and dispersal groups during the nestling feeding period. Sample sizes are given in parentheses.

Sex	Treatment group	Status	Metabolic rate (kJ/day)	ROM concentrations 2012 (mM H_2O_2)	ROM concentrations 2013 (mM H_2O_2)	Antioxidant capacity 2012 (mM HCl)	Antioxidant capacity 2013 (mM HCl)	Body mass (g)	Fat-free mass (g)	Fat mass (g)
F	Control	Philopatric	54.5 ± 10.7 (6)	0.95 ± 0.42 (26)	0.30 ± 0.26 (26)	177 ± 31 (25)	212 ± 46 (26)	13.4 ± 0.6 (56)	12.3 ± 0.7 (37)	0.8 ± 0.4 (37)
		Dispersing	50.9 ± 9.6 (7)	1.40 ± 1.04 (7)	0.25 ± 0.19 (20)	173 ± 30 (8)	182 ± 47 (20)	13.3 ± 0.7 (33)	12.2 ± 0.7 (26)	0.8 ± 0.6 (26)
	Manipulated	Philopatric	61.3 ± 10.6 (9)	1.01 ± 0.58 (29)	0.30 ± 0.23 (23)	177 ± 34 (27)	212 ± 54 (23)	13.4 ± 0.8 (62)	12.4 ± 0.6 (35)	0.7 ± 0.5 (35)
		Dispersing	57.7 ± 10.8 (4)	0.86 ± 0.28 (13)	0.22 ± 0.22 (14)	166 ± 26 (14)	174 ± 44 (14)	13.2 ± 0.7 (31)	12.0 ± 0.5 (19)	0.7 ± 0.4 (19)
M	Control	Philopatric	-	0.99 ± 0.72 (22)	0.17 ± 0.11 (36)	175 ± 16 (23)	192 ± 45 (36)	13.1 ± 0.6 (61)	-	-
		Dispersing	-	1.40 ± 1.40 (7)	0.22 ± 0.19 (12)	171 ± 27 (6)	180 ± 28 (12)	13.0 ± 0.6 (20)	-	-
	Manipulated	Philopatric	-	1.03 ± 0.81 (34)	0.17 ± 0.13 (30)	164 ± 26 (32)	200 ± 37 (30)	13.0 ± 0.8 (65)	-	-
		Dispersing	-	0.86 ± 0.56 (12)	0.18 ± 0.14 (14)	171 ± 21 (11)	212 ± 45 (14)	13.0 ± 0.7 (26)	-	-

Table S3: Final linear mixed-effects models describing female field metabolic rate, body mass, body composition and markers of adult oxidative state during nestling feeding. The effect of year is expressed as 2013 compared to 2012, and the effect of dispersal status as philopatric individuals compared to dispersing ones.

Effect	Estimate ± S.E.	F	Num. d.f.	Den. d.f.	P
Field metabolic rate (2013 only) $V_{residual}$ = 73 (N = 26 observations), $V_{habitat}$ = 18 (N = 9 plots)					
Wing load manipulation	8.7 ± 3.5	6.10	1	18.91	0.023
Brood size (hatchlings)	-4.0 ± 1.6	6.54	1	19.93	0.019
Body mass $V_{residual}$ = 0.18 (N = 182 observations), $V_{indiv.}$ = 0.23 (N = 156 females), $V_{habitat}$ = 0.01 (N = 9 plots)					
Year	-0.17 ± 0.08	4.19	1	82.23	0.044
Nestlings age	-0.09 ± 0.02	19.11	1	131.40	< 0.001
Tarsus length	0.48 ± 0.10	20.81	1	159.29	< 0.001
Fat free mass $V_{residual}$ = 0.01 (N = 117 observations), $V_{indiv.}$= 0.23 (N = 106 females), $V_{habitat}$ = 0.01 (N = 9 plots)					
Year	-0.59 ± 0.05	119.73	1	14.20	< 0.001
Nestlings age	-0.05 ± 0.02	8.44	1	19.98	0.009
Tarsus length	0.60 ± 0.10	37.48	1	106.58	< 0.001
Wing load manipulation	0.18 ± 0.07	6.39	1	30.27	0.017
Plot density	1.10 ± 0.38	8.53	1	21.94	0.008
Fat mass $V_{residual}$ = 0.10 (N = 117 observations), $V_{indiv.}$= 0.03 (N = 106 females), $V_{habitat}$ = 0.01 (N = 9 plots)					
Year	0.49 ± 0.07	54.56	1	64.27	< 0.001
Nestlings age	-0.05 ± 0.02	9.13	1	109.50	0.003
Reactive oxygen metabolites (ROM) concentration $V_{residual}$ = 0.46 (N = 129 observations), $V_{indiv.}$ = 0.00 (N = 116 females), $V_{habitat}$ = 0.03 (N = 9 plots), V_{plate} = 0.06 (N = 11)					
Wing load manipulation	1.33 ± 0.71	5.32	1	115.55	0.023
Dispersal status	1.35 ± 0.73	5.36	1	115.25	0.022
Plot density	2.27 ± 1.10	0.12	1	10.48	0.731
Dispersal status x Manipulation	0.61 ± 0.26	5.60	1	112.01	0.020
Manipulation x Plot density	-2.56 ± 1.04	6.01	1	115.78	0.016
Dispersal status x Plot density	-2.45 ± 1.07	5.28	1	115.95	0.023
Total antioxidant capacity $V_{residual}$ = 1.09 (N = 157 observations), $V_{indiv.}$ = 0.00 (N = 139 females), $V_{habitat}$ = 0.07 (N = 9 plots), V_{plate} = 0.11 (N = 8)					
Dispersal status	0.56 ± 0.18	9.65	1	150.18	0.002

Table S4: Final generalized linear mixed-effects models and linear mixed-effects models describing nestling probability of fledging and body mass at 12 days of age, respectively, as a function of the foster mother characteristics. The effect of year is expressed as 2013 compared to 2012, and the effect of dispersal status as philopatric individuals compared to dispersing ones.

Effect	Estimate ± S.E.	Test statistic	P
Nestling fledging probability N = 1116 nestlings, V_{foster}= 12.18 (N = 197 pairs), $V_{genetic}$= 1.49 (N = 239 pairs), $V_{habitat}$ = 0.00 (N = 9 plots)			
Year	-3.96 ± 0.74	$X^2_1 = 28.58$	< 0.001
Dispersal status	1.75 ± 0.66	$X^2_1 = 7.11$	0.008
Nestling body mass at day 12 $V_{residual}$ = 0.55 (N = 898 nestlings), V_{foster}= 1.12 (N = 167 pairs), $V_{genetic}$= 0.49 (N = 203 pairs), $V_{habitat}$ = 0.35 (N = 9 plots)			
Year	-1.82 ± 0.23	$F_{1,164} = 64.28$	< 0.001
Brood size	-0.31 ± 0.09	$F_{1,152} = 10.60$	0.001
Time of weighting	2.57 ± 0.86	$F_{1,156} = 8.99$	0.003
Dispersal status	3.18 ± 1.25	$F_{1,149} = 6.45$	0.012
Plot density	4.07 ± 1.98	$F_{1,31} = 1.75$	0.196
Dispersal status x Plot density	-4.17 ± 1.86	$F_{1,151} = 5.05$	0.026

Table S5: Final mixed-effects models describing male body mass and markers of adult oxidative state, as well as nestling probability of fledging and body mass at 12 days of age as a function of the foster father characteristics. The initial full models were the same as those described for females in the text. The effect of year is expressed as 2013 compared to 2012.

Effect	Estimate ± S.E.	Test statistic	P
Body mass $V_{residual}$ = 0.17 (N = 171 observations), $V_{indiv.}$ = 0.17 (N = 144 males), $V_{habitat}$ = 0.03 (N = 9 plots)			
Year	-0.20 ± 0.08	$F_{1,95}$ = 6.26	0.014
Nestlings age	-0.09 ± 0.02	$F_{1,140}$ = 21.25	< 0.001
Tarsus length	0.32 ± 0.09	$F_{1,149}$ = 13.15	< 0.001
Reactive oxygen metabolites (ROM) concentration $V_{residual}$ = 0.60 (N = 148 observations), $V_{indiv.}$ = 0.00 (N = 130 males), $V_{habitat}$ = 0.00 (N = 9 plots), V_{plate} = 0.00 (N = 11)			
Year	-0.32 ± 0.13	$F_{1,144}$ = 5.75	0.018
Nestlings age	0.07 ± 0.03	$F_{1,144}$ = 7.09	0.009
Plot density	-1.90 ± 0.55	$F_{1,144}$ = 11.89	0.001
Total antioxidant capacity $V_{residual}$ = 0.71 (N = 164 observations), $V_{indiv.}$ = 0.03 (N = 140 males), $V_{habitat}$ = 0.00 (N = 9 plots), V_{plate} = 0.08 (N = 10)			
No significant effect	-	-	-
Nestling fledging probability N = 988 nestlings, V_{foster}= 7.28 (N = 174 pairs), $V_{genetic}$= 0.42 (N = 216 pairs), $V_{habitat}$ = 0.22 (N = 9 plots)			
Year	-3.36 ± 0.62	X^2_1 = 29.48	< 0.001
Nestling body mass at day 12 $V_{residual}$ = 0.72 (N = 830 nestlings), V_{foster}= 1.04 (N = 157 pairs), $V_{genetic}$= 0.32 (N = 193 pairs), $V_{habitat}$ = 0.21 (N = 9 plots)			
Year	-1.85 ± 0.20	$F_{1,152}$ = 84.71	< 0.001
Brood size	-0.32 ± 0.09	$F_{1,155}$ = 12.50	< 0.001
Time of weighting	1.61 ± 0.80	$F_{1,156}$ = 4.04	0.046

Chapter V – Responses of dispersing and philopatric individuals to experimentally relieved energetic constraints

PAPER 1 – FOOD SUPPLEMENTATION MITIGATES HABITAT-DEPENDENT DIFERENCES BETWEEN DISPERSING AND NON-DISPERSING INDIVIDUALS IN COLLARED FLYCATCHERS.

Charlotte Récapet, Pierre Bize, Blandine Doligez

In preparation

Keywords: dispersal, food supplementation, energetic constraint, density dependence, body mass, reactive oxygen metabolites, antioxidant defences, oxidative stress, reproductive output, *Ficedula albicollis*

Dispersal is a crucial trait affecting many ecological and evolutionary processes. Dispersal allows individuals to respond to environmental variation, but it entails costs and recent research shows that dispersal could be associated with phenotypic and life-history traits that allow dispersers to reduce dispersal costs. Differences in resulting life-history strategies between dispersing and philopatric individuals may thus represent alternative strategies. However, the role of environmental variation in shaping these differences has often been overlooked. Our previous results in a patchy population of a small passerine, the collared flycatcher, suggest that dispersing individuals invest less in reproduction but more in maintenance with decreasing local density, a proxy of habitat quality in this population, whereas philopatric individuals maintain a high reproductive investment in all habitats at the expense of increased oxidative stress. These differences could result from different responses to habitat quality. Alternatively, dispersing (resp. philopatric) individuals breeding in low quality habitats could also be phenotypically different from those in high quality habitats. To investigate the importance of environmental conditions in mediating differences in life-history traits between dispersing and philopatric individuals, we experimentally manipulated habitat quality by providing additional food during the nestling rearing period and we measured subsequent body mass, plasmatic markers of oxidative state and reproductive effort and output for experimental parents. Density-dependent differences between dispersing and philopatric individuals in body mass and fledging success were observed in control nests, but these differences disappeared in supplemented nests. Most differences between control and supplemented nests were observed in low-density plots, thus confirming that density reflects habitat quality in this population. Our results confirm the importance of environmental conditions in shaping differences in life-history traits according to dispersal. Considering that reaction norms rather than fixed differences shape traits associated to dispersal may help improving our understanding of dispersal syndromes.

1. Introduction

Dispersal has long been recognized as a key process in ecology and evolution in the wild. In particular, it may allow individuals to escape adverse conditions especially in degrading environments, reduces local extinction risk in metapopulations and maintains gene flow between populations, shaping local adaptation and speciation processes (Clobert *et al.* 2001). At the individual level, dispersal has been shown to entail costs, e.g. the development of morphological structures for mobility, the mortality risks during movement and settlement in new habitats or the loss of familiarity with the habitat and social environment (Bonte *et al.* 2012). Therefore, selective pressure should favour dispersal of individuals bearing traits allowing them to reduce such costs (Duckworth 2012). In the last decade, a growing number of studies have shown that between-individual variation in dispersal behaviour often correlates with variation in behavioural, morphological and physiological traits (Clobert *et al.* 2009; Ronce & Clobert 2012; Debeffe *et al.* 2014). Such covariation can strongly affect population dynamics (Bowler & Benton 2005; Phillips *et al.* 2006; Duckworth & Badyaev 2007; Clobert *et al.* 2009; Hanski 2012) and evolution (Garant *et al.* 2005; Edelaar & Bolnick 2012), because dispersers are not a random sample of the population with respect to many phenotypic traits.

Because of such correlations between dispersal and phenotypic traits reducing dispersal costs, dispersing and philopatric individuals might differ in their investment in other life-history traits, and thus in their life-history strategies, even though they might eventually reach the same lifetime individual fitness (Bélichon *et al.* 1996; Julliard *et al.* 1996; Marr *et al.* 2002). Such differences would result in "life-history syndromes" associated to dispersal. Correlations between dispersal and life history traits have been well described at the interspecific level (Stevens *et al.* 2013, 2014), although patterns are often inconsistent between taxa (Stevens *et al.* 2014). However, the existence and direction of correlations at the intraspecific level between dispersal and other life-history traits such as reproductive performance or survival remains controversial, because no general pattern emerges from

empirical studies comparing life-history traits between dispersing and philopatric individuals (reviewed in Bélichon *et al.* 1996; Doligez & Pärt 2008).

Correlative studies may however fail to find expected relationships between dispersal and other life-history traits, or may even find opposite relationships, if the expression of the traits expected to covary with dispersal depends on environmental conditions and dispersing and philopatric individuals are found in different habitats. Accordingly, it is often reported that dispersing individuals use ephemeral habitat, newly created habitat or range edges more frequently than philopatric individuals (Duckworth 2008; Sexton *et al.* 2009). Because environmental conditions in such habitats are often less favourable than in long-term occupied habitat (e.g. Gaston 2009), the covariation between dispersal and the traits considered may mostly be mediated by habitat quality. Alternatively, dispersing and philopatric individuals may occupy similar habitats but respond differently to the same environmental conditions (van Noordwijk & de Jong 1986; Price, Kirkpatrick & Arnold 1988; Stearns 1989). We however know little about the differential life-history responses of dispersing and philopatric individuals to environmental variation. The modulation of the covariation between dispersal and other life-history traits depending on environmental conditions has only been investigated correlatively in a few studies so far (Tschirren *et al.* 2007; Duckworth & Badyaev 2007; Germain 2014). Whether environmental conditions could directly alter the difference in breeding strategies between dispersing and philopatric individuals still needs to be tested, by manipulating habitat quality without influencing habitat choice. Among factors determining habitat quality, food availability seems a privileged target for such experiments because energy requirements shape many life-history trade-offs. Furthermore, because both food availability and dispersal decisions can be strongly modulated by population density (Doligez *et al.* 2004; Matthysen 2005; Le Galliard *et al.* 2012), variation in local density seems an important factor to take into account when addressing the role of environmental conditions.

To experimentally test the importance of habitat quality in shaping differences between dispersing and philopatric individuals in breeding decisions, we provided additional food during the nestling feeding period in a patchy population of collared

flycatchers *Ficedula albicollis*. Food supplementation has previously been shown to improve reproductive output in pied flycatchers *Ficedula hypoleuca*, a sister species (Verhulst 1994; Siikamäki 1998). We measured body mass and plasmatic markers of oxidative state (reactive oxygen metabolites and antioxidant capacity) for experimental parents, as well as their reproductive effort, measured through nestling provisioning rate, nestlings' body mass and fledging success. Previous results in this species suggest that dispersing individuals produce less offspring when breeding density decreases, but incur lower physiological costs in terms of oxidative stress, than philopatric individuals (cf. Chapter IV). In this population, local breeding density could potentially reflect habitat quality, because it is positively correlated with average breeding success (Doligez *et al.* 2004), but it could also reflect the level of intraspecific competition during breeding. If the differences previously observed between dispersing and non-dispersing individuals are the result of fixed phenotypic differences or breeding strategies, the food supplementation treatment should not affect them. However, if environmental conditions mediate these differences, we predict that density-dependent differences between dispersing and philopatric individuals will be reduced in food-supplemented nests compared to the control situation. If low breeding density reflects low food availability, as the main factor affecting breeding success in this population, the effect of the food supplementation should be stronger in low-density habitats. Conversely, if high breeding density reflects high intraspecific competition for food resources, the effect of the food supplementation should be stronger in high-density habitats.

2. Material and methods

2.1.1 Study population and definition of dispersal

The study was conducted during spring 2014 on the island of Gotland, Sweden (57°07'N, 18°20'E). Collared flycatchers bred in artificial nest boxes erected in eight spatially discrete forest plots, with 17 to 42 breeding pairs per plot. Nests were surveyed every second day starting from 27 April to estimate laying date, clutch size, incubation date and hatching date. Males and females were caught when nestlings were six to 12 days old (mean nestling

age when catching the parents ± S.E. = Females: 9.7 ± 1.2; Males: 9.3 ± 1.3). Birds were weighed (to the nearest 0.1g), their tarsus length was measured (to the nearest 0.1mm by a single observer, C.R.) and a blood sample was taken (see below). Birds could be aged as yearlings or older adults (2 years old or more) based on plumage characteristics (Svensson 1992) or previous capture history. Nestlings were ringed when eight days old and weighed when 12 days old. Nests were checked at the end of the season to evaluate fledging success for each nestling.

Local breeding density was measured as the proportion of available nest boxes occupied by flycatchers in a plot during the year considered. A nest box was considered available to flycatchers when it was empty (i.e. contained no nest from another species, mainly great tit *Parus major* and blue tit *Cyanistes caeruleus)* up to five days after the earliest egg laying date for flycatchers in the same plot. This measure of density is positively correlated with reproductive success at the plot level (Doligez *et al.* 2004) and was previously found to influence differences in oxidative balance and reproductive success between dispersing and non-dispersing individuals (Chapter IV).

The landscape of the study area is spatially fragmented, with breeding forest plots separated by fields, pastures and meadows unsuitable for breeding in this species. Dispersal was defined as a change of breeding plot between birth and the first capture as a breeder (natal dispersal) or between two consecutive captures as a breeder on different years (breeding dispersal; see Doligez *et al.* 1999 for a discussion of this definition of dispersal in this population). We excluded from our analyses all individuals that were caught for the first time on a given year (i.e. previously unringed individuals) and were thus of unknown dispersal status. Our dataset included 87 females (29 dispersing, 58 philopatric) and 77 males (14 dispersing, 63 philopatric) caught when feeding nestlings, coming from 121 different nests. Only one male was caught twice on two different nests (i.e. polygynous). As previously found in this (and other passerine) species, dispersal was lower in males than in females (Chi-squared test: $X^2_1 = 4.28$, $P = 0.038$) and in adults than in yearlings: 9 out of 12 yearlings were dispersers, but only 34 out of 152 older adults ($X^2_1 = 13.32$, $P = 0.0003$). However, dispersal status was independent of early breeding parameters such as of laying

date (Wilcoxon rank-sum test; females: W = 862, P = 0.86; males: W = 549, P = 0.19) and clutch size (females: W = 782, P = 0.55; males: W = 415, P = 0.63).

2.1. Parental provisioning rate

When nestlings were five-days old, parental feeding rate was recorded during one hour using a camouflaged camera located at least six meters from the nest box. At the beginning of the recording period, the experimenter went to the nest, emptied the feeders if necessary (see below) and determined the number of nestlings as well as their level of hunger, as their response to the experimenter whistling: (0) nestlings sleeping; (1) up to two nestlings begging but stopping within a few seconds; (2) most nestlings begging but stopping within a few seconds; (3) most nestlings begging continuously. Feeding rate was calculated for each parent as the number of feeding events per hour and per nestling, starting from the first feeding event until the end of the recording. This accounted for the parents' latency to resume feeding visits, which could be influenced by the birds' response to the disturbance when setting the camera: feeding rate calculated by excluding this latency was not correlated with latency (r = -0.05, $CI_{95\%}$ = [-0.21 – 0.11], t_{142} = -0.63, P = 0.53). Three nests for which the feeders had not been properly emptied before the test were excluded from the analyses. Three birds that resumed feeding visits less than 20min before the end of the recording were excluded, because their feeding rate could not be calculated with confidence. Individuals that did not resume feeding visits during the hour of recording were also excluded because it was not possible to know whether they were absent from the surroundings during that time or too disturbed by the presence of the camera (but including them with a feeding rate of zero did not qualitatively change the results).

2.2. Cross-fostering

The dispersal behaviour of breeding adults might influence the quality of their offspring through (i) genetic effects, (ii) pre-hatching effects (including breeding habitat choice and maternal effects) or (iii) post-hatching investment in nestling care. We were here interested in post-hatching parental responses to the supplementation treatment in relation

to their dispersal status. Therefore, nestlings were cross-fostered when two days old to dissociate pre-treatment effects of the parental dispersal status from effects linked to the treatment. Nestling body mass and survival were then analysed in relation to their foster parents' dispersal status and experimental treatment only (i.e. we did not account for parents' dispersal status in the nest of origin). Nestlings were cross-fostered within triplets of broods hatched on the same day and with similar body mass (i.e. with a difference in brood average body mass < 1g on day 2). Whenever possible, all nestlings were exchanged with nestlings from the two other broods in the triplet, so that experimental parents reared none of their own nestlings, but nestlings coming from two other broods. Partial cross-fostering were conducted when only one suitable other brood was available or when brood sizes differed too much. For practical reasons, exchanges were performed within breeding plots whenever possible (663 out of 1017 nestlings) or between nearby plots. Nests with a high early mortality (3 or more dead hatchlings on day 2) were not cross-fostered but were included in the supplementation experiment and the analyses if there was still at least one nestling alive.

2.3. Food supplementation

Food availability was manipulated at the nest level by providing additional food. When nestlings were two days old, transparent plastic containers were attached to the front side of the nest box, below the nest entrance. For supplemented nests, 30g of live maggots were placed in the containers daily until nestlings were 12 days old (included), i.e. over 11 days in total. Each experimental nest therefore received a total of 330g maggots over the whole nestling phase. Control nests received no food but were visited daily to control for disturbance linked with human presence. Nests that hatched the same day were assigned either to the control or supplemented treatment group alternatively in space, to distribute treatments homogeneously according to space and hatching date within study plots.

The supplemented (N = 60) and control nests (N = 61) did not differ in laying date (W = 1926, P = 0.62), female body mass during incubation (Student t-test: $t_{111} = 1.28$, P = 0.20) or brood size at the beginning of the experiment (W = 2031, P = 0.27).

2.4. Blood markers of oxidative state

A 40μL blood sample was taken from the brachial vein into heparin-coated Microvette tubes (Sarstedt, Nümbrecht, Germany) from both parents when the nestlings were six to 12 days old. Blood samples were maintained at 5°C in the field before being centrifuged in the evening to separate plasma from red blood cells. Plasma and red blood cells were then stored at -80°C until analyses in the laboratory.

The oxidative balance is multidimensional. First, the different types of oxidative damage, on the one hand, and different mechanisms of antioxidant defences, on the other hand, do not always correlate positively (Dotan, Lichtenberg & Pinchuk 2004). Second, the level of antioxidant defences may not predict accurately the level of oxidative damages and these two components should always be measured simultaneously (Costantini & Verhulst 2009). Because of the low amount of plasma available per individual, we chose to focus on two markers of oxidative state, describing oxidative damages and non-enzymatic oxidative defences, independently of uric acid (Costantini 2011): the reactive oxygen metabolites (ROM) and the plasma total antioxidant capacity. These markers have been successfully used in birds to assess the cost of reproduction (Beaulieu *et al.* 2011; Stier *et al.* 2012). Here, we followed protocols successfully used in a previous study in the collared flycatcher (Supplementary Information S1 and Chapter IV). In our analyses, the inter-plate CVs were 8% and 13% on 11 duplicates for the d-ROMS and OXY tests respectively and the intra-plate CVs were both 6% on 11 duplicates.

2.5. Statistical analyses

To test whether food supplementation altered the dispersal-dependent responses to breeding density, we examined the three-way interaction of dispersal status, food supplementation and breeding density on adult body mass, markers of oxidative balance and feeding rate using linear mixed models (LMM). Adult age (yearling vs. older), nestlings' age at parents' sampling and brood size at hatching were included as fixed covariates, as well as parents' body mass (when analysing markers of oxidative balance and feeding

behaviour) or tarsus length (when modelling adult body mass). Sex was included in interaction with all other main effects (except for age, see below), to account for potential sex-dependent responses. Due to the very small number of yearlings in 2014 (12 yearlings out of 164 parents), the interaction between age and other variables could not be tested. Pair and breeding plot were included as random effects to account for the non-independence of the two members of a pair and for spatial environmental variation. For the markers of oxidative balance, the assay was also included as a random effect. Finally, for feeding rate, nestlings' hunger level and the starting hour of the recording were added as fixed effects and the recording day was added as a random effect to account for climatic variation between days.

We then examined the three-way interaction of dispersal status, food supplementation and breeding density on nestlings' body mass when 12-days old, which is a predictor of future survival and recruitment in the collared flycatcher (Lindén *et al.* 1992), and on the fledging probability of nestlings. Nestling body mass was analysed using a linear mixed model, while fledging probability was analysed using a generalised linear mixed model (GLMM, with a logit link function and a binomial error distribution). We analysed males and females separately, because dispersal status was not always known for both members of a pair. We modelled the three-way interaction between the foster parent' dispersal status, food supplementation of the foster nest and breeding density. Parent body mass, hatching date and brood size were included as covariates and foster nest, nest of origin and plot as random effects. Age was only included as a covariate in the models for females, because only three yearling males were found among experimental birds in this study. When analysing nestlings' body mass, the time of weighing was also included as a covariate.

All models were run using the R software (R Core Team 2014). Fixed effects were selected by stepwise backward elimination. Selection criteria were the p-values of type-III F-tests for LMM, with denominator degrees of freedom calculated using Satterthwaite's approximation (R package 'lmerTest', function anova, Kuznetsova, Brockhoff & Christensen 2013) and the p-values of type-III Wald chi-square tests for GLMM (R package 'car', function Anova, Fox & Weisberg 2011). No selection was performed on random effects, which were thus kept in all final models.

3. Results

3.1. Adult body mass

The three-way interaction between dispersal status, breeding density and food supplementation treatment on adult body mass was significant ($F_{1,149}$ = 8.75, P = 0.004, Figure 8). Among dispersing birds, adult body mass increased with breeding density in control nests (post-hoc test: $F_{1,18}$ = 11.71, P = 0.003; estimate ± SE: 2.61 ± 0.76) but not in supplemented nests ($F_{1,11}$ = 1.51, P = 0.25). Conversely, the body mass of philopatric birds did not depend on density whatever the treatment group (both P > 0.32). The relation between body mass and breeding density was also sex-dependent (interaction between breeding density and sex: $F_{1,99}$ = 5.69, P = 0.019). Body mass increased with density in females ($F_{1,7}$ = 7.79, P = 0.025; estimate ± SE: 1.39 ± 0.50) but not in males ($F_{1,58}$ = 0.38, P = 0.54). Body mass also increased with tarsus length ($F_{1,151}$ = 19.37, P < 0.001; estimate ± SE: 0.43 ± 0.10) and was higher in yearlings than older adults ($F_{1,149}$ = 4.16, P = 0.043; estimate ± SE for yearlings compared to older adults: 0.39 ± 0.19). Finally, body mass tended to decrease with nestling age at parents' capture ($F_{1,115}$ = 3.11, P = 0.080; estimate ± SE: -0.07 ± 0.04) and did not depend on brood size at hatching ($F_{1,96}$ = 0.12, P = 0.73).

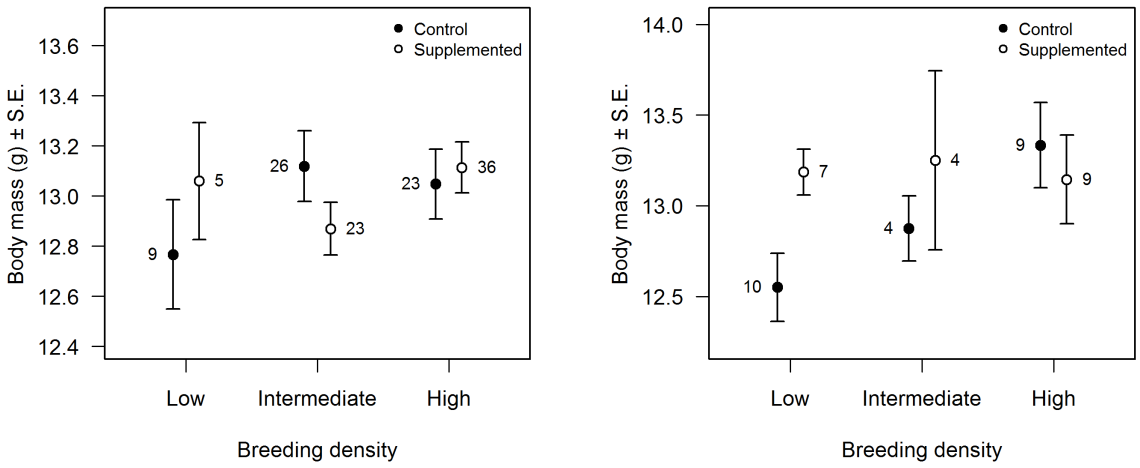


Figure 8: Body mass as a function of breeding density and food supplementation for philopatric (left) and dispersing (right) individuals. Three classes of breeding density were defined here for the sake of illustration, based on the distribution of breeding density in the study plots: "low" (from 43 to 50% of available nest boxes occupied), "intermediate" (from 65 to 73%) and "high" (from 80 to 91%).

3.2. Adult oxidative balance

ROM concentration depended on plot density differently between dispersing and philopatric birds (interaction between dispersal status and plot density: $F_{1,132} = 4.49$, $P = 0.036$, Figure 9). ROM concentration tended to increase with breeding density in philopatric individuals ($F_{1,81} = 3.49$, $P = 0.065$; estimate ± SE : 0.184 ± 0.098) but not in dispersing ones ($F_{1,6} = 0.20$, $P = 0.67$). ROM concentrations did not depend on the food supplementation treatment ($F_{1,95} = 0.13$, $P = 0.72$; all two- and three-way interactions with dispersal and density were not significant: all $P > 0.29$). ROM concentration depended on brood size differently between males and females ($F_{1,81} = 4.22$, $P = 0.043$), with a tendency for decreasing ROM with increasing brood size in females ($F_{1,67} = 2.98$, $P = 0.09$; estimate ± SE: -0.028 ± 0.016) and a reverse but non-significant pattern in males ($F_{1,61} = 0.64$, $P = 0.43$; estimate ± SE: 0.018 ± 0.022). ROM concentration did not depend on age ($F_{1,122} = 0.11$, $P = 0.74$), body mass ($F_{1,121} = 0.03$, $P = 0.86$) or nestling age ($F_{1,106} = 1.24$, $P = 0.27$).

We found a significant interaction of sex and dispersal status on antioxidant capacity ($F_{1,157} = 4.12$, $P = 0.044$). The relative values of antioxidant capacity for dispersing and philopatric individuals were reversed between males and females, leading to the interaction despite no significant within-sex difference (tendency for higher antioxidant capacity in philopatric compared to dispersing females: $F_{1,83} = 3.64$, $P = 0.060$; estimate ± SE: 16.2 ± 8.5; and no difference in males: $F_{1,74} = 0.93$, $P = 0.34$; estimate ± SE: -9.4 ± 9.7). The supplementation treatment had a positive effect on antioxidant capacity ($F_{1,157} = 4.18$, $P = 0.043$; estimate ± SE for supplemented compared to control adults: 11.1 ± 5.4). Antioxidant capacity did not depend on breeding density ($F_{1,156} = 0.01$, $P = 0.93$), adult age ($F_{1,155} = 0.92$, $P = 0.34$), body mass ($F_{1,157} = 0.01$, $P = 0.91$), nestling age ($F_{1,158} = 1.41$, $P = 0.24$) or brood size ($F_{1,156} = 0.24$, $P = 0.62$). All interactions between dispersal status, food supplementation and/or breeding density were not significant (all $P > 0.23$).

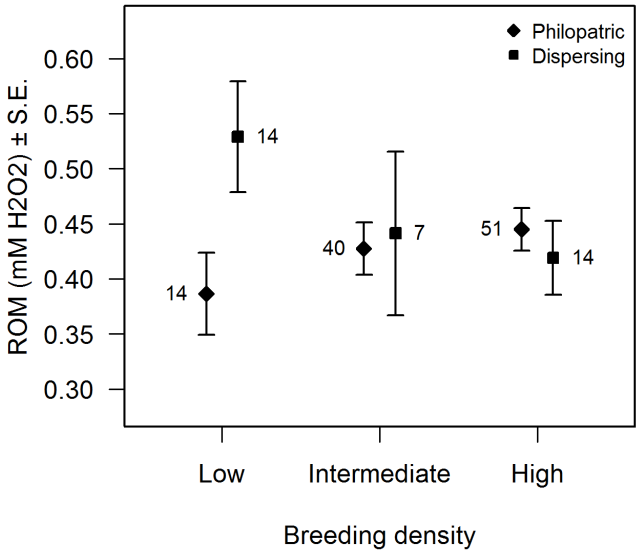


Figure 9: Concentration of reactive oxygen metabolites (ROM) as a function of breeding density and dispersal status (dispersing vs. philopatric individuals). Three classes of breeding density were defined here for the sake of illustration (cf. Figure 8).

3.3. Feeding rate

Overall, feeding rate per nestling was higher for dispersing compared to philopatric individuals ($F_{1,126}$ = 10.90, P = 0.001; estimate ± SE for dispersing compared to philopatric individuals: 0.5 ± 0.2). Feeding rate also decreased with plot density in control nests ($F_{1,70}$ = 8.79, P = 0.004; estimate ± SE: -1.8 ± 0.6) but not in supplemented ones ($F_{1,40}$ = 0.83, P = 0.37), leading to a significant interaction between plot density and supplementation treatment on feeding rate ($F_{1,68}$ = 6.85, P = 0.011, Figure 10). Feeding rate per nestling also decreased with increasing brood size ($F_{1,84}$ = 29.32, P < 0.001; estimate ± SE: -0.3 ± 0.1). Feeding rate did not depend on parent's age ($F_{1,119}$ = 0.39, P = 0.53), body mass ($F_{1,114}$ = 0.03, P = 0.86), nestlings' hunger level ($F_{3,95}$ = 1.40, P = 0.25) or the time of the day when feeding rate was recorded ($F_{1,72}$ = 1.84, P = 0.18).

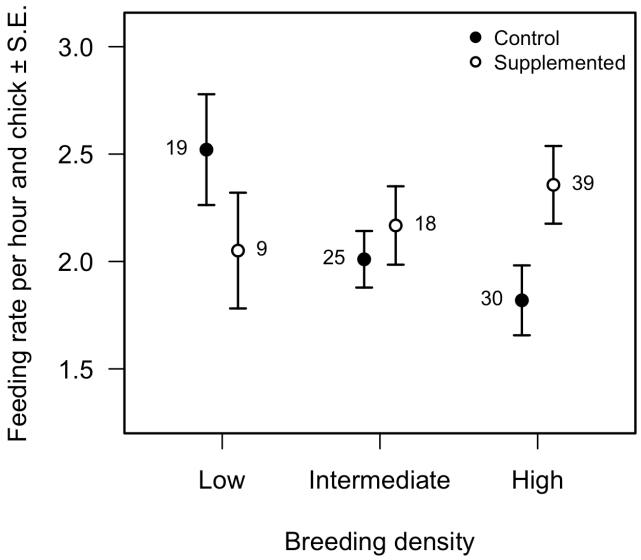


Figure 10: Feeding rate per hour and per nestling as a function of breeding density and food supplementation treatment. Three classes of breeding density were defined here for the sake of illustration (cf. Figure 8)

3.4. Nestling body mass

Nestling body mass when 12 days old did not differ between dispersing and philopatric foster mothers ($F_{1,65}$ = 0.07, P = 0.80; all two- and three-way interactions with food supplementation treatment and/or breeding density were not significant). It did not depend on the foster female's age ($F_{1,69}$ = 0.40, P = 0.53) or body mass ($F_{1,62}$ = 2.84, P = 0.10) either. Nestling body mass tended to be higher for dispersing compared to philopatric foster males ($F_{1,54}$ = 3.43, P = 0.069; estimate ± SE for dispersing compared to philopatric males: 1.04 ± 0.56; all two- and three-way interactions with food supplementation treatment and/or breeding density were not significant). Nestling body mass did not depend on the foster father's body mass ($F_{1,53}$ = 0.19, P = 0.66). For both analyses, nestling body mass did not depend on the supplementation treatment (P > 0.37), breeding density (P > 0.86) or their interaction (P > 0.40). Nestling body mass decreased with hatching date (P < 0.02; Table S3) but did not depend on brood size (P > 0.12) or the time of the day when the nestlings were weighed (P > 0.36).

3.5. Fledging success

Fledging probability did not depend on the foster female's dispersal status ($X^2_1 = 1.26$, $P = 0.26$; all two- and three-way interactions with food supplementation treatment and/or breeding density were not significant). Fledging probability did not depend either on the female's age ($X^2_1 = 0.63$, $P = 0.43$) or body mass ($X^2_1 = 2.23$, $P = 0.14$). Fledging probability was higher in supplemented compared to control nests ($X^2_1 = 6.53$, $P = 0.011$; estimate ± SE for supplemented compared to control nests: 1.86 ± 0.73) but it did not depend on breeding density ($X^2_1 = 1.01$, $P = 0.31$; the interaction with food supplementation was not significant). Conversely, fledging probability differed between foster dispersing and philopatric males but this effect depended on both plot density and supplementation treatment (three-way interaction: $X^2_1 = 6.26$, $P = 0.012$, Figure 11). Among philopatric males, fledging probability increased with breeding density in control nests ($X^2_1 = 8.84$, $P = 0.003$; estimate ± SE: 9.76 ± 3.28) while it was equally high in supplemented nests ($X^2_1 = 0.01$, $P = 0.93$), leading to a significant interaction between supplementation treatment and plot density for philopatric fathers ($X^2_1 = 4.44$, $P = 0.035$). Among dispersers, fledging probability did not depend on supplementation treatment ($X^2_1 = 1.44$, $P = 0.23$), breeding density ($X^2_1 = 0.15$, $P = 0.70$) or their interaction ($X^2_1 = 2.56$, $P = 0.11$) (but sample sizes were low). Fledging success also decreased with the foster father's body mass ($X^2_1 = 5.72$, $P = 0.017$; estimate ± SE: -1.40 ± 0.59). For both analyses, fledging probability decreased with increasing hatching date ($P < 0.001$, Table S2) but did not depend on brood size at hatching ($P > 0.51$).

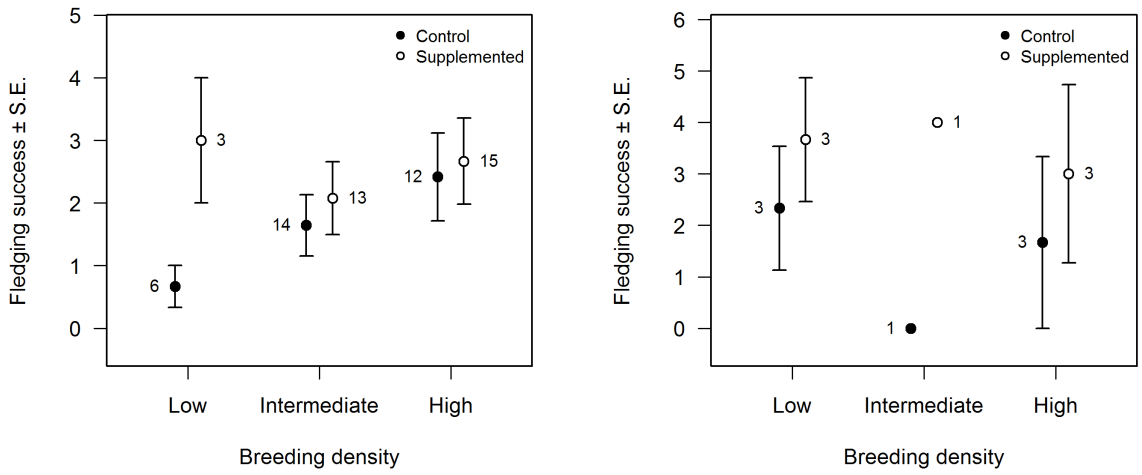


Figure 11: Number of fledged nestlings as a function of breeding density and food supplementation treatment for philopatric (left) and dispersing (right) males. Three classes of breeding density were defined here for the sake of illustration (cf. Figure 8).

4. Discussion

Differences in life-history traits between dispersing and philopatric individuals are frequently observed, but their origin is yet poorly understood. Previous results in the collared flycatcher have shown that differences between dispersing and philopatric individuals in oxidative balance and reproductive output depended on local breeding density. These differences may result from differences in habitat quality and/or competition for food and breeding sites. Through a manipulation of food availability after the onset of breeding, i.e. during the nestling feeding period, we investigated whether those phenotypic differences between dispersing and philopatric birds were due to habitat quality effects or were explained by fixed individual differences associated with dispersal. In control nests, we observed density-dependent differences in body mass and fledging success between dispersing and philopatric individuals, but these differences disappeared in supplemented nests. The food supplementation treatment only interacted with dispersal through a three-way interaction with breeding density, indicating that only traits that are already differently sensitive to natural environmental conditions according to dispersal were also differently sensitive to the experimental treatment. This confirms that the differences observed between

dispersing and philopatric individuals are not fixed but depend on environmental conditions, which emphasizes that the description of dispersal syndromes needs to account for environmental variation.

Differences between dispersing and philopatric birds in non-supplemented conditions

Dispersing and philopatric individuals differed in their feeding behaviour and antioxidant capacity (for females), independently of plot breeding density or food supplementation. The higher antioxidant capacity of philopatric females compared to dispersing ones is consistent with previous results in that species (cf. Chapter IV). It could be result from an advantage of breeding in a familiar habitat, which facilitates both the access to good quality breeding sites and the search for food (Pärt 1995). Alternatively, dispersing individuals could be lower quality individuals that were constrained to disperse because of their low competitive ability and could not secure a good site and/or are less efficient in searching for high quality food. Philopatric individuals also had a lower feeding rate per nestling than dispersing ones. Although we do not have information on the type of preys brought by the parents here, longer foraging trips have been shown to associate with larger or more numerous prey items brought in a single feeding visit in many passerine species (Nour *et al.* 1998; Grieco 2002; Schwagmeyer & Mock 2008) and prey size is an important determinant of the total volume of prey brought to a nest (Grundel 1987) and eventually of reproductive success (Schwagmeyer & Mock 2008). In our study, the absence of differences in nestling body mass and survival between dispersing and philopatric individuals in control conditions suggest that philopatric birds were able to reach the same reproductive output despite a lower feeding rate than dispersing ones. Philopatry thus seems to confer an advantage in finding high quality food. This could be associated with a higher selectivity for larger prey items (Naef-Daenzer, Naef-Daenzer & Nager 2000), or more generally more profitable, higher quality prey items, in philopatric individuals.

When considering only control nests, fledging success increased with breeding density in philopatric males only, whereas body mass increased with plot density in dispersing individuals (males and females) only. The general directions of these effects

confirm that high-density habitats are also high quality breeding habitats, because measures of adult condition or success increase when density increases. Nevertheless, the fact that either reproductive success (in philopatric males) or body mass (in dispersing individuals) increases but not both simultaneously, as well as the negative relationship between fledging success and body mass observed in males, suggest a trade-off between parental condition and nestling survival until fledging, so that both may not increase simultaneously. In this case, philopatric individuals would increase their investment in maintenance when habitat quality increases, while dispersing males increase their investment in reproduction.

ROM level also increased with breeding density in philopatric individuals only, independently of the food supplementation. This result is in contradiction with the decrease of ROM level with increasing breeding density observed in philopatric individuals in 2012 and 2013 (cf. Chapter IV). Environmental conditions in 2014 differed however very strongly from previous years, with highly unfavourable meteorological conditions (high rain and low temperatures), which resulted in very high nestling mortality rate and thus poor reproductive output in 2014 compared to previous years (mean fledging success in control nests ± S.D. = 4.7 ± 2.6 in 2012, 3.0 ± 2.4 in 2013 and 1.5 ± 2.0 in 2014, to be compared to the average fledging success from 1980 to 2011 = 3.6 ± 0.6). Although the effect of breeding density on ROM level was reversed in 2014 compared to previous years, an interaction between breeding density and dispersal status was found in all three years. This confirms the importance of environmental conditions in determining the differences between dispersing and philopatric individuals in phenotypic traits. This result is also in line with the hypothesis that, in non-manipulated conditions, dispersing individuals maintain a constant reproductive investment whatever the environmental conditions whereas philopatric individuals adjust their investment to environmental conditions, and notably invest more in reproduction in high-density/high-quality habitats. The increase in ROM concentration with breeding density in philopatric individuals may thus be a consequence of the increase in reproductive effort to feed larger broods under constrained conditions, at least in males.

Food supplementation mitigated the density-dependent effects on body mass and reproduction

Consistently with previous studies in pied flycatchers (Verhulst 1994; Siikamäki 1998), our food supplementation was successful in increasing nestlings' survival. Moreover, supplemented birds had a higher non-enzymatic antioxidant capacity. Non-enzymatic antioxidant capacity has been shown to positively correlate with the amount of antioxidant-rich food in the diet in humans (Talegawkar *et al.* 2009; Wang *et al.* 2012b; a) and birds (Cohen & McGraw 2009). The higher antioxidant capacity of supplemented birds could be due to a diet richer in antioxidants if supplemented birds are more selective in their food choice than control birds or to lower antioxidant consumption due to a decreased foraging effort. However there was no effect of breeding density on antioxidant capacity, suggesting that individual variation rather than environmental variation drives antioxidant capacity, consistently with the results of chapter III. This higher antioxidant capacity did not translate into lower oxidative damages, as measured by ROM concentration. Enzymatic antioxidants, such as catalase and superoxide dismutase, could be alternative low-cost antioxidant mechanisms allowing non-supplemented individuals to compensate for the lower dietary intake of exogenous antioxidants. Enzymatic antioxidants are active in bird blood (Kurhalyuk *et al.* 2009; Oropesa *et al.* 2013; Cram *et al.* 2015b), which allows their measure in wild populations of small-size animals monitored on the long-term, for which blood is often the only tissue that can be sampled. Importantly, however, markers of oxidative state in the plasma do not always correlate with values in other tissues (Veskoukis *et al.* 2009; Speakman & Garratt 2014), which may limit the interpretation of patterns observed based on blood samples. Here, it was not possible to quantify enzymatic antioxidants because of the small quantity of plasma available.

In supplemented nests, the effect of breeding density on adult body mass and on fledging success was no more significant. The decrease in feeding rate with density in control nests, independently of dispersal status, was also cancelled in supplemented nests, mainly because of an increase in feeding rate in high-density habitats (Fig. 3). Food availability thus played a role in mediating the density-dependence of these traits and in particular the differences between dispersing and philopatric individuals in patterns of density-

dependence on body mass and fledging success. Interestingly, for both traits, the difference between control and supplemented nests was observed at low densities (Fig. 1 and 4). This confirms that food availability was more constrained in low-density than high-density habitats and that density reflects habitat quality rather than intraspecific competition.

Although the small number of dispersing males prevented us to directly investigate sex differences in the observed patterns, results across studies suggest that males and females might respond differently to breeding density and food availability. In control nests, philopatric males did not adjust reproductive effort even in challenging conditions, resulting in a lower reproductive output, but maintained their body mass. The reverse pattern was observed in dispersing males, but the sample size was too low to conclude on the differences in fledging success. These results contrast with the relationships found in females in previous years. Indeed, in chapter IV, we found that fledging success was constant with density whereas ROM concentrations decreased with increasing in philopatric females, but no such interaction was found in males. Such sex by dispersal interaction on life-history traits, also observed in other bird species (Clobert *et al.* 1988), could be related to different life-history strategies associated with dispersal in males and females, due to different costs of dispersal between the sexes (Pärt & Gustafsson 1989; Pärt 1990, 1994, 1995).

Differences in life-history traits in response to variation in habitat quality between dispersing and philopatric individuals could correspond to different life-history strategies or mainly reflect differences in individual quality. Discriminating these alternatives requires assessing the effect of these differences on long-term individual fitness, in particular lifetime reproductive success. The important contribution of fledging success to lifetime reproductive success has been shown in this population (Gustafsson 1986) and dispersing males breeding in low-quality habitats might gain fitness compared to philopatric males in the same habitat, a conclusion limited by the small number of dispersing males. However, the impact of the lower body mass of dispersing individuals in low-quality habitats on future survival and reproductive success remains unclear. If body mass is not an important predictor of survival, the differences between dispersing and philopatric males might only reflect a lower quality of philopatric males, which produce less fledglings than dispersing males, in low quality

habitat. Thus, investigating the fitness consequences of these differences between dispersing and philopatric individual in their responses to habitat quality requires a estimating their survival rate as a function of breeding density or, indirectly, as a function of body mass. However, estimating differences in survival between dispersing and non-dispersing individuals can be challenging due to the higher dispersal propensity of dispersing individuals out of the study area and thus their lower probability of recapture (Doligez & Pärt 2008).

This study experimentally demonstrated that, in collared flycatchers, differences in maintenance and reproductive success between dispersing and philopatric individuals are habitat-dependent, and not simply fixed differences determined by phenotypic differences. The food supplementation increased the body mass of dispersers and the fledging success of philopatric males in low-quality habitats, thus mitigating the dispersal-dependent effects of habitat quality observed in control nests. Most studies of dispersal syndromes (reviewed in Ronce & Clobert 2012) have described one-time differences, without investigating their within-individual variation and in particular the role of environmental variation, beyond their effect on the dispersal decision itself (but see Ims 1990; Meylan *et al.* 2009; Hoset *et al.* 2010). Investigations of dispersal syndromes should pay more attention to the potential confounding effects of habitat quality and seek to measure or manipulate it before drawing conclusions from observed phenotypic differences between dispersing and non-dispersing individuals.

Acknowledgements.

Many thanks to Jukka Forsman and the landowners of Gotland for access to the study sites; Lars Gustafsson for logistics on the field; to Myriane Chalopin, Arthur Colliot, Justine Le Vaillant, Pierre-Lou Marion, Joëlle Taroni and Murielle Vergniol for their help on the field; and to Imogen Lowes for extracting the data from most of the video recordings. This work was supported by grants from the CNRS (PICS France-Switzerland to BD). CR was supported by a PhD fellowship from the French Ministry of Research, and mobility grants

from the Région Rhône-Alpes (Explora'doc), the Rectors' Conference of the Swiss Universities and the Fondation pour l'Université de Lausanne.

Supplementary Information S1: Protocols for the measure of oxidative state markers

The concentration of Reactive Oxygen Metabolites in the plasma was measured using the d-ROMs test (MC 0001 kit, Diacron International, Grosseto, Italy). 4μL of plasma were mixed with 198μL acidic buffer and 2μL chromogenic substrate (N, N-diethylparaphenilendiamine) and left to incubate for 75mn at 37°C, before measuring OD twice at 550nm. The values were averaged to get the concentration in the sample. To control for the natural opacity of some samples, OD at 800nm was measured and samples with OD_{800}> 0.100 were excluded from the analysis.

Plasma total antioxidant capacity was estimated as the capacity of plasma to oppose the oxidative action of the hypochlorous acid HClO (OXY adsorbent test, MC 434 kit, Diacron International, Grosseto, Italy). Each plasma sample was diluted at 1/100 in ultra-pure water. 5μL diluted sample were incubated 10 mn at 37°C with 200μL HClO solution. 2μL chromogenic substrate (N, N- diethylparaphenilendiamine) were then added and OD at 550nm was measured twice and averaged to quantify HClO excess.

Table S1: Linear mixed-effect models best describing adults' body mass, oxidative state markers and feeding rate. The sample size and the partition of the variance are given for each model. The estimate of the effect of age is expressed as yearlings vs. older birds, and that of sex as males vs. females.

Effect	Estimate ± S.E.	F	Num. d.f.	Den. d.f.	P
Body mass					
$V_{residual}$ = 0.29 (N = 164 adults), V_{pair} = 0.04 (N = 120 pairs), $V_{habitat}$ = 0.00 (N = 9 plots)					
Age	0.39 ± 0.19	10.54	1	100.57	0.002
Sex	0.77 ± 0.45	10.54	1	100.57	0.002
Tarsus length	0.42 ± 0.10	18.13	1	154.89	< 0.001
Density	1.10 ± 0.25	3.32	1	109.69	0.071
Dispersal	-1.51 ± 0.66	0.48	1	140.06	0.492
Supplementation	-0.33 ± 0.60	5.25	1	106.30	0.024
Density x Sex	-1.45 ± 0.61	0.33	1	147.28	0.564
Density x Dispersal	1.84 ± 0.92	0.33	1	147.28	0.564
Density x Supplementation	0.41 ± 0.79	5.05	1	109.90	0.027
Dispersal x Supplementation	3.02 ± 0.97	8.24	1	142.05	0.005
Density x Dispersal x Suppl.	-3.97 ± 1.34	7.47	1	148.40	0.007
Reactive oxygen metabolites					
$V_{residual}$ = 0.0152 (N = 140 adults), V_{pair} = 0.0052 (N = 108 pairs), $V_{habitat}$ = 0.0000 (N = 9 plots), V_{plate} = 0.0016 (N = 5 plates)					
Sex (Male vs. female)	-0.365 ± 0.158	5.31	1	82.60	0.024
Brood size	-0.029 ± 0.016	0.06	1	101.69	0.810
Density	0.136 ± 0.101	0.30	1	114.03	0.585
Dispersal	0.296 ± 0.127	5.42	1	130.45	0.021
Sex x Brood size	0.052 ± 0.025	4.22	1	81.49	0.043
Density x Dispersal	-0.374 ± 0.177	4.49	1	131.82	0.036
Total antioxidant capacity					
$V_{residual}$ = 1178 (N = 164 adults), V_{pair} = 0.00 (N = 120 pairs), $V_{habitat}$ = 0.00 (N = 9 plots), V_{plate} = 270 (N = 5 plates)					
Sex (Male vs. female)	-0.1 ± 6.3	4.21	1	156.74	0.042
Dispersal	-14.1 ± 7.9	0.02	1	156.69	0.896
Supplementation	11.1 ± 5.4	4.18	1	157.47	0.042
Sex x Dispersal	26.6 ± 13.1	4.12	1	157.41	0.044
Feeding rate per nestling					
$V_{residual}$ = 0.56 (N = 140 adults), V_{pair} = 0.07 (N = 106 pairs), V_{date} = 0.09 (N = 15 dates), $V_{habitat}$ = 0.00 (N = 9 plots)					
Brood size	-0.3 ± 0.1	29.32	1	84.32	< 0.001
Density	-1.6 ± 0.6	0.53	1	76.40	0.467
Dispersal	0.5 ± 0.2	10.90	1	126.12	0.001
Supplementation	-1.6 ± 0.7	4.87	1	65.64	0.031
Density x Supplementation	2.5 ± 1.0	6.85	1	68.12	0.011

Table S2: Generalized linear mixed-effect models best describing fledging success. The sample size and the partition of the random variance are given beside each model.

Effect	Estimate ± S.E.	X^2	P
Fledging probability (foster mother of known dispersal status) *N = 585 nestlings, V_{foster} = 7.62 (N = 102 nests), V_{origin} = 0.31 (N = 121 nests), $V_{habitat}$ = 1.41 (N = 9 plots)*			
Hatching date	-1.46 ± 0.42	12.23	< 0.001
Supplementation	1.86 ± 0.73	6.53	0.011
Fledging probability (foster father of known dispersal status) *N = 451 nestlings, V_{foster} = 4.99 (N = 78 nests), V_{origin} = 0.004 (N = 101 nests), $V_{habitat}$ = 0.17 (N = 9 plots)*			
Hatching date	-2.06 ± 0.48	18.58	< 0.001
Body mass	-1.40 ± 0.59	5.72	0.017
Supplementation	9.51 ± 3.94	5.83	0.016
Density	9.73 ± 3.89	6.27	0.012
Dispersal	14.98 ± 5.82	6.63	0.010
Supplementation x Density	-11.42 ± 5.26	4.72	0.030
Supplementation x Dispersal	-16.89 ± 7.92	4.55	0.033
Density x Dispersal	-22.91 ± 8.66	6.99	0.008
Suppl. x Density x Dispersal	30.19 ± 12.07	6.26	0.012

Table S3: Linear mixed-effect models best describing the body mass of 12-day old nestlings. The sample size and the partition of the random variance are given beside each model.

Effect	Estimate ± S.E.	F	Num. d.f.	Den. d.f.	P
Nestling body mass (foster mother of known dispersal status) *$V_{residual}$ = 1.08 (N = 346 nestlings), V_{foster} = 0.91 (N = 70 nests), V_{origin} = 0.51 (N = 90 nests), $V_{habitat}$ = 0.44 (N = 9 plots)*					
Hatching date	-0.14 ± 0.05	6.78	1	66.23	0.011
Nestling body mass (foster father of known dispersal status) *$V_{residual}$ = 1.26 (N = 288 nestlings), V_{foster} = 1.87 (N = 62 nests), V_{origin} = 0.47 (N = 82 nests), $V_{habitat}$ = 0.20 (N = 9 plots)*					
Hatching date	-0.21 ± 0.07	8.29	1	59.89	0.006

PAPER 2 – FOOD SUPPLEMENTATION MITIGATES DIFFERENCES IN NEST-DEFENCE BEHAVIOUR BETWEEN DISPERSING AND NON-DISPERSING INDIVIDUALS IN A PASSERINE BIRD.

Charlotte Récapet, Joëlle Taroni, Grégory Daniel, Pierre Bize and Blandine Doligez

In preparation

Keywords: dispersal, habitat quality, anti-predator behaviour, reproductive investment, personality, Collared flycatcher, *Ficedula albicollis*

Dispersal behaviour is a major response to environmental spatio-temporal variability and has been shown to be associated with individual variation in behavioural and life-history traits. However, to what extent these differences between dispersing and non-dispersing individuals are fixed or depend on external conditions remains poorly known. To explore this question, we manipulated local habitat quality by providing additional food during the nestling feeding period in a patchy population of collared flycatchers and we compared the subsequent nest defence against a dummy predator between dispersing and non-dispersing parents. Dispersing birds showed more intense nest defence behaviour in supplemented compared to control nests, whereas non-dispersing birds showed an equally strong response in both treatments. These effects were independent of brood value. The difference between dispersing and non-dispersing individuals may result from a dispersal cost, with dispersers being normally constrained to invest more in foraging behaviour but supplementation releasing this constraint. It may also reflect two distinct behavioural strategies associated with dispersal in this population, with dispersers adjusting their investment when breeding in low-quality habitat while non-dispersers show a less flexible reproductive investment. More attention should therefore be given to the influence of environmental variation on behavioural and life-history responses of dispersing and non-dispersing individuals when assessing the costs and benefits of dispersal.

1. Introduction

Dispersal, defined as the movement of individuals between breeding sites or between the birth site and the site of first breeding (Greenwood & Harvey 1982), is a fundamental process in ecology, allowing rapid individual responses to environmental variation, in particular the deterioration of local conditions (Clobert *et al.* 2001). Dispersal decisions are frequently driven by interactions between environmental factors and phenotype (Ims & Hjermann 2001), but recent studies have shown that dispersal is partly heritable in different taxa (e.g. (Roff & Fairbairn 2001; Doligez *et al.* 2009; Zera & Brisson 2012)). Furthermore, natural selection might favour the functional integration of dispersal with various phenotypic traits that allow individuals to reduce dispersal costs (Clobert *et al.* 2009), resulting in genetic correlations at the population level (Duckworth & Kruuk 2009; Korsten *et al.* 2013). In particular, temerity and exploratory propensity are the behavioural traits most consistently associated with dispersal (Ronce & Clobert 2012; Debeffe *et al.* 2013) (but see (Cote *et al.* 2010)). Thus dispersal is often predicted to be associated with fixed differences in behavioural and physiological traits, defining a "dispersal syndrome" (Duckworth & Kruuk 2009; Meylan *et al.* 2009). However, natural selection is also expected to favour flexibility, allowing individuals to adjust decisions to environmental conditions. Thus, dispersing individuals may differ in their response to environmental conditions and in this case, the variation observed between dispersing and non-dispersing individuals would be conditional on the environment rather than fixed (Pennekamp *et al.* 2014). Because most studies on dispersal syndromes so far did not manipulate environmental conditions independently of habitat selection, they cannot discriminate between these alternatives.

To explore whether differences between dispersing and non-dispersing individuals in behavioural traits were fixed or conditional on environmental conditions, we manipulated habitat quality by providing additional food during the nestling rearing period in a patchy population of collared flycatchers *Ficedula albicollis*. Just before fledging, we measured nest defence behaviour against a natural nest predator, the red squirrel, for individuals that previously bred in the patch and for new comers, while controlling for brood value.

2. Material and methods

The study was conducted in spring 2014 on a patchy population of collared flycatchers breeding in nest boxes on the island of Gotland, Sweden (57°07'N, 18°20'E). Nest boxes in our eight experimental plots were monitored regularly throughout the season to record breeding data (laying and hatching dates, clutch and brood size, mass and tarsus length of nestlings on day 12). Parents were caught inside boxes, aged (yearlings vs. older adults) based on plumage characteristics (Svensson 1992) and previous capture history, and weighed when chicks were six to 12 days old. Dispersal was defined as a change of breeding patch between birth and the first capture as a breeder (natal dispersal) or between successive captures as a breeder (breeding dispersal). Non-dispersing individuals did not change plot between successive captures (see (Doligez *et al.* 1999) for a discussion of this binary definition of dispersal in the collared flycatcher). We excluded all previously unringed adults, which were of uncertain dispersal status because a fraction of local breeders are missed every year. Permission for catching and ringing adult and young birds was granted by the Ringing Centre from the Museum of Natural History in Stockholm (licence number 471:M009 to CR).

Food availability was manipulated by providing additional food to half of our nests (N = 86 supplemented nests) from day two to day 12 post-hatching. 30g of live maggots were provided once a day, between 07:00 and 19:00, in transparent containers attached to nest boxes. Control nests (N = 82) received no food, but were also visited daily to be subjected to the same level of disturbance. Nests that hatched the same day were assigned either to the control or supplemented treatment group alternatively in space, to distribute treatments homogeneously according to space and hatching date within study plots. Breeding density in the plot, i.e. the fraction of available nest boxes that were occupied by flycatchers in the plot, was calculated to account for natural variation in habitat quality between plots. Breeding density is positively correlated with breeding success in our population (Doligez *et al.* 2004), but could also reflect varying levels of competition.

Nest defence was measured when chicks were 13-days old by placing a stuffed red squirrel on the entrance of the nest box, mimicking a nest predator attack on nestlings. To

avoid premature fledging, nest box entrance was closed during the test. The stuffed squirrel was left for no longer than 5min from the arrival of the second parent and no longer than 15 min from the observer's arrival. If no adult was seen, it was removed after 10 min. An observer hidden under a camouflage net and sitting at least 10 meters from the nest box recorded the behaviour of the pair. Recorded behaviours are described in Table S1. Because some parents arrived less than 5 min before the end of the test, behavioural responses during the 4.5 min only following an individual first sighting were available for all individuals and used in the analyses. One individual that arrived only 16 s before the end of the test was considered absent during the test. Following a multivariate analysis of the data exploring correlations between behavioural responses (Supplementary material S1), a nest defence score was computed based on the behaviours that best described the intensity of the response: (i) time spent within 2 meters of the box, (ii) total number of moves and (iii) presence/absence of direct attacks on the dummy (Table 1). Similar scoring system are used in other studies of nest defence (Hakkarainen & Korpimaki 1994; Duckworth 2006).

Table 5: Construction of the nest defence score. Tertiles of the distribution for the whole population were used as cut-off values for the two quantitative variables. Individuals that were not seen during the test were assigned a score of 0. If individuals attacked the dummy, the score was increased by 1. Thus the final score varied between 0 and 6.

		Time spent within 2m of the nest box		
		$t \leq 29.9\%$	$29.9\% < t \leq 81.1\%$	$t > 81.1\%$
Number of moves	$a \leq 14$	1	2	3
	$14 < a \leq 26$	2	3	4
	$a < 26$	3	4	5

In total, nest defence score was obtained for 128 individuals whose dispersal status was known, 67 females (25 dispersing, 42 non-dispersing) and 61 males (10 dispersing, 51 non-dispersing) from 101 different nests. The supplemented (N = 51) and control nests (N = 40) differed neither in brood size at the beginning of the treatment (Wilcoxon rank-sum test: W = 886, P = 0.257) nor in laying date (W = 903, P = 0.350).

The influence of individual dispersal status, supplementation treatment and breeding density, as well as their interactions, on individual nest defence score was investigated using a linear mixed-effects model. Sex, age (yearling vs. older), adult body mass, brood size on the day of the nest defence test and average nestling body mass were included as fixed covariates as well as their interaction with the supplementation treatment; nest and observer were included as random effects to control for the non-independence of the two pair members and for variation between observers. To test whether nest defence behaviour was measured on a biased sample of breeders with respect to dispersal and supplementation treatment, the effect of supplementation treatment and dispersal status were investigated on (i) the probability of total brood failure (using GLM with a binomial error distribution), (ii) brood size on the day of the nest defence test and (iii) average nestling body mass (with average tarsus length as covariate), on males and females separately to avoid pseudo-replication of the breeding data. We also tested the effect of dispersal in interaction with the supplementation treatment, which was non-significant in all three models and thus not reported in the results. All analyses were run using the R software. Non-significant effects (starting with interactions) were removed based on type-III F-tests with denominator degrees of freedom calculated using Satterthwaite's approximation (R package 'lmerTest' (Kuznetsova *et al.* 2013)).

3. Results

The mean (± SE) nest defence score was 2.9 ± 1.7. Nest defence score differed between dispersing and non-dispersing individuals and this difference depended on the food supplementation treatment (interaction between dispersal status and supplementation treatment: $F_{1,108} = 3.95$, $P = 0.049$, Figure 12). In control nests, dispersing birds had a lower score than non-dispersing ones (post-hoc test: $F_{1,50} = 7.61$, $P = 0.008$; estimate ± SE for dispersing compared to non-dispersing individuals: -1.32 ± 0.48), whereas the score of non-dispersing and dispersing birds did not differ anymore in supplemented nests ($F_{1,68} = 0.57$, $P = 0.45$). The difference between dispersing and non-dispersing individuals also depended on breeding density (interaction between dispersal status and breeding density: $F_{1,106} = 6.38$,

P = 0.010, Figure 13). Among dispersing individuals, nest defence decreased with increasing density (post-hoc test: $F_{1,21}$ = 5.11, P = 0.030; estimate ± SE: -3.65 ± 1.62), while no relation was observed in non-dispersing birds ($F_{1,85}$ = 2.34, P = 0.13).

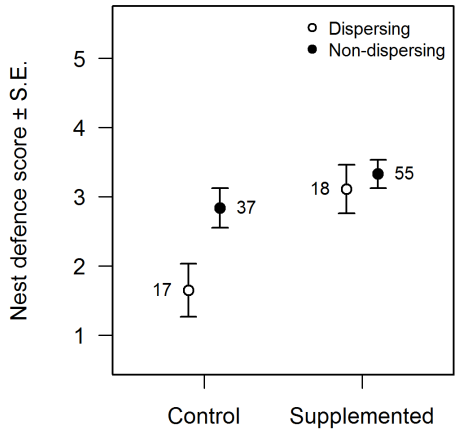


Figure 12: Effect of the food supplementation treatment on the nest defence score of dispersing and non-dispersing individuals.

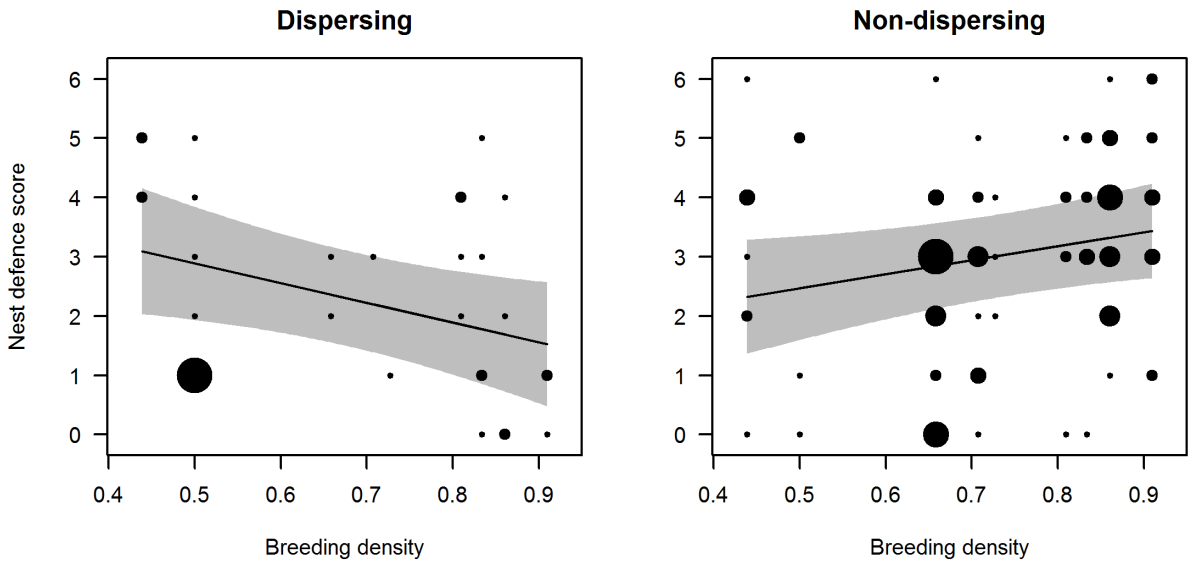


Figure 13: Effect of breeding density on the nest-defence score of dispersing and non-dispersing individuals.

Furthermore, brood size affected nest defence score depending on supplementation treatment (interaction between treatment and brood size: $F_{1,61} = 4.10$, $P = 0.047$): the nest defence score tended to increase with brood size in control nests ($F_{1,49} = 3.42$, $P = 0.070$; estimate ± SE: 0.23 ± 0.12), but not in supplemented nests ($F_{1,43} = 0.38$, $P = 0.54$). Individual's age, either alone or in interaction with dispersal status, body mass and sex, as well as average nestling body mass, had no effect on nest defence score (all $P > 0.18$, Table S3).

The probability of total brood failure between the start of the supplementation treatment and the nest defence test was lower in supplemented nests, although only marginally so in females (males: $F_{1,76} = 6.19$, $P = 0.020$; females: $F_{1,100} = 3.27$, $P = 0.070$; estimates ± SE for supplemented nests compared to control ones: -1.48 ± 0.63 and -0.78 ± 0.43 for males and females respectively). Brood failure was independent of dispersal status (males: $F_{1,75} = 0.46$, $P = 0.50$; females: $F_{1,99} = 0.22$, $P = 0.64$). Brood size was higher in supplemented nests (males: $F_{1,59} = 9.90$, $P = 0.003$; females: $F_{1,65} = 8.31$, $P = 0.005$; estimates ± SE for supplemented nests compared to control ones: 1.16 ± 0.40 and 1.23 ± 0.39 for males and females respectively), but did not differ between dispersing and non-dispersing individuals (males: $F_{1,58} = 0.06$, $P = 0.81$; females: $F_{1,64} = 0.36$, $P = 0.55$). Nestling body mass was higher for dispersing individuals compared to non-dispersing ones, although only marginally so in males (males: $F_{1,58} = 3.91$, $P = 0.052$; females: $F_{1,64} = 4.83$, $P = 0.030$; estimates ± SE for dispersing compared to non-dispersing individuals: 0.72 ± 0.36 and 0.52 ± 0.24 for males and females respectively) Nestling body mass did not differ between supplemented and control nests (males: $F_{1,57} = 0.81$, $P = 0.37$; females: $F_{1,63} = 0.07$, $P = 0.80$).

4. Discussion

Dispersing individuals increased the intensity of nest defence when food availability was increased compared to control conditions. In contrast, non-dispersing individuals showed a high intensity of nest defence whatever the supplementation treatment. Consistently, food abundance was found to be positively linked to nest defence in Ural owls (Kontiainen *et al.* 2009) and great tits (Rytkönen 2002), controlling for brood size. There was no evidence of a bias in our sample of nest defence scores due to higher early failure of a

non-random fraction of individuals with respect to dispersal, because the probability of breeding failure before the nest defence test did not depend on the interaction between supplementation treatment and dispersal status. Intra-individual flexibility rather than inter-individual differences might therefore explain a large part of the observed variation in nest defence behaviour.

The parental investment theory postulates that parental care should increase with current reproductive value, which increases with nestling number and condition, and decrease with residual reproductive value, i.e. future breeding prospect of the individual (Winkler 1987). Here, nest defence was positively related to brood size, reflecting current reproductive value, in control nests only, but was not related to fledging mass. There was no effect of parent age or body mass, possibly reflecting residual reproductive value, on nest defence behaviour. As brood size was independent of dispersal, the observed variation in nest defence behaviour was not mediated by differential effects of the supplementation on nestling survival and growth or on adult condition.

The observed difference in nest defence behaviour between dispersing and non-dispersing birds could result either from a constraint on dispersers or from an adaptive adjustment by dispersers. In control conditions, dispersing individuals may not be able to invest as much time and energy in nest defence as non-dispersing individuals. They may indeed not be able to exploit their habitat as efficiently due to unfamiliarity with the environment (Pärt 1995) and need to reallocate time and energy to foraging and nestling provisioning. However, when such constraint is released, here via food supplementation but also naturally when density increases, possibly reflecting increasing quality habitat, dispersing individuals can increase their investment in other parental care behaviours, such as nest defence. Under this hypothesis, the reduced nest defence in control nests would reflect a cost for dispersers in terms of immediate increased risk of nest predation. Alternatively, our results could suggest the existence of different investment strategies, with dispersing individuals adjusting their level of parental care depending on resource availability while non-dispersing ones show a constantly high investment in nest defence. Under this hypothesis, the reduced nest defence behaviour in control nests would reflect a

beneficial adjustment by dispersing individuals. In line with this idea, dispersing individuals reared heavier nestlings than non-dispersing individuals independently from the supplementation treatment, and thus seemed to benefit from adjusting their investment in parental care. Our study population is characterised by a low level of nest predation compared to other similar passerine populations, thanks to the absence of mustelid species on Gotland (Doligez & Clobert 2003). Because the most important factor determining reproductive success in our population is thus the availability of resources for nestlings, selection may have favoured high investment in nestling provisioning at the expense of other parental care behaviours. In this case, the reason why non-dispersing individuals maintain a high level of nest defence whatever environmental conditions remain to be explored.

Our study experimentally showed the role of environmental conditions in mediating differences in behaviour during breeding between dispersing and non-dispersing individuals. Characterising dispersal syndromes without taking environmental variability into account might thus be lead to intrinsically flawed inferences. To better understand the processes at play on the evolution of dispersal strategies, we encourage integrating measures of habitat quality variation or manipulating this quality when studying behavioural syndromes, and more generally differences in life-histories, associated to dispersal.

Acknowledgements.

Many thanks to Jukka Forsman and the landowners of Gotland for access to the study sites; Lars Gustafsson for logistics on the field; and to Myriane Chalopin, Arthur Colliot, Justine Le Vaillant, Pierre-Lou Marion, and Murielle Vergniol for their help on the field. This work was supported by grants from the CNRS (PICS France-Switzerland to BD). CR was supported by a PhD fellowship from the French Ministry of Research, student mobility grant from the Région Rhône-Alpes (Explora'doc) and the Rectors' Conference of the Swiss Universities and a grant from the Fondation pour l'Université de Lausanne. GD was supported by the Région Rhône-Alpes (CIBLE - PhD scholarship grant and Explora'doc mobility grant) and a stipend from the University of Uppsala. JT was supported by a grant from the Doctoral School of the Faculty of Biology and Medicine, University of Lausanne.

Table S1: Ethogram used to code the behavioural tests. Alarm calls were also frequent, but could rarely be attributed to one of the parents, especially when these remained far from the nest box. They were therefore not included in the score.

Behaviour	Description
+ 10 meters	The bird flies and lands more than ten meters from the nest box or out of view.
- 10 meters	The bird flies and lands between ten and five meters from the nest box.
- 5 meters	The bird flies and lands between five and two meters from the nest box.
- 2 meters	The bird flies and lands within two meters of the nest box.
Attack	The bird attacks the dummy squirrel by giving it a peck.
Hovering	The bird does a stationary flight in front of the nest box.
Swooping	The bird plunges toward the dummy squirrel to chase it.

Supplementary information S2: Comparison of the nest defence score with a Principal Component Analysis of behaviour

Excluding individuals that were not seen, and one individual that arrived 16s before the end of the test, a Principal Component Analysis was performed on the following variables using the function *dudi.pca* of the 'ade4' package (Dray & Dufour 2007):

- latency between the start of the test and the bird's arrival
- time spent within 2 meters of the nest
- time spent within 5 meters of the nest, but further than 2 meters
- time spent within 10 meters of the nest, but further than 5 meters
- time spent alarming
- activity = number of moves
- number of attacks and swooping (cf. Supplementary Table S1).

The first principal component explained 33.4% of the variance in behaviour. This figure dropped to 17.9% for the second component. The loadings of the first principal axes showed that the latency and alarms did not contribute much to this axis (Supplementary Table S2.1). Alarms contributed positively to the first axis and were negatively correlated to activity and the time spent within 2 meters, as did the time spent within 5 meters and within 10 meters. The intensity of the nest defence response was thus best described by the time within 2 meters, the activity, and the attacks. As the correlation coefficients between these three variables were moderate, we kept them all to build the nest defence score.

Table S2.1: Loadings of the behavioural variable on first two principal axes of the PCA. Their correlations with the axes are given into parentheses.

Loadings (Correlations)	**Principal axis 1**	**Principal axis 2**
Latency	0.041 (0.063)	-0.102 (-0.114)
Time within 2 meters	-0.553 (-0.846)	-0.419 (-0.469)
Time within 5 meters	0.348 (0.533)	0.552 (0.618)
Time within 10 meters	0.387 (0.591)	0.048 (0.053)
Alarms	0.293 (0.448)	-0.372 (-0.416)
Activity	-0.489 (-0.748)	0.367 (0.411)
Attacks and swooping	-0.309 (-0.473)	0.483 (0.541)

Table S2.2: Correlation between the behavioural variables

	Time within 2 meters	Time within 5 meters	Time within 10 meters	Alarms	Activity	Attacks and swooping
Latency	0.014	0.074	-0.062	-0.086	-0.115	-0.099
Time within 2 meters		-0.651	-0.556	-0.173	0.431	0.121
Time within 5 meters			-0.031	0.048	-0.132	-0.118
Time within 10 meters				0.159	-0.316	-0.072
Alarms					-0.346	-0.171
Activity						0.458

Using the first Principal Component as a response variable instead of the calculated score yielded similar results, although the power was lower due to the individuals excluded (Supplementary Table S3).

The probability not to be seen during the test (including the late individual) could not be properly modelled due to the low number of individuals concerned. However, it followed a similar pattern with a higher proportion of missed individuals in dispersing birds from control nests compared to philopatric birds or dispersing birds from supplemented nests:

Table S2.3: Number of individuals that were seen (present) or not (absent) during the test. The proportion of absent individuals is given in parentheses.

Treatment	**Control**		**Supplemented**	
Dispersal status	Dispersing	Philopatric	Dispersing	Philopatric
Absent:Present (Proportion)	3:14 (0.18)	5:32 (0.14)	1:17 (0.06)	3:52 (0.05)

Using the score thus allowed us to include these individuals in the analyses, while maintaining the contribution of the three major variables.

Table S2.4: Distribution of the nest defence score

Score	0	1	2	3	4	5	6
N	12	19	16	30	24	21	5

Table S3: Linear mixed-effect model best describing the sources of variation in nest defence score (white cells), and non-significant marginal effects (grey cells). A similar model was then applied to the score and the first Principal Component for individuals present during the test. The sample size and the partition of the random variance are given beside each model. The initial full model included age, sex, brood size during the test, adult body mass, average body mass of nestlings, and their interaction with the supplementation.

Effect	Estimate ± S.E.	F	Num. d.f.	Den. d.f.	P
Nest defence score $V_{residual}$ = 1.97 (N = 127 adults), V_{pair}= 0.24 (N = 91 pairs), $V_{observation}$ = 0.31 (N = 3 experimenters)					
Brood size	0.26 ± 0.12	0.79	1	60.05	0.378
Breeding density	1.61 ± 1.15	0.62	1	70.34	0.435
Dispersal	1.88 ± 1.35	3.46	1	97.04	0.066
Supplementation	1.92 ± 0.91	7.61	1	62.24	0.008
Brood size x Supplementation	-0.37 ± 0.18	4.10	1	60.98	0.047
Dispersal x Breeding density	-4.70 ± 1.86	6.38	1	106.28	0.013
Dispersal x Supplementation	1.20 ± 0.60	3.95	1	108.27	0.049
Age class (Yearlings)	0.83 ± 0.62	1.79	1	112.13	0.183
Age class (Yearlings) x Suppl.	0.90 ± 1.21	0.56	1	111.81	0.456
Sex (Males)	0.11 ± 0.27	0.18	1	59.64	0.677
Adult body mass	-0.06 ± 0.22	0.07	1	115.20	0.795
Nestlings average body mass	0.01 ± 0.10	0.01	1	57.01	0.915
Nest defence score (excl. absent individuals) $V_{residual}$ = 1.35 (N = 115 adults), V_{pair}= 0.25 (N = 84 pairs), $V_{observation}$ = 0.42 (N = 3 experimenters)					
Brood size	0.18 ± 0.11	0.90	1	65.73	0.346
Breeding density	1.01 ± 1.01	0.03	1	75.20	0.864
Dispersal	0.21 ± 1.20	0.43	1	93.87	0.514
Supplementation	0.97 ± 0.82	3.50	1	69.45	0.066
Brood size x Supplementation	-0.21 ± 0.16	1.58	1	67.03	0.213
Dispersal x Breeding density	-2.32 ± 1.70	1.86	1	99.23	0.176
Dispersal x Supplementation	1.15 ± 0.55	4.40	1	96.66	0.038
First Principal Component (opposite to homogenize the signs) $V_{residual}$ = 1.20 (N = 115 adults), V_{pair}= 0.41 (N = 84 pairs), $V_{observation}$ = 0.45 (N = 3 experimenters)					
Brood size	0.23 ± 0.12	0.53	1	74.05	0.468
Breeding density	1.18 ± 1.02	0.29	1	82.39	0.593
Dispersal	0.93 ± 1.21	1.37	1	98.98	0.244
Supplementation	1.59 ± 0.83	5.97	1	77.26	0.017
Brood size x Supplementation	-0.34 ± 0.17	3.96	1	75.21	0.050
Dispersal x Breeding density	-3.30 ± 1.71	3.73	1	102.58	0.056
Dispersal x Supplementation	0.96 ± 0.55	2.99	1	100.87	0.087

Chapter VI – Evaluation of three potential predictors of fitness prospects in a passerine with natal and breeding dispersal.

Assessing survival prospects in a spatially limited study site can be biased with respect to dispersal if dispersing individuals are more prone to disperse again and thus leave the site. Therefore, assessing individual long-term fitness may require using indirect markers. We investigated three traits known to depend on physiological condition and to relate to survival in other species: body mass, telomere length and glycated haemoglobin. Telomeres, short repeated sequences that protect the end of chromosomes, are linked to survival in various non-model species. High glycaemia can result in haemoglobin glycation, which is associated with poor survival and health outcomes in humans and model species, but has rarely been investigated in wild animals. We investigated whether these markers predict return rate, subsequent dispersal and reproduction prospects in the collared flycatcher and could mediate the dispersing-dependent responses to environmental conditions described in this population. We showed that telomere length decreased with increasing breeding density in dispersing birds, but not in philopatric ones, whereas body condition decreased with increasing breeding density for all individuals. Conversely, glycated haemoglobin was independent of breeding density and dispersal. Glycated haemoglobin negatively related to survival, suggesting the selective disappearance of individuals with poor glucose homeostasis. In contradiction with the positive relationship often reported between telomere length and survival, longer telomeres were associated with lower local survival. Our three markers were not related to subsequent dispersal within the study area and their relationship to local survival did not depend on the dispersal status in 2009. These results suggest that telomere length and glycated haemoglobin could be used as markers of future survival. Longitudinal analyses of both markers in a capture-mark-recapture framework will now be necessary to validate their use.

Authors: Charlotte Récapet, Blandine Doligez and Pierre Bize

Keywords: return rate, telomere length, HbA1, glycated haemoglobin, glucose regulation, *Ficedula albicollis*

1. Introduction

Dispersal is a key life-history trait that allows individuals to respond to spatio-temporal variation in the environment (Clobert *et al.* 2001, 2012), but could entail fitness costs and benefits. The eventual success of dispersal strategies will depend on the differences in survival and reproductive success according to dispersal. Despite many empirical studies comparing life-history traits between dispersing and non-dispersing individuals (reviewed in Bélichon *et al.* 1996; and Doligez & Pärt 2008), no general pattern of association emerges, with only half of them (i.e. 65 out of 133 studies up to 2008) reporting a significant link between dispersal and at least one life-history trait. When a link was reported, dispersers showed a lower value as often as a higher value for the trait considered compared to non-dispersers. Whether these differences modify the success of dispersal strategies will ultimately depend on the relative fitness of dispersing and non-dispersing individuals. Dispersers were found to reach lower lifetime reproductive success (LRS) than non-dispersers at least for one sex or in some environmental conditions in a majority of correlative studies (i.e. 16 out of 21; Wauters, Matthysen & Dhondt 1994; Doligez & Pärt 2008; Pärn *et al.* 2009; Bouwhuis *et al.* 2010; Gienapp & Merilä 2011; Serrano & Tella 2012; Nevoux *et al.* 2013; García-Navas *et al.* 2014; Germain 2014; Peterson *et al.* 2014), but an equal number of studies showed no difference in at least one sex or in some environmental conditions (Wauters *et al.* 1994; Spear, Pyle & Nur 1998; Doligez & Pärt 2008; Pärn *et al.* 2009; Gienapp & Merilä 2011; Waser, Nichols & Hadfield 2013; Nevoux *et al.* 2013; Germain 2014; Peterson *et al.* 2014) and a positive relationship between dispersal and LRS was even found in three studies (Spear *et al.* 1998; Maccoll & Hatchwell 2004; García-Navas *et al.* 2014). Importantly, both local survival and recruitment estimates for dispersing individuals can be underestimated in spatially limited study areas if dispersers and/or their offspring are more likely to disperse again and thus leave the study area, which would create a downward bias on the LRS of dispersers (Orell *et al.* 1999; Doligez & Pärt 2008). The hypothesis thus emerged that dispersal could be associated with different life-history strategies that would be ultimately balanced in terms of fitness, through a differential investment either in the

quantity and quality of offspring or in individual breeding success and survival (Bélichon *et al.* 1996; Julliard *et al.* 1996; Spear *et al.* 1998; Marr *et al.* 2002).

The lack of general pattern in empirical studies on associations between life-history traits and dispersal could also result from an effect of environmental characteristics on the observed differences between dispersers and non-dispersers. Accordingly, dispersers and non-dispersers were sometimes found to respond differently to environmental variation in terms of life-history traits (Fjerdingstad *et al.* 2007; Bestion *et al.* 2015). Correlative and experimental studies also confirmed that local adaptation and habitat selection could play a large role in determining fitness differences according to dispersal (Hansson, Bensch & Hasselquist 2004; Peterson *et al.* 2014; Bonte *et al.* 2014).

Directly assessing subsequent survival and reproductive prospects according to dispersal is challenging because, as explained above, survival estimates in a spatially limited study site can be biased with respect to dispersal (Doligez & Pärt 2008), but also because of the need of longitudinal long-term data individual and because the probability of capture of breeding individuals is often linked to their reproductive success (Doligez *et al.* 2012). Therefore, using markers of long-term fitness-related traits could be a better strategy than attempting to estimate these traits directly, especially in cases when obtaining unbiased estimates is difficult. As the decision to disperse often depends on the health and condition of the individual (Ims & Hjermann 2001), dispersal decisions could also be biased according to the chosen markers and the relationship between those markers and future survival and dispersal could differ according to past dispersal events. Assessing the usefulness of potential markers will require testing them in a context were dispersal can be observed in a large area and thus be more easily distinguished from survival.

Among such markers, physiological parameters that could integrate the effects of metabolism and stress over time and have been associated to survival or reproductive prospects in other species are good candidates. Three such markers are of particular interest: body mass, telomere length and the level of glycated haemoglobin. First, body mass, when corrected for skeletal size, reflects the energetic balance of an individual and its energy stores at a given point in time (Schulte-Hostedde *et al.* 2005; Peig & Green 2009). A higher body

mass has therefore often been found to associate with higher survival prospect in wild animals. Energy stores are an insurance against food shortage (Ricklefs & Schew 1994) and accordingly factors that lessen body reserves such as diseases (Booth, Clayton & Block 1993; Neuhaus 2003; Hawlena *et al.* 2006) or physical exhaustion (Winkler & Allen 1995; Carrascal & Polo 2006; Harding *et al.* 2009; Harrison *et al.* 2011) are known to reduce survival. Second, telomere length reflects the decrease over replications, i.e. cell divisions, of these repeated sequences that protect the end of chromosomes (Chan & Blackburn 2004), which can be accelerated by various stressors such as lower habitat quality (Young *et al.* 2013; Angelier *et al.* 2013) and higher competition for food within a brood (Nettle *et al.* 2013, 2015; Boonekamp *et al.* 2014; Reichert *et al.* 2015). Environmental conditions could influence telomere length via oxidative stress (Houben *et al.* 2008). Indeed, oxidative stress has been experimentally shown to increase telomere shortening in cell cultures (von Zglinicki 2002; Richter & von Zglinicki 2007). Moreover, an experimental increase of immune and psychological stress (Hau *et al.* 2015) or corticosterone exposure (Haussmann *et al.* 2012) are associated with both higher oxidative stress and telomere attrition. Longer telomeres have therefore been linked to higher survival and lifespan in various animal species in the wild (reviewed in Stier *et al.* 2015b). Third, the level of glycated haemoglobin reflects the irreversible glycation of hemoglobin proteins linked to a poor control of blood glucose levels. The level of glycated haemoglobin was found to correlate positively with markers of oxidative stress and oxidative damage (Rytter *et al.* 2009) and negatively with telomere length in humans (Campa *et al.* 2014). Disruption of glucose homeostasis is associated with detrimental health outcomes in various model species (Harwood, Listrani & Wagner 2012) and with physiological and pathological aging in humans (López-Otín *et al.* 2013). Conversely, glycated haemoglobin was positively correlated to individual condition in wild birds (Andersson & Gustafsson 1995; Ardia 2006), probably because it reflects a high food availability. Its association with survival in wild populations has never been investigated.

Here, we explored whether body mass, telomere length, and glycated haemoglobin differ between dispersing and philopatric individuals and whether they reflect future local survival and reproductive success depending on dispersal behaviour in a patchy population

of collared flycatchers (*Ficedula albicollis*). Previous results have shown that differences between dispersing and non-dispersing individuals in reproductive success were dependent on habitat quality, which is positively correlated with breeding density in this population (Chapter IV and V). Lower reproductive success was however often paralleled with lower exposure to oxidative stress or higher body mass, suggesting compensation between the reproductive and maintenance costs of low habitat quality. Such compensation will only be effective if lower reproductive damages or higher body mass translate into higher survival and thus contribute to increasing lifetime reproductive success, compensating for the negative effect of lower reproductive success on LRS. The long-term consequences of the observed variation in oxidative balance and body mass, in terms of survival and future reproductive prospects, therefore need to be examined. We explored whether local breeding density of conspecifics differentially impacted these physiological markers between dispersing and philopatric individuals, possibly suggesting different life-history strategies in response to environmental conditions depending on dispersal behaviour.

2. Material and methods

2.1. Study site and definition of dispersal

The study was conducted in May-June 2009 on a breeding population of collared flycatchers of the island of Gotland, Sweden (57°10'N, 18°20'E). Collared flycatchers are hole-nesting migratory birds that readily accept to breed in artificial nest boxes. The study site comprises 17 spatially discrete forest plots of varying size. Nest boxes were checked regularly to record breeding data (laying and hatching date, clutch size and brood size at hatching and fledging). Breeding adults were trapped within nest boxes during incubation (day 6 to 12) for females or during nestling feeding (day 5 to 12) for males and a few females missed during incubation. Upon capture, adults were identified with aluminium rings, aged based on plumage characteristics (yearlings vs. older adults; Svensson 1992), weighed (to the nearest 0.1g), measured (tarsus length to the nearest 0.1mm) and blood sampled (100 to 130 µl from the brachial vein in EDTA-coated Microvettes, Sarstedt, Nümbrecht, Germany).

Blood samples were maintained at 5°C in the field before being centrifuged in the evening to separate plasma from red blood cells. Plasma and red blood cells were then stored at -80°C.

The exact age of each individual was subsequently determined from previous records in the population for birds ringed as nestlings or first caught as yearlings. Individuals for which exact age was not known were excluded from the analyses. We defined dispersal as a change of breeding plot, either between birth and the first breeding event (natal dispersal) or between two consecutive breeding events (breeding dispersal; see Doligez *et al.* 1999 for a discussion of this binary definition of dispersal in this population).

Brood size manipulations were performed two to eight days after hatching in 11 plots as part of another study. This manipulation could not directly impact our measures of markers in females, which were sampled during incubation, but could potentially affect measures in males. Because glycated haemoglobin integrates blood glucose levels over the past 3 to 5 weeks in birds (Andersson & Gustafsson 1995), it seemed unlikely that the manipulation could have a strong effect on this marker. However, variation in telomere length can be measured over approximately a week (Stier *et al.* 2015a). We checked whether brood size manipulation could affect our markers by including the number of added/removed nestlings in our models as well as its interaction with sex. We found no effect of the manipulation on any of the studied markers and it did not impact the effects of other variables either. Therefore, we ignored this manipulation in all analyses presented here.

2.2. Measure of relative telomere length

DNA samples were extracted from red blood cells in the laboratory using the Qiagen blood tissue kits and were stored at 4°C in AE buffer (10 mM Tris-HCl, 0.5 mM EDTA, pH = 9.0). Measures of DNA concentration and purity tests were performed using a NanoDrop spectrophotometer (Supplementary Information S1).

Telomere length was quantified using a real-time quantitative PCR assay developed to measure relative telomere length in humans and later validated for birds (Criscuolo *et al.* 2009). This technique estimates relative telomere length by determining the ratio (T/S) of

telomere repeat copy number (T) to a non-variable copy number gene (S) used as a reference, here 18S, in focal samples when compared with a reference sample. The primers used also amplify interstitial telomere-like sequences (Delany *et al.* 2003). However, measures of telomere length including these repeats has been shown to correlate well to measures excluding them in different bird species (Foote, Vleck & Vleck 2013; Young *et al.* 2013). Therefore, this should not be a problem here. We followed the same protocol and used the same equipment as Voillemot *et al.* (2012) and the detailed qPCR protocol is reproduced in Supplementary Information S1.

Telomere and 18S amplifications were carried out on different 396-well plates, each plate containing 376 samples, one serial dilution run in duplicate (two fold-dilution from 16 ng down to 0.0625 ng of DNA, i.e. 9 wells per series) and two negative controls. Each plate was replicated twice to obtain two telomere and two 18S measurements for each sample. Serial dilutions were used to set up the threshold of fluorescence at which amplification would be measured and to produce a standard curve allowing assessing the efficiency and goodness of fit of each PCR reaction using the SDS software (version 2.4, Applied Biosystems, Foster City, USA). Efficiency was also calculated individually for each well using LinReg software (Ramakers *et al.* 2003), with a common window of linearity for each plate that was checked visually and changed manually for a few wells based on the goodness of fit of a linear curve. The Ct values were calculated in SDS as the number of PCR cycles needed for the sample to reach the threshold of fluorescence.

Mean amplification efficiency estimated by the standard curves was 111.3% and 127.6% for the two 18S plates and 101.5% and 102.7% for the two telomeres plates, with goodness of fit between 0.983 and 0.999. Mean amplification efficiency estimated by LinReg over all samples after quality control was 79.8% and 83.4% for 18S and 65.9% and 65.8% for telomeres. This is consistent with methodological studies than found systematically higher efficiencies with standard curves methods than with LinReg (Ruijter *et al.* 2013). The minimum efficiency of individual wells over both plates was 69.4% for 18S and 52.7% for telomeres. For each sample within a plate, the telomeres or 18S ratio relative to the standard were calculated as:

$$\frac{eff_{std}{}^{Ct_{std}}}{eff_{sample}{}^{Ct_{sample}}}$$

where eff_{std} and eff_{sample} were the efficiencies estimated through LinReg for the standard and the sample, respectively, and the standard was a sample chosen as a point of reference for the comparison of other samples between plates (Pfaffl 2004). The T/S ratio relative to the standard was then calculated as the mean telomeres ratio for the duplicates (i.e. the two values for a given sample on both plates) divided by the mean 18S ratio for the duplicates. For seven individuals, we had two different samples and used their mean T/S ratio in the statistical analyses.

After quality control, the mean CV on 348 samples was 6.7% for the Ct of telomere amplification, 1.7% for the Ct of 18S amplification and 32.1% for the T/S ratio.

2.3. Measure of glycated haemoglobin

The level of glycated haemoglobin was measured using the Biocon Diagnostik© HbA1 kit (Biocon Diagnostik, GmbH, Burbach, Germany), with a protocol adapted to small samples. 5μl red blood cells were suspended in 150μl PBS, then 100μl of this suspension was mixed with 500μl of the lysis reactant and centrifuged. To quantify total haemoglobin, 40μl supernatant was diluted in 1000μl ultrapure water before reading the absorbance at 440 nm ($A_{Hbtotal}$). To quantify glycated haemoglobin, 100μl supernatant was mixed with 1.2mL of cation-exchange resin before separation by filtration. The absorbance was read at 440nm (A_{HbA1}). The resulting absorbance ratio ($A_{HbA1}/A_{Hbtotal}$) was standardized using the kit calibrator. Each sample could be analysed three times in total: in duplicate in a first assay, then once in a second assay, usually within the same day. The inter- and intra-assay CV were respectively 13.7% and 7.9% (364 samples).

2.4. Statistical analyses

To study the effect of dispersal in interaction with breeding density on body mass, telomere length and levels of glycated haemoglobin, linear models including dispersal status, breeding density and their interaction as fixed effects, age, squared age, sex, tarsus

length as covariates. Because the distance between nest boxes was similar in the different plots (except one plot with much higher box density, which was excluded from the analyses), breeding density was defined as the proportion of nest boxes occupied by flycatchers in a plot during the year considered (Doligez et al. 2004). Using the proportion of nests available to flycatchers by excluding those occupied by tits species (Chapter IV and V) yielded similar results. The distribution of residuals for both telomere length and the level of glycated haemoglobin was skewed toward high values but log-transforming these variables yielded normally distributed residuals. These variables were therefore log-transformed for the analyses. The sample sizes and distributions according to sex, age, and dispersal for each marker are given as Supplementary Information S2.

The return rate, i.e. probability to be caught again in 2010 or 2011, was used as a proxy of survival. To test whether it could be strongly biased by dispersal out of the study area, we tested whether return rate varied between plots that were at the centre or periphery of the study area and we checked whether the factors that influenced return rate also influenced the probability of dispersal within the study area. The probability of return, the probability of dispersing to a new plot between 2009 and 2010 and the probability to fledge at least one young in 2010 were analysed using generalized linear models with a logit link and a binomial distribution of errors, as a function of log-transformed telomere length, log-transformed level of glycated haemoglobin, body mass, breeding density, dispersal, tarsus length, age, squared age, sex, the position of the plot and the interaction between dispersal and breeding density. Because body mass strongly differed between sexes, the interaction of sex and body mass was also included. Among nests that succeeded to fledge at least one young in 2010, the number of fledged young was analysed using a linear model with the same effects as above. Seven individuals were caught again in 2011 but not in 2010, only one of which dispersed between 2009 and 2011. Telomere length measures were available for five of them and glycated haemoglobin and body mass measures for all of them. Studying return rate in 2010 only (excluding them) or dispersal between 2009 and the first recapture in 2010 or 2011 (including them) did not change our results, but there was then no significant effect of the explanatory variables on reproductive success when including these individuals.

We did not include breeding plot or cohort as random effects in the models because preliminary analyses showed that the random variances for these effects were null in all models considered. Similarly, the interactions between dispersal and respectively body mass, telomere length and glycated haemoglobin were not significant when modelling return rate, future dispersal and future reproductive success, and are thus not described here.

All models were run using the R software (R Core Team 2014). Statistical inferences were based on type-III F-tests calculated with the function *Anova*, in the package *car* (Fox & Weisberg 2011).

3. Results

3.1. Factors affecting body mass, telomere length and the level of glycated haemoglobin

Body mass did not differ according to dispersal status ($F_{1,240} = 0.47$, $P = 0.49$), breeding density ($F_{1,240} = 1.19$, $P = 0.28$) or their interaction ($F_{1,238} = 0.41$, $P = 0.53$). Body mass followed a quadratic relationship with age (mean ± s.e. estimate for the linear effect of age: 0.37 ± 0.11, $F_{1,241} = 10.60$, $P = 0.001$; quadratic effect of age: -0.05 ± 0.02, $F_{1,241} = 8.30$, $P = 0.004$; Figure S3.1). Body mass increased with tarsus length (0.41 ± 0.09, $F_{1,241} = 19.54$, $P < 0.001$) and was lower in males than in females (-2.73 ± 0.10, $F_{1,241} = 793.87$, $P < 0.001$).

Log-transformed telomere length differed between dispersing and philopatric individuals, but this difference depended on breeding density (interaction between dispersal status and breeding density: $F_{1,209} = 7.24$, $P = 0.008$; Figure 14). Among dispersing individuals, telomere length decreased with increasing breeding density (post-hoc test: -1.70 ± 0.69, $F_{1,87} = 6.07$, $P = 0.016$), but no effect of density was found among philopatric individuals (0.78 ± 0.58, $F_{1,122} = 1.82$, $P = 0.18$). Log-transformed telomere length was independent of linear and quadratic age ($F_{1,208} = 0.48$, $P = 0.49$ and $F_{1,207} = 0.01$, $P = 0.91$, respectively; Figure S3.2), sex ($F_{1,208} = 0.74$, $P = 0.39$) or tarsus length ($F_{1,208} = 0.08$, $P = 0.78$).

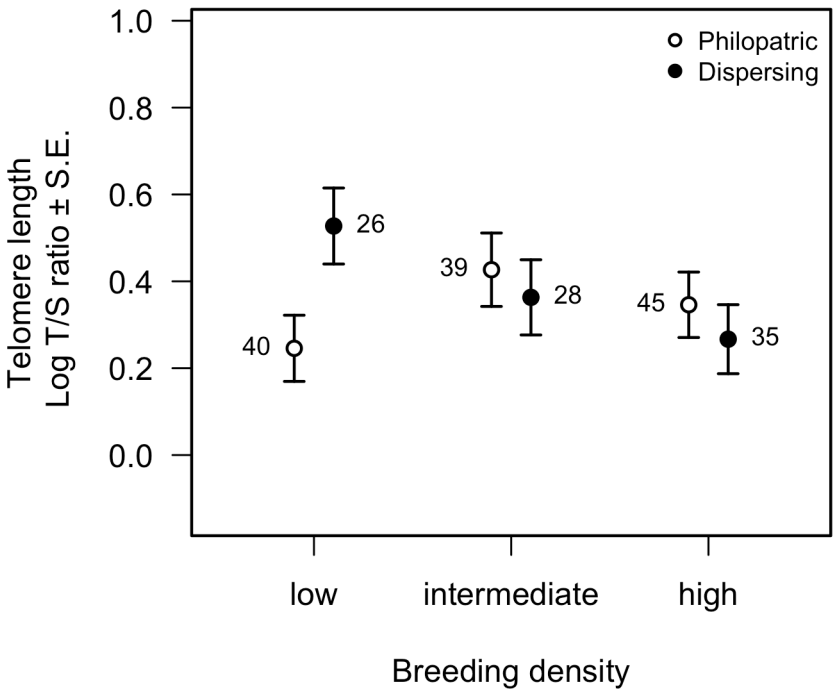


Figure 14: Log-transformed telomere length in 2009 as a function of dispersal status and breeding density. We used plot density quantiles here to define three density classes for the sake of illustration (low density: < 32% of nest boxes occupied, high density: ≥ 40%).

The level of glycated haemoglobin did not differ according to dispersal status ($F_{1,227}$ = 0.02, P = 0.88), breeding density ($F_{1,227}$ = 0.52, P = 0.47) or their interaction ($F_{1,225}$ = 0.03, P = 0.87). Log-transformed levels of glycated haemoglobin followed a quadratic relationship with age (Figure S3.3), showing a strong significant decline between 1 and 5 years of age (mean ± s.e. estimate for the linear effect of age: -0.468 ± 0.093, $F_{1,228}$ = 25.39, P < 0.001) and a significant increase between 5 and 8 years of age (quadratic effect of age: 0.043 ± 0.013, $F_{1,228}$ = 10.80, P = 0.001). The level of glycated haemoglobin did not depend on sex ($F_{1,227}$ = 0.26, P = 0.61) or tarsus length ($F_{1,227}$ = 0.43, P = 0.51).

Finally, body mass in either sex was not correlated with telomere length (Pearson's product-moment correlation test, females: r = 0.068, t_{126} = 0.77, P = 0.44, males: r = 0.012, t_{82} = 0.11, P = 0.91) or glycated haemoglobin (r = 0.032, t_{134} = 0.36, P = 0.72, males: r = 0.011, t_{92} = 0.10, P = 0.92). Telomere length was not correlated with the level of glycated haemoglobin (r = 0.118, t_{199} = 1.68, P = 0.10).

3.2. Link between physiological markers and return rate, subsequent dispersal and reproductive success

Return rate was 40.1% on 248 individuals (95% confidence interval: [33.9% – 46.5%]). Return rate decreased with increasing telomere length (-0.710 ± 0.308, $F_{1,198} = 5.45$, P = 0.021; Figure 15) and with increasing levels of glycated haemoglobin (-0.659 ± 0.248, $F_{1,198} = 7.35$, P = 0.007; Figure 16). Return rate was independent of body mass ($F_{1,196} = 1.72$, P = 0.19), sex ($F_{1,197} = 0.46$, P = 0.50) and their interaction ($F_{1,194} = 0.09$, P = 0.76), dispersal status ($F_{1,197} = 0.35$, P = 0.56), breeding density ($F_{1,197} = 0.61$, P = 0.44) and their interaction ($F_{1,195} = 1.51$, P = 0.22), linear and quadratic age ($F_{1,197} = 1.06$, P = 0.30 and $F_{1,196} = 0.05$, P = 0.82 respectively), tarsus length ($F_{1,197} = 2.10$, P = 0.15) or the position of the breeding plot at the centre or periphery of the study area ($F_{1,160} = 0.77$, P = 0.38).

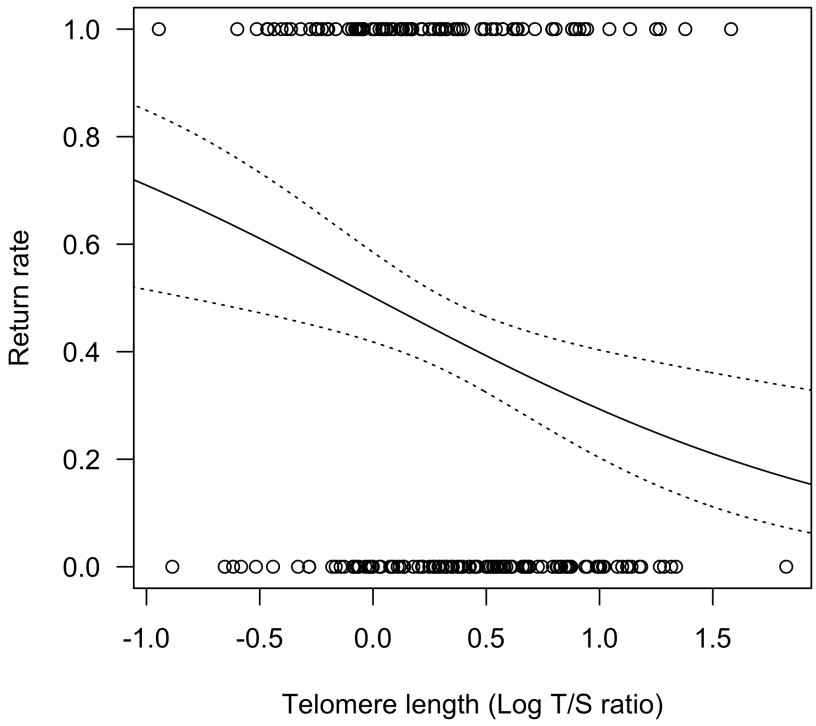


Figure 15: Return rate, used as a proxy of survival, as a function of log-transformed telomere length, with predicted values from a generalized-linear model with binomial error distribution (solid line) and their 95% confidence interval (dotted lines).

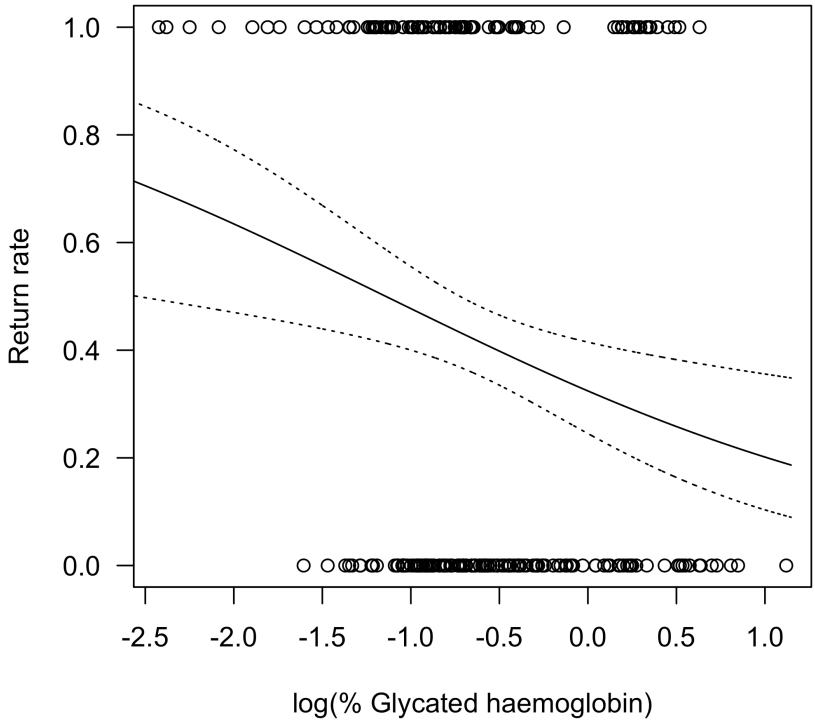


Figure 16: Return rate as a function of log-transformed level of glycated haemoglobin, with predicted values from a generalized-linear model with binomial error distribution (solid line) and their 95% confidence interval (dotted lines).

For individuals caught again in 2010 (N = 92), the mean probability of dispersing between 2009 and 2010 was 23.9% (95% confidence interval: [15.6% – 33.9%]). As expected, dispersal probability was lower in males (estimate ± s.e. for males compared to females: -2.25 ± 0.78, $F_{1,90} = 12.49$, $P < 0.001$), but it did not depend on telomere length ($F_{1,83} = 0.01$, $P = 0.92$), glycated haemoglobin ($F_{1,86} = 0.85$, $P = 0.36$), body mass ($F_{1,89} = 2.80$, $P = 0.10$) and its interaction with sex ($F_{1,88} = 0.57$, $P = 0.45$), dispersal status ($F_{1,89} = 0.82$, $P = 0.37$), breeding density ($F_{1,89} = 0.01$, $P = 0.94$) and their interaction ($F_{1,87} = 1.56$, $P = 0.22$), linear and quadratic age ($F_{1,89} = 0.51$, $P = 0.48$ and $F_{1,88} = 0.11$, $P = 0.74$ respectively), tarsus length ($F_{1,89} = 0.54$, $P = 0.46$) or the position of the breeding plot at the centre or periphery of the study area ($F_{1,73} < 0.01$, $P = 1.00$).

Fledging success for the individuals caught again in 2010 varied between 0 and 8 fledglings (N = 89, median = 5, mean ± SE = 5.0 ± 1.9). Only 12 were paired together and thus

not independent. The probability to fledge at least one young was independent of telomere length ($F_{1,81}$ = 0.32, P = 0.57), glycated haemoglobin ($F_{1,84}$ = 0.33, P = 0.56), body mass ($F_{1,87}$ = 3.14, P = 0.08), sex ($F_{1,87}$ = 3.22, P = 0.08) and their interaction ($F_{1,85}$ = 0.53, P = 0.47), dispersal status ($F_{1,87}$ = 0.67, P = 0.42), breeding density ($F_{1,87}$ < 0.01, P = 0.99) and their interaction ($F_{1,85}$ = 0.47, P = 0.49), linear and quadratic age ($F_{1,87}$ = 0.47, P = 0.49 and $F_{1,86}$ = 1.57, P = 0.21 respectively) or tarsus length ($F_{1,87}$ = 0.91, P = 0.34).

For individuals that fledged at least one young (N = 81), the number of fledged young was higher for dispersing individuals (0.67 ± 0.29, $F_{1,77}$ = 5.80, P = 0.018), decreased with the body mass in 2009 (-0.22 ± 0.07, $F_{1,77}$ = 9.34, P = 0.003) and increased with age (0.24 ± 0.10, $F_{1,77}$ = 5.44, P = 0.022) but was independent of telomere length ($F_{1,70}$ = 1.96, P = 0.17), glycated haemoglobin ($F_{1,73}$ = 1.28, P = 0.26), sex ($F_{1,76}$ = 2.96, P = 0.09) and its interaction with body mass ($F_{1,75}$ = 0.41, P = 0.52), breeding density ($F_{1,76}$ = 1.15, P = 0.29) and its interaction with dispersal status ($F_{1,75}$ = 2.52, P = 0.12), quadratic age ($F_{1,76}$ = 1.44, P = 0.23) or tarsus length ($F_{1,76}$ = 0.16, P = 0.69). When removing the six non-independent pairs, the effects of body mass (-0.17 ± 0.08, $F_{1,66}$ = 4.28, P = 0.042) remained, but dispersal (0.52 ± 0.30, $F_{1,66}$ = 2.99, P = 0.09) and age (0.19 ± 0.11, $F_{1,66}$ = 2.87, P = 0.10) were no more significant.

4. Discussion

Because dispersal is supposed to entail energetic costs, dispersing and philopatric individuals may be expected to adjust their investment in life-history traits, and thus their life-history strategies, differently (Ronce & Clobert 2012; Cotto *et al.* 2014), and such differences may depend on environmental conditions. To assess long-term consequences of differential investment in life-history traits, it may be helpful to use indirect but integrative markers of energetic management. Here, we explored in a patchy population of collared flycatchers whether three potential physiological markers of survival and future reproductive success differed according to individual dispersal status in plots of varying breeding density and whether these markers potentially related to long-term fitness traits, i.e. a proxy of survival and future reproductive success. Among the three markers, only telomere length differed between dispersing and philopatric individuals in low breeding

density plots. Nevertheless, shorter telomeres and lower level of glycated haemoglobin were all associated with higher return rate, a proxy of local survival in this population. Conversely, these markers were in general not associated with future reproductive success (except body mass, which was negatively linked to fledgling number in successful nests). Overall, the results suggest that these physiological markers may be useful to investigate long-term consequences of energetic management on future survival, but revealed only limited differences between dispersing and philopatric individuals in our population.

Differences in telomere length depending on breeding density: consequence of dispersal or phenotypic quality?

Telomere length decreased with increasing density in dispersing birds but not in philopatric ones. Such differences could result either from a differential response to breeding density linked to dispersal status (i.e. a post-dispersal effect on telomere length) or from a difference in habitat choice according to telomere length in dispersing compared to philopatric individuals (i.e. a pre-dispersal effect on telomere length). Our correlative and cross-sectional study does not allow distinguishing between these alternatives. In the first case, the decreasing telomere length with increasing density could suggest that dispersers are more affected by density than philopatric individuals, for instance via competition. Breeding density could be a proxy of the level of competition for nesting sites and/or food (Van Horne 1983; Newton 1994). Although fledging success decrease with breeding density in the pied flycatcher *Ficedula hypoleuca*, a species closely related to the collared flycatcher (reviewed in Lundberg & Alatalo 2010), the relationship rather seems reversed in the Gotland population of collared flycatchers. Indeed, breeding density positively correlates with average reproductive success in this population (Doligez *et al.* 2004) as well as in the majority of temperate bird species (Bock & Jones 2004). Breeding density could thus have an indirect negative effect on telomere length through increased reproductive effort at high breeding density. Although shorter telomeres in wild populations have been associated with low quality habitat, as measured through population dynamics and food availability (Young *et al.* 2013) or individual survival and reproductive success (Angelier *et al.* 2013), increased

breeding effort can have a negative effect on telomere length (Heidinger *et al.* 2012; Reichert *et al.* 2014). The effect of breeding density may thus signal higher reproductive effort, due to higher brood sizes and/or higher competition. Several mechanisms could explain the higher sensitivity to competition and increased reproductive effort in dispersers compared to philopatric individuals. If dispersing individuals are of lower quality and less competitive than philopatric ones, they could need a reproductive effort to reach the same provisioning rate. Philopatric individuals have a higher familiarity with their habitat, which could decrease the costs of acquiring food and thus mitigate the impact of increased brood size or competition (Pärt 1995). Differences in personality traits according to dispersal could also increase the sensitivity to competition. If dispersers are more aggressive, they might engage more in competitive interactions and thus use more energy in denser habitats (Marler *et al.* 1995; Careau *et al.* 2010). Contrarily, dispersers could be more reactive and more socially stressed in dense habitats, which has been negatively related to telomere length (Kotrschal *et al.* 2007; Lewin *et al.* 2015).

The observed differences could also stem from difference in habitat choice according to telomere length and dispersal. In this case, dispersers would settle in less dense areas when their telomeres are longer. If telomere length is an indicator of condition, the observed difference may be explained by high condition individuals choosing to settle in less dense habitats to avoid competition with local high quality breeders. This is however not in line with previous results suggesting that higher competitive individuals prefer, while lower competitive ones avoid, attractive plots as measured by high local density and reproductive success (Doligez et al. 1999, 2004). Here, we found no relation between telomere length and body mass, and return rate decreased with increasing telomere length. These observations do not support the assumption that longer telomere length would indicate higher condition. Thus it remains unclear whether the alternative of a pre-dispersal difference in telomere length and differential habitat choice can explain the observed interaction between dispersal status and breeding density on telomere length. Experimental studies manipulating local density would be needed to investigate the origin of this result.

Longer telomeres, an indicator of lower condition and lower survival prospect?

Here, the relation between telomere length and our proxy of survival was negative, which is opposite to the positive relation observed in many previous studies (Haussmann & Marchetto 2010; Foote *et al.* 2010; Olsson *et al.* 2011; Heidinger *et al.* 2012; Vera *et al.* 2012; Boonekamp *et al.* 2013, 2014; Caprioli *et al.* 2013; Barrett *et al.* 2013; Angelier *et al.* 2013; Aydinonat *et al.* 2014; Bauch *et al.* 2014). Two studies on captive or domestic animals, however, found no relation (Aydinonat *et al.* 2014; Reichert *et al.* 2014) and one study found a negative relation between telomere length and return rate, although it did not account for potential differences in the probability of recapture and/or dispersal out of the study area (Ujvari & Madsen 2009). The negative relation observed here was not due an age effect (i.e. longer telomere and lower survival in young individuals) because neither telomere length nor return rate depended on age. The relationship between telomere length and local survival or dispersal was independent of the dispersal status, excluding that telomere length could have very dissimilar implications in dispersing and non-dispersing individuals. We could however not fully exclude that longer telomere length might be associated with higher dispersal out of the study area or with early breeding failure, thus explaining the lower local survival. Notably, males are only caught after hatching in this population and show very limited breeding dispersal, thus early breeding failure is an important component of their probability of recapture when older than one year (Doligez *et al.* 2012). Whether the decrease in local survival occurs through actual survival or breeding failure, telomere length would still be negatively related to residual reproductive value and thus indicate a fitness cost. On the contrary, dispersal out of the study area would not be associated with fitness costs *per se* and could confound our results. As telomere length was not related to future dispersal within the study area or subsequent breeding success, and birds breeding at the edge and at the centre of the study area had a similar return rate, the effects of dispersal out of the study area or breeding failure on return rate are likely to be low and the effect of telomere length would mainly reflect differences in survival. Further analyses in a capture-mark-recapture framework and on a larger sample would however be necessary to confirm this relationship, but could not be conducted here because of the very low number of individuals caught in

2011 but not 2010 (5 out of the 91 individuals caught again after 2009 for which telomere length was measured).

In our study population, the negative relation could show that telomere length may not reflect survival and breeding prospects as expected from previous results. If, on the contrary, longer telomeres are an indicator of poorer condition and residual reproductive success, the decrease in telomere length with increasing breeding density in dispersing individuals would express either (i) the higher cost they pay in low-quality, i.e. here low-density, habitat compared to high-quality habitat or (ii) the settlement of high-quality dispersers in high-quality habitat and low-quality dispersers in low-quality habitats (Garant *et al.* 2005). However, no physiological mechanism among those described to date that link telomere shortening to cell and tissue functioning can account for a negative relation between telomere length and survival. A recent study on the great reed warbler *Acrocephalus arundinaceus* showed that the link between telomere length and lifespan could be modulated by environmental conditions, such as malaria infection (Asghar *et al.* 2015), but not the point of obtaining significant slopes in opposite direction. Whether such environmental modulation may drive the negative relation observed in our population may require longitudinal data on telomere length to explore telomere shortening in response to the variation in environmental conditions.

Selective disappearance of individuals with high levels of glycated haemoglobin

Increased anabolism due to the deregulation of glucose sensing pathways results in sustained high blood glucose levels and consequently a higher glycation of haemoglobin proteins (Yki-Järvinen 1992; Braun & Sweazea 2008). It is associated with aging and has been experimentally shown to reduce longevity (López-Otín *et al.* 2013). Thus a negative relation is expected between the level of glycated haemoglobin and future survival. Here, individuals with high levels of glycated hemoglobin indeed had a lower return rate and, for the same reasons stated above for telomere length, this is likely due to a link of glycated haemoglobin with actual survival rather than with dispersal out of the study area or increased breeding failure. Our study suggests that poor control of glucose homeostasis comes at a cost of

survival in this natural bird population. Consistently with a previous study in that population (Andersson & Gustafsson 1995), we found no relationship between the level of glycated haemoglobin and body mass. This previous study concluded that glycated haemoglobin could still be a measure of body condition because individuals with higher levels of glycated haemoglobin started breeding earlier and fledged more young than individuals with lower levels (Andersson & Gustafsson 1995). Our results show that the level of glycated haemoglobin is also negatively associated with a proxy of survival. Consequently, the level of glycated haemoglobin might in fact reflect the trade-off between current reproductive investment and future survival. However, this marker did not differ between dispersing and non-dispersing individuals, whatever the environmental condition, but was dominated by age-related changes. Indeed, our results show a significant decrease in glycated haemoglobin levels among individuals aged one to five years and a marginally significant increase thereafter. Selective disappearance from the population of individuals with the highest glycation levels is thus probably accounting for the counter-intuitive age-related decline in glycated hemoglobin levels in the early age categories.

5. Conclusion

Of the three markers used here, only telomere length may reflect environmentally-mediated differences between dispersing and non-dispersing individuals in life-history strategies. To better understand the influence of environmental variation on the investment by dispersing and philopatric individuals in different life-history traits and the lifetime consequences of this differential investment, we could (i) use longitudinal data on these physiological markers and (ii) experimentally manipulate environmental conditions, e.g. here density. Longitudinal data would give us information on within-individual variation in the markers, but remains correlative, which limits the ability to test for the alternative role of differential settlement in driving a between-individual correlation between habitat characteristics and physiological markers. Although the present study was restricted to adults, the studied markers could predict survival differently in juveniles, which would deserve additional investigation. Notably, body mass at fledging is a good predictor of local survival in the

collared flycatcher (Lindén *et al.* 1992), although we found no effect in adults. Similarly, telomere length as juvenile, but not as adult, predicted lifespan in zebra finches (Heidinger *et al.* 2012).

Taken together, our results suggest that differences in energy management in response to environmental variation between dispersing and non-dispersing individuals might impact telomere length with potential consequences on further survival. The level of haemoglobin glycation could be another physiological marker of survival prospects, but it did not differ between dispersing and non-dispersing individuals, even when accounting for environmental conditions. Importantly, all both markers combined explained only a very small proportion of the observed variation in return rate (5.3% of the observed deviance on the logit scale). Their actual predictive power is thus very low. Combining multiple biomarkers in a multivariate framework might allow better prediction of individual condition, and thus survival and reproductive prospects (Cohen *et al.* 2013; Milot *et al.* 2014), which could be particularly important in cases when such prospects cannot be estimated directly.

Supplementary information S1: Methodological details of the measure of telomere length

1 DNA quality

After quality control on qPCR values (see below), DNA concentrations of the retained samples ranged from 34.71 to 246.30 mg.L^{-1} (mean ± S.D. = 134.00 ± 41.37). The 260/280 ratio ranged from 1.760 to 2.360 (mean ± S.D. = 1.868 ± 0.062) and the 260/230 ratio ranged from 1.595 to 2.450 (mean ± S.D. = 2.198 ± 0.149).

2 qPCR reaction

For the quantitative PCR assay, forward and reverse telomere primers were 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′ (Tel-1b) and 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′ (Tel-2b), respectively, and forward and reverse 18S primers were 5′-GAGGTGAAATTCTTGGACCGG-3′ and 5′-CGAACCTCCGACTTTCGTTCT-3′. We used 1 ng of DNA, 156 nM of each primer and 5 µl of SYBR Green (and 0.09 µl of betaine for the telomeres amplification to improve efficiency) per reaction, for a total reaction volume of 10 µl. PCR conditions for the telomeres were 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, 30 s at 60°C and 30 s at 72°C, and those for 18S were 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 30 s at 60°C. Both reactions ended with a dissociation program of 15 s at 95°C, 1 min at 60°C and 15 s at 95°C. PCR plates of 396 wells were loaded with a robot (Tecan, Mannedorf, Switzerland), to increase consistency and repeatability among plates, and qPCRs were performed using a Realtime qPCR ABI 7900 HT (Applied Biosystems, Foster City, USA).

3 Quality control

The samples that did not meet the following criteria were excluded from the analyses:

1. The well Ct value should be lower than 85% of the lower blank Ct value on the same plate (1 sample).
2. The coefficient of variation between the Ct values for the two 18S measures for a given sample should be under 5% (4 samples).
3. The difference between any of the 18S Ct values for a given sample and the median Ct value for 18S for the same plate should be under 1.5 (10 samples).

The maximum coefficient of variation between the Ct values for the telomeres was 8.6%.

Supplementary Information S2: Age distribution for the body mass, telomere and glycated haemoglobin data

Table S2.1: Sample size and age distribution of the measures of body mass

Status	Sex	Age								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**All**
Philopatric	F	2	22	28	17	10	2	0	1	82
Philopatric	M	2	8	16	20	6	4	3	1	60
Dispersing	F	24	20	16	1	0	2	0	0	63
Dispersing	M	19	12	6	1	3	0	0	0	41

Table S2.2: Sample size and age distribution of the measures of telomere length

Status	Sex	Age								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**All**
Philopatric	F	2	19	27	16	6	1	0	1	72
Philopatric	M	2	6	15	18	6	2	2	1	52
Dispersing	F	21	18	17	0	0	1	0	0	57
Dispersing	M	16	9	5	1	1	0	0	0	32

Table S2.3: Sample size and age distribution of the measures of glycated haemoglobin

Status	Sex	Age								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**All**
Philopatric	F	1	21	26	17	10	2	0	0	77
Philopatric	M	2	8	15	18	6	4	3	1	57
Dispersing	F	22	19	17	1	0	1	0	0	60
Dispersing	M	18	10	6	0	3	0	0	0	37

Supplementary Information S3: Variation of the markers with age

Figure S3.1: Variation of body condition with age in females (left) and males (right)

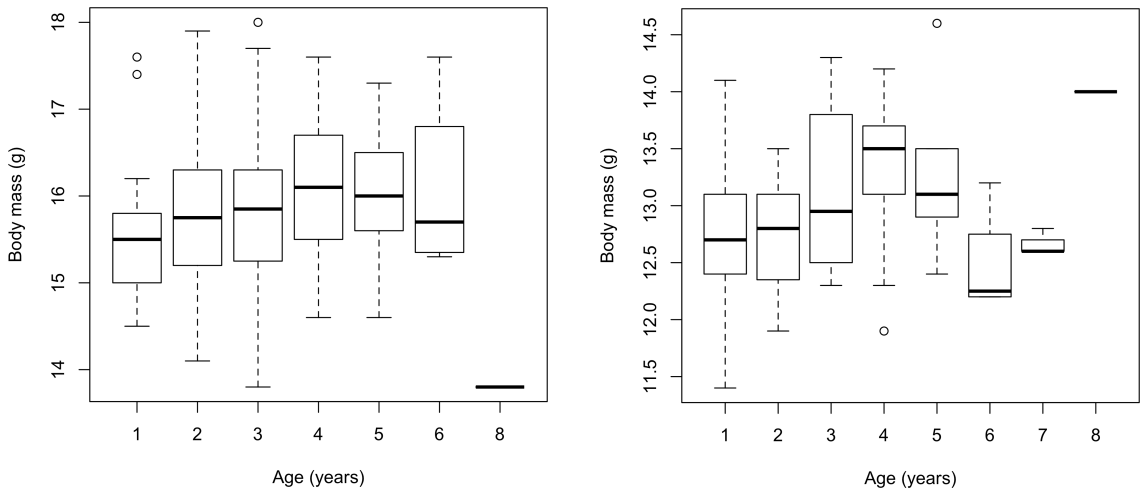


Figure S3.2: Variation of log-transformed telomere length with age

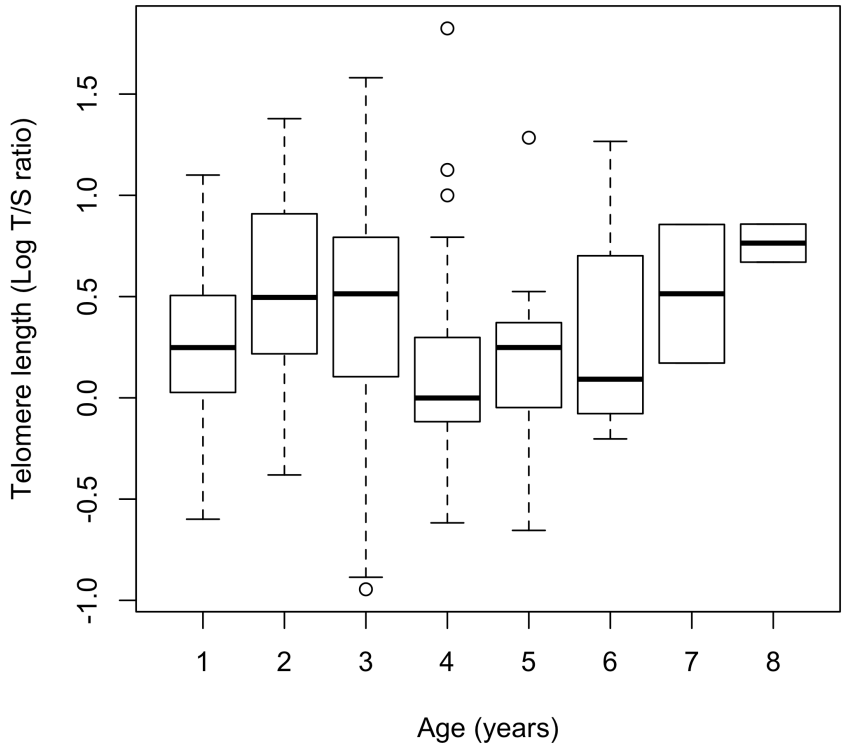
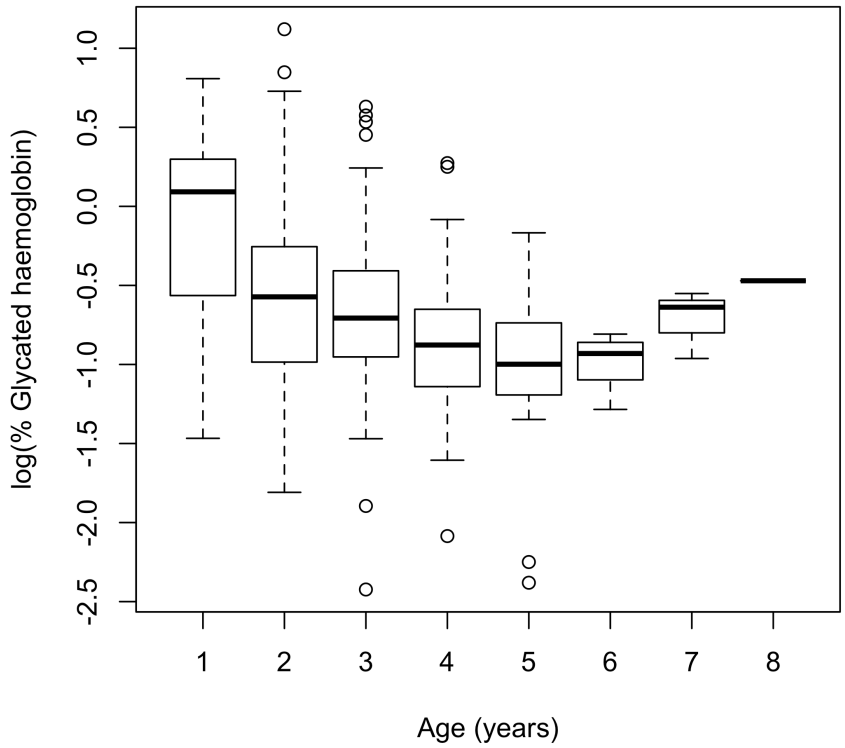


Figure S3.3: Variation of log-transformed percentage of glycated haemoglobin with age



Chapter VII – Main results and general discussion

1 MAIN RESULTS

Differences in life-history traits between dispersing and philopatric individuals have been put forward as an important determinant of the success of dispersal strategies and the maintenance of variation in dispersal behaviour (Clobert *et al.* 2009). In particular, differences in the response of dispersing and non-dispersing individuals to environmental variation might allow different strategies to co-exist within a population (alternative strategies hypothesis). Alternatively, the observed differences might mainly express environmentally determined differences in individual quality, with dispersal to low quality habitats being the "best of a bad job" for non-competitive individuals but dispersal to high quality habitats a "change for the best" for competitive ones (individual quality hypothesis). In this work, we investigated the response of dispersing and philopatric individuals to energetic constraints in terms of oxidative balance, reproductive success and parental behaviour. We then examined whether their management of oxidative balance could compensate for their differential responses to increased energy demands in terms of reproductive output (alternative strategies hypothesis), or whether reproduction and oxidative balance showed parallel responses to energetic constraints (individual quality hypothesis).

In chapter III, we explored the correlations between field metabolic rate, pro-oxidants and antioxidant capacity, independently of dispersal, to determine the importance of individual processes and environmental variation in driving these markers and their relationships. Antioxidant defences were positively correlated to ROM concentration and this relation was stronger under energetic constraint: when the bird was handicapped to increase its flight costs or when the breeding season was particularly poor (i.e. in 2014 compared to 2012 and 2013). Yet, the food supplementation in 2014 failed at relieving the correlation between markers, maybe because it was not sufficient to offset the demanding environmental conditions (low temperatures and low food availability). Despite the low sample sizes, our results also suggest a positive correlation between metabolic rate and both antioxidant capacity and ROM concentration in handicapped females. Those individuals with higher metabolic rate seemed to produce more ROS and thus suffer higher oxidative

damage, but responded by also increasing antioxidant protection. As antioxidant defences, but not ROM concentrations, were repeatable between years, the stronger correlation between these two markers, observed under energetic constraints, implies that metabolic rate could be constrained by the ability of a given individual to acquire antioxidants. Overall, this study confirmed the importance of individual and environmental variation in the management of oxidative balance.

We then examined the differential response of dispersing and philopatric females to an increase in metabolic rate induced through a reduction of the wing area (Chapter IV). ROM concentrations were increased in handicapped philopatric females compared to control females, whereas no effect of the handicap was found in dispersing females. As philopatric females also had higher antioxidant capacity independently of the experimental handicap, one hypothesis is that philopatric females may afford to pay higher oxidative costs. However, because philopatric females showed no increased reproductive output, an alternative hypothesis is that philopatric females may simply be less able to cope with increased energetic constraints and maintained their reproductive output at the cost of oxidative damage. These results are opposite to a similar handicapping experiment in the pied flycatcher *Ficedula hypoleuca* showing no effect on female body condition and reproductive effort, but a significant reduction of chick growth and survival (Moreno *et al.* 1999). This suggests that breeding females can adopt two different strategies by paying the costs either through their own condition or their chicks' condition. Whether the long-term fitness consequences of these two strategies are similar remains to be tested.

Dispersing and philopatric females also differentially managed their oxidative balance and reproductive investment in response to naturally varying local breeding density, which is positively correlated to habitat quality in this population (Doligez *et al.* 2004; Chapter IV). ROM concentrations decreased with increasing density in philopatric females, but were uniformly low in dispersing ones. These differences in oxidative markers were paralleled by a lower success for dispersing individuals, especially in constrained conditions: the fledging success and, in low-density habitats, body mass of nestlings were higher for a philopatric mother compared to a dispersing mother. Therefore, the differential management

of metabolic processes and reproductive investment by dispersing and philopatric individuals did not allow dispersing individuals to reach the same reproductive success than philopatric ones. However, because philopatric individuals suffered higher oxidative damages in low-density habitats, the costs in terms of future survival and reproductive success, and ultimately differences between dispersing and philopatric individuals in lifetime reproductive success, remain to be assessed.

The direct effect of habitat quality on the differences between dispersing and philopatric individuals was confirmed by a food supplementation experiment (Chapter V). The food supplementation indeed cancelled the interaction between dispersal and breeding density on adult body mass and male fledging success (Chapter V, Paper 1). These interactions were therefore not only due to differential dispersal decisions of high and low quality individuals with respect to habitat quality, but were directly influenced by habitat quality post-dispersal. The food supplementation had however no effect on the response of ROM concentrations of dispersing and philopatric individuals to breeding density. Most differences between control and supplemented nests were observed in low-density plots, thus confirming the positive association between density and habitat quality in this population. Our results also confirmed the role of food availability in constraining antioxidant capacity, since supplemented individuals had higher antioxidant capacity than controls.

The food supplementation also cancelled the difference in nest defence behaviour between dispersing and philopatric individuals (Chapter V, paper 2). Dispersing individuals had lower nest defence that philopatric ones in control nests, but they increased nest defence behaviour when supplemented to reach the same level as philopatric individuals. However, the supplementation did not modify the interaction between dispersal and density on nest defence behaviour, the intensity of nest defence decreasing with density in dispersers only. Behavioural traits associated with dispersal are thus not fixed but can be modulated by environmental variation.

Chapters IV and V yielded contradicting results on the differential response to density according to dispersal. In 2012 and 2013 (Chapter IV), philopatric individuals

suffered higher pro-oxidants levels with decreasing density but maintained their reproductive success, whereas in 2014, dispersing individuals suffered higher pro-oxidants levels and body mass loss at low densities (Chapter V). These differences could stem from the large variation in environmental conditions observed between these years. Because of meteorological conditions, control nests in 2014 had a much lower fledging success than in previous years and adult body mass was also more sensitive to habitat quality. Follow-up studies over several years are necessary to describe the effects of temporal variation in habitat quality.

Finally, the effect of breeding density on telomere length also differed according to dispersal (Chapter VI): it decreased with density in dispersing individuals but not in philopatric ones, resulting in longer telomeres in dispersing compared to philopatric individuals in low-density habitats. This suggested that dispersing individuals either settled in different habitats according to telomere length or that they were subjected to lower oxidative damages in low-density habitats. However, telomere length was negatively related to the individual's return rate and another predictor of recapture probability, glycated haemoglobin, did not differ according to breeding density or dispersal. Capture-mark-recapture analyses on the long-term database might help us understand whether habitat quality impact the future survival and reproductive success differently according to dispersal status.

2 PAYING THE COST OF DISPERSAL?

2.1 In most cases, differences in reproduction were compensated by differences in body mass or oxidative damages.

Despite the differences in the responses of dispersing and philopatric individuals to breeding density between years, reproductive costs were always balanced by maintenance costs. The dispersal status, in which reproductive success (fledging success and/or nestling body mass) decreased with density, showed constant body mass and ROM concentrations. Reversely, the dispersal status, in which reproductive success remained constant, had lower

body mass and/or higher ROM concentrations with increasing density. This suggested a trade-off between these two components of fitness, although the longer term consequences of lower body mass or oxidative stress are still to be examined. Indeed, body mass was unrelated to future survival and negatively related to future reproductive success, whereas telomere length, a potential marker of oxidative damage, was negatively related to a proxy of survival (Chapter VI). The higher antioxidant capacity of philopatric individuals, irrespective of energetic or environmental constraints, could in particular allow them to offset the costs of higher hydroperoxides production in the long term. Despite this limitation, our results suggest that dispersing and philopatric individuals favour different fitness components when environmental conditions are constraining. Compensations between different life-history traits have been proposed to explain the observed differences between dispersing and non-dispersing individuals. For example, dispersing blue tits *Cyanistes caeruleus* raise less but heavier offspring (Julliard *et al.* 1996), which might also be more dispersive (Riddington 1992, cited in Julliard *et al.* 1996) and/or disperse to higher quality habitats (Verhulst *et al.* 1997; Garant *et al.* 2005). In song sparrows *Melospiza melodia*, immigrant and residents eventually reach the same lifetime reproductive success though different combinations of life-history traits (Marr *et al.* 2002). Immigrant females laid later and fewer clutches that were compensated by a higher fledging success per egg than resident females. Immigrant males in the same population had higher survival but higher breeding failure than resident males. Although a comprehensive investigation of reproductive traits in our population did not find clear compensations between the various components of annual reproductive success between natal dispersers and philopatric individuals (Germain 2014), our results suggest that such compensation could occur between reproductive success and future survival. The main limitation to formally assess the survival of individuals from our study was that, at the time of writing, we had not yet access to sufficient recapture data and subsequent dispersal and reproductive success for the individuals included in the experimental studies (Chapters III to V) and could not directly test potential differences in survival or future reproductive success. Because dispersal out of the study area can decrease the estimation of local survival of dispersing individuals if they are more prone to disperse

again, and of their offspring if there is a correlation of dispersal propensities between generations (Doligez & Pärt 2008), capture-mark-recapture analyses would be needed to minimise such biases and would thus require multiple years of post-experiment monitoring of the population.

In extremely constrained environmental conditions (low-density, i.e. low-quality habitats in 2014, chapter IV), dispersing females experienced lower body mass and higher oxidative damages than philopatric ones, without achieving higher reproductive success as observed in dispersing males compared to philopatric ones. Consistently, a previous comparison of lifetime reproductive success according to natal dispersal in this population found lower LRS for dispersing individuals only under particularly challenging conditions, i.e. experimentally increased brood size or, for females, being the secondary female of polygynous males, which provide little paternal care (Germain 2014). In some extreme cases, it thus seemed that there was an absolute and uncompensated cost to dispersers.

Although we found no differences between yearlings and older adults in interaction with dispersal, in the present datasets there were only few yearlings and most of them were dispersers. We can thus not fully exclude that differences between in reproduction, body mass and oxidative balance maintenance between dispersers and philopatrics were partly explained by age-related effects.

2.2 Experimental manipulations are crucial to detect the oxidative costs of reproduction.

Integrating a measure of environmental quality as explanatory variables and experimentally manipulating individual energy requirement or food availability allowed us to detect the opposite investments in maintenance and reproduction, which would not have been visible by only studying correlations between traits. The direct between-individual relationships between oxidative damages or body mass and reproductive success were indeed ambiguous. Body mass was negatively related to fledging success in males in 2014 (Chapter V) and positively related to nestling mortality (Chapter VI), which is consistent with a maintenance-reproduction trade-off. Previous results in this population have also

shown a negative link between male body condition and fledging success in dispersers (Germain 2014). No relationship was however found in 2012-2013 in males or in 2012, 2013 and 2014 in females. There was also no effect of brood size on body mass. The negative relationship between fledging body mass and oxidative damage in females (Chapter IV) is rather consistent with a correlation based on individual quality or resource acquisition: higher quality individuals or those that are in a better environment might both suffer lower oxidative damages and produce higher quality offspring. Further investigation of the within-individual vs. between-individuals correlation showed a negative correlation between pro-oxidants and fledging success at the within-individual level, confirming that resource availability, rather than permanent individual quality, explained the observed relationship (data not shown). This confirms the importance of taking into account environmental characteristics or experimentally manipulating them to detect costs of reproduction (Metcalfe & Monaghan 2013; Speakman & Garratt 2014), because differences in individual quality or resource acquisition can hinder the detection of trade-offs (Reznick, Nunney & Tessier 2000). The fitness costs of higher pro-oxidants should however be determined to conclude about the existence of a trade-off. For example, the frequently-observed variation in antioxidant defences (in both directions) but stability of oxidative damages under increased energetic constraints is usually interpreted as the expression of organisms favouring the maintenance of a low oxidative stress at the expense of developing a higher antioxidant protection. Evidence for long term costs of antioxidant protection however remains elusive (Isaksson *et al.* 2011). The classical justification for these costs is that antioxidant defences might be limited by the availability of dietary antioxidants (carotenoids, vitamins E and C) or of trace metals such as selenium, copper, zinc, and manganese which act as cofactors of antioxidant enzymes (Catoni *et al.* 2008; Limón-Pacheco & Gonsebatt 2009). Selenium could play a particularly important role as a cofactor of glutathione peroxidases (Isaksson *et al.* 2011). In that case, food supplementation and food restriction experiments would help uncover the costs of antioxidant protection. The positive effect of the food supplementation on antioxidant capacity in our population (Chapter V, Paper 1) confirms that dietary antioxidants acquisition might be limiting for non-enzymatic antioxidant defences: although

the food we provided was not antioxidant-rich, it might have relieved the need to find energetic food and individuals would have had more time to forage for antioxidant-rich food (Beaulieu & Schaefer 2014) and the higher antioxidant capacity of philopatric females could allow them to face an increased hydroperoxides production without long term negative consequences. Other studies also found decreased oxidative damages in food-supplemented animals compared to controls in American red-squirrels (Fletcher *et al.* 2013) and in great tits (Giordano *et al.* 2015). Although some antioxidant enzymes such as catalase or superoxide dismutase might be virtually "free" to produce in terms of energy requirements, increasing this single line of defence might not be sufficient to avoid oxidative stress. Whereas a reduced or increased expression of superoxide dismutase SOD1 has strong effects on oxidative damages in cells and whole organisms (Ho *et al.* 1998; Blander *et al.* 2003; Elchuri *et al.* 2005; Pérez *et al.* 2009), overexpression of other antioxidant enzymes had a much lower impact on oxidative damages (Pérez *et al.* 2009). Nevertheless, the costs of antioxidant protection need not be only energetic. For example, increased antioxidant protection may hinder the immune response in case of an infection (Cram *et al.* 2015a). Submitting individuals to an immune challenge could be another interesting way of unravelling the costs of antioxidant protection.

3 ENVIRONMENTAL VARIATION CAN MODULATE DISPERSAL SYNDROMES.

The observation of behavioural, morphological, physiological and life-history differences between dispersing and non-dispersing individuals as led to the concept of dispersal syndromes (Clobert *et al.* 2009; Ronce & Clobert 2012). Some studies investigated whether behavioural differences associated with dispersal were stable in time and among contexts (Meylan *et al.* 2009; Hoset *et al.* 2010; Quinn *et al.* 2011; Cote *et al.* 2013), but the potential effects of environmental variability on the relationships between dispersal and other, non-behavioural traits have often been overlooked in empirical studies, beyond the effect of the environment on dispersal decisions. Environmental variation could however affect the expression of other traits associated with dispersal, such as life-history traits. Here,

we showed experimentally that dispersing and philopatric individuals responded differently to habitat quality and to energetic constraints, in terms of behaviour, oxidative balance, and reproductive success. Differences between dispersing and philopatric birds thus had a strong environmental component distinct from the settlement in habitat of varying quality of dispersing and philopatric individuals.

3.1 Environmental variation: proximal or ultimate cause of dispersal syndromes?

The proximate causes of dispersal are often (but not systematically) related to the ultimate causes of dispersal, e.g. variation in habitat quality, social and genetic contexts. Whether this holds true for the proximate and ultimate causes of dispersal syndromes remains an unsettled question (Ronce & Clobert 2012). The alternative responses of dispersing and philopatric individuals to environmental variation might merely reflect differential constraints. Philopatric females had a higher antioxidant capacity whatever their local breeding density or experimental group (Chapters IV and V), which they might owe to their higher familiarity with their habitat compared to dispersing ones. In collared flycatchers, antioxidant capacity appears to limit the level of pro-oxidants, and thus probably the energy expenditure an individual can afford (Chapter III). Philopatric females could afford to increase their reproductive effort to maintain their reproductive success in low-quality habitats, at the cost of higher oxidative damages in the short-term (Chapter IV). In that case, we would expect philopatric females to suffer no long-term consequences of this transient increase in oxidative damages thanks to their high antioxidant capacity. Conversely, dispersing females are constrained by their low antioxidant ability: they cannot increase their reproductive effort with decreasing habitat quality, because this would increase oxidative damages, and thus they suffer a decrease in reproductive success.

Alternatively, these different responses might have been shaped by selection on dispersal phenotypes. They could be respectively advantageous to philopatric and dispersing individuals in a spatially variable but temporally autocorrelated environment (Doligez *et al.* 1999). In that case, the reproductive costs endured by dispersing females

would be balanced by oxidative costs in terms of survival or future reproductive success in philopatric females. Because dispersing individuals breeding in low-quality habitats have a higher probability to change habitat between years and thus to breed in a better habitat later, maintaining or even decreasing their reproductive effort to limit their oxidative damages and thus increase their probability of future reproduction might be adaptive. Philopatric individuals will reversely maximise their fitness by getting the most out of their current habitat, given that their reproductive lifespan will rarely exceed three years (Brommer, Wilson & Gustafsson 2007).

Knowledge of the fitness consequences of each "strategy" will be necessary to determine whether they are adaptive or only by-products of environmental constraints. It will however not be sufficient to conclude that variation in habitat quality can be an ultimate cause of the covariation between dispersal and life-history traits in response to the environment. Whether adaptive covariation between traits can actually be selected will depend on the genetic basis of the correlation between traits. The study of behavioural syndromes or personalities has recognized the need to partition the observed (phenotypic) covariation into the underlying levels of variation to assess the demographic and evolutionary significance of the syndromes (Dingemanse & Dochtermann 2014). Phenotypic covariation is indeed a weighted average of between-individual covariation and residual (within-individual) covariation, where the sources of between-individual variation can in turn be separated into genetic and permanent environment effects.

The ability of natural selection to favour some combination of traits will depend on the existence of additive genetic covariation between these traits. Additive genetic covariation between dispersal and behavioural and life-history traits has been reported only in a few cases, such as fecundity and lifespan in the light brown apple moth *Epiphyas postvittana* (Gu & Danthanarayana 1992), fecundity and diapause in the sand cricket (Roff *et al.* 1999; Bégin & Roff 2002), aggressiveness in western bluebirds (Duckworth & Kruuk 2009) or exploratory behaviour in the great tit (Korsten *et al.* 2013). Other studies however found no additive genetic covariation between dispersal and life-history traits despite significant between-individual variation due to non-additive genetic effects and maternal effects (Li &

Margolies 1993). Some strong covariation patterns, even associated with fitness differences, actually lay at the within-individual level. For example, although it does not involve dispersal, the phenotypic correlation between early laying date and boldness in nest defence behaviour in female tawny owls *Strix aluco* could indirectly select for boldness through the higher recruitment of early laying females, but the correlation is entirely due to within-individual variation and does thus not constraint selection on boldness (Brommer *et al.* 2014).

In a more complex way, the dispersal, behavioural and life-history responses to environmental variation can also be genetically related in an adaptive way. In the side-blotched lizard, the settlement decisions in response to social environment is genetically associated with morphological and behavioural traits through linkage disequilibrium and this association provide fitness benefits by facilitating mutualistic interactions between genetically similar individuals (Sinervo *et al.* 2006).

3.2 Consequences for the study of dispersal syndromes

Phenotype-by-environment interactions decrease our ability to evaluate the fitness associated with a particular phenotype, such as dispersal behaviour. Indeed the fitness differences will then be conditional on the environment in which they are measured, and several pitfalls will arise if the relevant environmental variables are not identified. If life-history traits are only measured in a subset of the possible environments that the population experiences, the observed differences will not reflect the actual selection pressure acting at the whole population level (see Ducatez *et al.* 2014 for an example at the interspecific level). Even when measuring the whole possible range of environmental conditions, pooling together individuals that do not experience the same environment will often obscure the existing differences. When measuring differences between dispersing and philopatric individuals, the plasticity of the observed traits according to realistic environmental variation should be tested when feasible, through experimental manipulations of environmental variables and/or through the measure of within-individual responses to environmental variation.

Delayed responses to environmental conditions are even more difficult to assess. For example, natal habitat can have long-term effects on both dispersal propensity and phenotype, thus generating environmentally-driven dispersal syndromes (Benard & McCauley 2008). They can however be masked by current environmental variation if the conditions experienced are correlated throughout life (Descamps *et al.* 2008), for example because of habitat selection or temporal environmental trends. Longitudinal data over the lifespan of multiple individuals will then be needed to identify such bias, as discussed below (§ 5.2).

Relevant environmental variation should also be taken into account when estimating the genetic (or at least between-individual) basis of traits associated with dispersal. Indeed differences in the response to environment can generate differences in genetic correlation of the traits between habitats (Donohue, Polisetty & Wender 2005). Selective pressures on dispersal syndromes can thus vary temporally and spatially within a (meta)population. Estimating the genetic basis of different plastic responses can however be challenging when genotype cannot be directly assessed through phenotypic traits (Donohue *et al.* 2005; Jacquin *et al.* 2012) or by using genetic lines bred in laboratory conditions (Pennekamp *et al.* 2014). Technically, when a trait cannot be measured as a point estimate for a given individual, but is rather a response to extrinsic factors, the statistical partition of variances and covariances described in § 3.1 requires random regression models (Dingemanse *et al.* 2010; Hayward *et al.* 2014; see § 5.2), which are limited by their low power for most ecological datasets (Martin *et al.* 2011).

Overall, our results confirm that environmental conditions define not only the probability of dispersal but also its association with different life-history traits. Although our system did not allow for a detailed dissection of the reaction norms as is possible in laboratory settings (Pennekamp *et al.* 2014), other approaches detailed below might help better describing these interactions in the collared flycatcher and other wild populations. We thus suggest a shift from a definition of dispersal syndromes as a multivariate dispersal phenotype to multivariate reaction norms shaping phenotypes in response to the environment. Such definition would encompass the different mechanisms for the association

between dispersal and life-history traits described in the introduction (§ 1.4). Through a better understating of the multiple dimensions of the syndromes (genetic, environmental, inter-generational) and thus the constraints that underlie them (e.g. linkage disequilibrium, temporal or spatial autocorrelation), they would allow to better discriminate between these mechanisms.

4 TESTING THE IMPORTANCE OF OXIDATIVE STRESS AS A LIFE-HISTORY CONSTRAINT

The importance of the oxidative balance as a mechanism in life-history trade-offs and thus a driver, and not merely a marker, of senescence is hotly debated (Pérez *et al.* 2009; Selman *et al.* 2012; Speakman & Garratt 2014). The inconsistency of the evidence to date, described in chapter I (Part 2.2), have indeed cast many doubts on the generality of the increase of oxidative damages with energy expenditure and the effects of those damages on life-history traits. As evidence from laboratory studies will remain fundamental to describe in details the mechanisms involved, I do think that such debate will only be meaningful if it takes into account the relevance of the oxidative balance for the life histories of natural populations. This work illustrates some of the difficulties of integrating oxidative balance in a study of life-history evolution in the wild, which I discuss below.

4.1 Describing a complex network of interactions

Although we were able to measure two markers of oxidative balance, reactive oxygen metabolites and plasma antioxidant capacity, they were restricted to one tissue (plasma) and do not fully describe the whole network of pro- and anti-oxidant mechanisms co-occurring in the organism. The relevance of plasmatic markers of oxidative stress to infer oxidative costs or benefits in other tissues could be low. Empirical data show a large variability of oxidative balance markers across tissues (Veskoukis *et al.* 2009; Speakman & Garratt 2014). This is because the exposure to reactive oxygen species might vary greatly between tissues and because variations of molecules in the blood do not only reflect their specific activity in the blood, but also depend on their dietary acquisition and their elimination. In our case,

higher hydroperoxides probably reflects a higher production, but could also be linked to the dietary intake of lipids (Pérez-Rodríguez *et al.* 2015). Damages to relevant biomolecules, such as the membrane phospholipids, would then not be linked to plasma hydroperoxides. However, preliminary data do not support a general link between plasma triglycerides and hydroperoxides in our study (Chapter III, Supplementary Information) and the contribution of dietary hydroperoxides to plasmatic concentrations is low because of their degradation in the stomach (Kanazawa & Ashida 1998) and their low uptake by intestinal cells (Maestre *et al.* 2013). In that regard, the use of telomere length as a potential marker of long-term oxidative damages is interesting because variation in telomere length as been found to be correlated across tissues, including blood cells, in humans and in birds (Reichert *et al.* 2013; Daniali *et al.* 2013).

Another caveat is that two markers only offer an incomplete view of the pro- and anti-oxidant systems. Antioxidant capacity as measured here only reflects low-weight non-enzymatic antioxidants, whose exact contribution to oxidative balance *in vivo* is discussed (Sies 2007). As different components of antioxidant protection do not systematically correlate positively (Cohen & McGraw 2009; Sepp *et al.* 2012; Romero-Haro & Alonso-Alvarez 2014) and can interact positively or negatively (Hulbert *et al.* 2007; Seifried *et al.* 2007; Catoni *et al.* 2008; Babin *et al.* 2014), combining measures of non-enzymatic antioxidant activity with measures of specific non-enzymatic antioxidants and of the activity of antioxidant enzymes is therefore advocated (Monaghan *et al.* 2009; Lowe 2014). In this study, measures of the activity of antioxidant enzymes would help better interpret the observed changes in non-enzymatic antioxidant capacity. Similarly, damages on different types of molecules do not always correlate, especially in the plasma where the concentration of lipids, proteins and DNA fragments depend on different mechanisms. Oxidised fatty acids and amino acids measured in the blood do not reflect much of the damages occurring in other tissues, as oxidised lipids and proteins are mostly degraded and recycled within the cells (Hulbert *et al.* 2007). Contrarily, oxidised DNA bases can be excised and eliminated in the general circulation (Lowe 2014).

Although studies of oxidative balance should ideally compare measures of multiple pro-oxidant and antioxidant markers and accurate methods have recently been developed to this end, the samples and funding available can still be limiting in most ecological studies.

4.2 Modifying the oxidative balance in the wild?

The experiments performed in this project indirectly manipulated the oxidative balance through manipulations of the energetic balance, i.e. by increasing energy expenditure or by increasing food availability. Although these manipulation were ecologically significant, simulating respectively the costs of early moult or a missed predation event and spatial variability in food availability, they do not provide much insight into the exact mechanisms involved. For example, food supplementation could increase antioxidant capacity either by directly increasing antioxidant acquisition, or by allowing individuals to spare antioxidants through a decrease in foraging costs and thus in the production of reactive oxygen species (if ROS production is positively related to metabolic rate). Such differences in the underlying mechanisms could have consequences on the individuals' responses: variation in foraging costs and variation in food restriction have different effects in mice (Schubert *et al.* 2008). To investigate the effects of the oxidative balance on life-history traits, another approach would be to directly target specific components of the oxidative balance. The only protocol routinely used in ecological studies is the supplementation in dietary antioxidants. However, antioxidant supplementation can have contradictory outcomes, depending on the duration and dose of supplementation as well as the sex and life stage of the individual and the environmental conditions it experiences (Hulbert *et al.* 2007; Banks *et al.* 2010; Pallauf *et al.* 2013; Marri & Richner 2014; Leclaire *et al.* 2015). Some dietary antioxidants could also have other non-redox biological activities (Blount 2004; Banks *et al.* 2010) and even exhibit a pro-oxidant activity under certain conditions (Rietjens *et al.* 2002). Because of these uncertainties, other protocols have been used on model species or on wild-caught animals in captivity to increase endogenous antioxidant defences or increase ROS production. They are however not easily implemented on wild free-ranging populations. Modifications of genes expression have yielded many

insights on the effects of pro- and anti-oxidant genes on the physiology, behaviour and especially lifespan of model species (Vanfleteren & Braeckman 1999; Pérez *et al.* 2009; Van Raamsdonk & Hekimi 2012). They are however still impracticable outside lab settings, although the development of therapeutic and commercial applications of RNA interference through injection or ingestion in humans and farm animals (Davidson & McCray 2011; Albina *et al.* 2013) has opened the way to their use in non-model species, including birds and fishes (Ubuka *et al.* 2013; Choi *et al.* 2013; Heath *et al.* 2014). ROS production can also be manipulated through various chemicals. Mitochondrial uncouplers can decrease the rate of ROS production for a given metabolic rate, but they also decrease the rate of ATP production (Salin *et al.* 2015a). In accordance, treated individuals may increase their metabolic rate to maintain energy acquisition, which cancels any effect on oxidative balance (Stier *et al.* 2014b; Salin *et al.* 2015a). Some herbicides, e.g. paraquat and diquat, have been extensively used on birds in captivity because of their ability to increase ROS production (Isaksson & Andersson 2008; Mougeot, Galván & Alonso-Alvarez 2012; Giraudeau *et al.* 2015), although the exact mechanisms are still discussed (Baltazar *et al.* 2015; Nisar *et al.* 2015). They can have strong deleterious consequences at high doses (Meitern *et al.* 2013). Due to their toxicity, the use of such pro-oxidants is restricted to captive animals, which limits their use in the investigation of life-history trade-off in the wild. Follow-up studies of individuals kept in captivity during the treatment and then released in their population of origin might still further our understanding of the long-term consequences of oxidative stress under realistic environmental constraints.

5 TOWARD LONGITUDINAL STUDIES OF LIFE-HISTORY AND PHYSIOLOGICAL TRAITS

Most of the results presented here focus on cross-sectional differences between individuals measured or observed over a very short time scale, which is a major limitation of this work for different reasons. First, the short- and long-term effects of environmental variation may differ not only in intensity, but also in direction. Second, as evidenced by our study of the correlations between metabolic, oxidative and behavioural traits, the strength,

slope and direction of the relationships between biological traits, but also between a phenotypic trait and an environmental variable, might not be the same when measured within individuals or between individuals.

5.1 Estimating the long-term effects and the evolutionary consequences of the differential responses to breeding densities

The environmental conditions and life-history decisions at a given point in time can have delayed effects (also named long-term or carryover effects) of a different nature, intensity, and sometimes direction compared to their short-term effects (Harrison *et al.* 2011; O'Connor *et al.* 2014). A well described example is the compensatory growth stimulated by a temporary nutritional deficit in early life, which is beneficial to the organism in the short term, but can have multiple negative effects on morphology, metabolism and aging in later life (Metcalfe & Monaghan 2001; Criscuolo *et al.* 2008). More relevant to our work, an oxidative challenge can impair immune responsiveness later in life (Mougeot *et al.* 2012).

The long-term database from the collared flycatcher population on the island of Gotland gathers 35 years of information on reproduction and biometry. It has been successfully used to study the differences in lifetime reproductive success according to natal dispersal status (Germain 2014). Few differences were found between natal dispersers and philopatric individuals, with a lower reproductive success of dispersers under naturally or experimentally increased reproductive effort only. Natal and breeding dispersal are however under different selective pressures (Bowler & Benton 2005). An investigation of the consequences of the observed differences in oxidative balance on breeding dispersers and non-dispersers would thus be necessary. Although the negative effect of oxidative damages are usually pointed out, we cannot rule out the possibility that such moderate oxidative stress might be harmless or even have a positive effect by increasing the ability of the antioxidant system to resist stronger stresses through a hormetic effect, although hormesis is mostly expected early in life (Ristow & Zarse 2010). A first step would thus be to determine the influence of natal and breeding dispersal in interaction with breeding density on subsequent survival and reproductive success. Comparing the fate of dispersing and non-

dispersing individuals is however subject to three main biases. First, the relationship between local survival and actual survival might differ according to dispersal, because dispersal can be repeatable and those individuals that dispersed might be more prone to disperse again, potentially out of the study area. In our case, the "study area" is fragmented with potential breeding sites in unmonitored gardens and forested plots between the monitored plots, so dispersal out of the "study area" can include short-distance dispersal movements (Figure 3, p. 34). Second, offspring can have a dispersal propensity matching that of their parents for various reasons, e.g. similar body condition or heritable variation (Doligez & Pärt 2008). For the same reason as above, offspring of dispersing parents would have a higher probability to settle in an unmonitored area, thus lessening the estimated recruitment success of dispersing parents. Finally, local survival can also be partially confounded with mating and breeding success in our population. Females are usually first caught in the middle of the incubation period, and males while rearing nestlings, so the probability of capture in case of mating failure or early breeding failure is lower for females and virtually null for males. On average 7.1% of females and 27.4% of males that start breeding are missed each year (Doligez *et al.* 2012). Because the long-term database covers a much longer period of time and a wider area than the work presented in this thesis, the influence of short- distance and long-distance dispersal on the probability of recapture and of breeding failure might be better estimated on this database, within a capture-mark-recapture framework (Doligez *et al.* 2012).

In the light of our findings, difference in the management of the oxidative balance between dispersing and philopatric individuals could in turn generate different selective pressures on senescence dynamics, in terms of survival and reproduction. A high reproductive investment early in life is often associated with quicker senescence in terms of survival and reproduction (Bouwhuis *et al.* 2010; Lemaître *et al.* 2015) and both oxidative stress and telomere dynamics have been proposed as mechanisms for such early-late life trade-offs (Dowling & Simmons 2009; Monaghan 2014), although the importance of these mechanisms is still hotly discussed (Pérez *et al.* 2009; Speakman & Selman 2011; Selman *et al.* 2012; Speakman & Garratt 2014). In our case, if dispersing individuals sacrifice their

reproductive output in low-quality habitats to achieve a stable oxidative balance whereas philopatric individuals maintain the same reproductive output at the cost of more oxidative damages, as was the case in 2012 and 2013, then dispersing individuals might experience lower senescence and/or a longer lifespan. Similarly, dispersal is associated with a "slow" life-history strategy in some insects (Gu & Danthanarayana 1992; Roff *et al.* 1999). Such results would be contrary to the most recent models investigating the co-evolution of senescence strategies and dispersal, which predict accelerated actuarial senescence and/or shorter lifespan in dispersing individuals compared to philopatric ones, even in the presence of strong competition (Cotto *et al.* 2014). These models are based on local extinction-colonisation dynamics within a metapopulation, where dispersing individuals often enjoy lower competition through the colonisation of empty patches that generate a "colonizing syndrome" of rapid development and high fecundity. This might not apply to models of balanced dispersal where dispersing and non-dispersing individuals do not systematically experience different levels of competition. A longitudinal study in the collared flycatchers could thus help apprehend empirically the links between dispersal decisions and life-history trajectories in such metapopulations, thanks to the development of adequate statistical methods to investigate variation in individual age-related trajectories (Nussey *et al.* 2008; Péron *et al.* 2010).

5.2 Disentangling individual plasticity from permanent environmental differences

This work demonstrated the important role of environmental variation in modulating dispersal-related traits, and we advocated that dispersal as well as dispersal-related traits might often be better understood as plastic responses to environmental variables. Longitudinal analyses of the collared flycatcher database would help discriminate the individual plastic response to environmental variation (reaction norm) from correlations generated by individual differences in habitat choice or by temporal trends in the environment. Within-individual centring of the relevant life-history traits and environmental variables would allow to model distinctly but within the same model these within- and

between-individual processes (van de Pol & Wright 2009; Figure 17) and determine the contribution of individual plasticity to the population average response to the environment (Nussey, Wilson & Brommer 2007). In the case of dispersal, the interaction between dispersal status and breeding densities could thus be modelled over the whole capture history of an individual, to test whether dispersing and non-dispersing individuals differ in their reaction norm to breeding density, or other relevant environmental variables. Alternatively, bivariate models considering in parallel the probability of dispersing and life-history traits in response to the environment can also evaluate the correlation between those traits, as the correlation between the random slopes, and would be a further step toward defining dispersal syndromes as more general reaction norm syndromes.

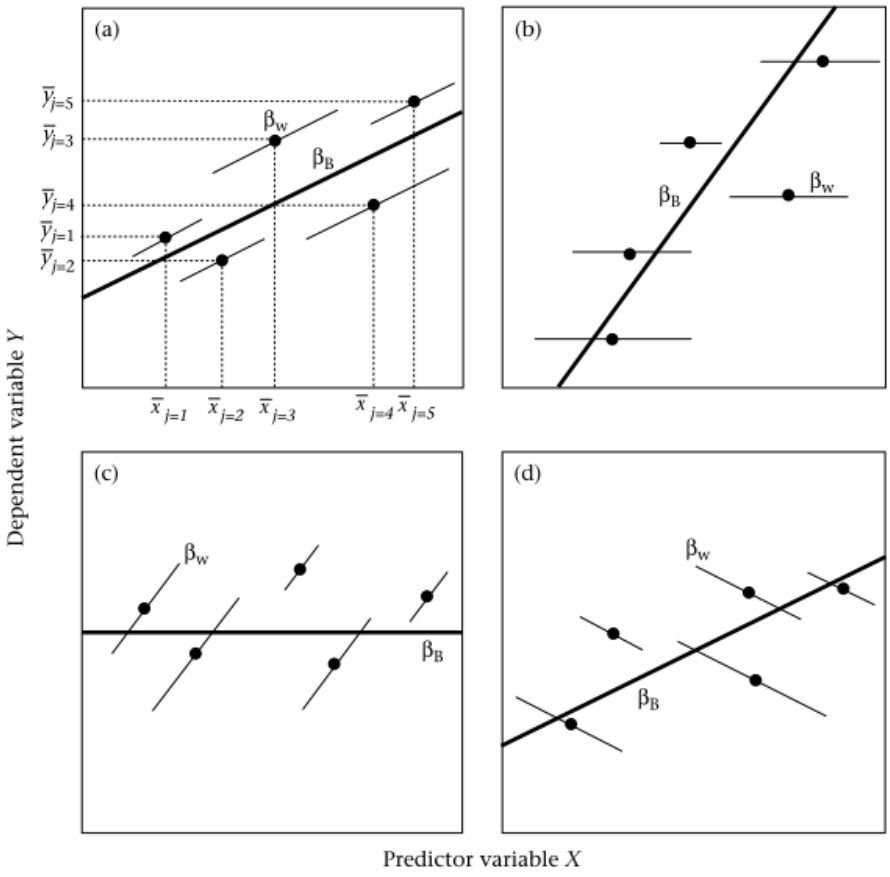


Figure 17: Four different combinations of within- and between-individual relationships between two variables, with respective slopes β_W and β_B. Within-individual centring consist in modelling the dependent variable y_{ij} as a function of the mean centred values $(x_{ij} - \bar{x}_j)$ and the within-individual mean $\bar{x}_j$ (from van de Pol & Wright 2009).

Although the original project involved longitudinal analyses of markers of oxidative balance and of telomere length over six years, many samples gathered from 2009 to 2011 could not be properly analysed and the longitudinal analyses of physiological variation were restricted to three years, from 2012 to 2014. They enabled us to separate the individual and environmental components of the variation and covariation of metabolism and markers of oxidative balance (Chapter III), but the sample sizes were not large enough to estimate the within-individual response of physiological traits and of a binary trait such as dispersal to environmental variation.

Finally, the probability of dispersal and life-history traits in response to breeding density could be studied in quantitative genetics multivariate models to evaluate the genetic basis of a potential covariation (Hayward *et al.* 2014), although the power to estimate the variance components might be limited by the lack of pedigree data for the numerous individuals that are not ringed as nestlings. Results from this population would be particularly valuable, because we lack clear theoretical predictions on how dispersal and other life-history traits should co-evolve, in the absence of strong environmental differences in the settlement of dispersing and non-dispersing individuals (balanced dispersal).

As discussed above, experimental manipulations of the environment or the energetic and oxidative balances are crucial in the study of dispersal, to fully dissociate the condition experienced by the individual from immigration and emigration decisions. Taking this approach, we identified promising research directions that would deserve further exploration using the long-term dataset and the continued sampling effort in this population but were beyond the timeframe of this project. More generally, as the longitudinal collection of physiological samples is developing in many non-model wild animal populations, they will provide amazing opportunities to uncover the mechanisms underlying life-history variation in wild populations, which remain largely unknown.

6 CONCLUSION

The work presented here aimed to clarify the impact of environmental variation on phenotypic differences between dispersing and non-dispersing individuals and to understand how the oxidative balance could underlie such correlations between life-history traits. Identifying the physiological mechanisms involved in evolutionary processes is indeed crucial for our comprehension of life histories evolution.

A few theoretical models have investigated the association of dispersal and other life-history traits at the between-individual scale (Ronce & Clobert 2012; Duputié & Massol 2013). They highlighted the importance of environmental variation in determining the direction and the strength of the association (Bonte & De La Peña 2009) and the consequences of coordinated responses to the environment across traits on the success of dispersal (Fogarty *et al.* 2011). Our results confirm the importance of environmental conditions in shaping differences in life-history traits, and potentially in fitness, according to dispersal. In the collared flycatcher, differences of behaviour and reproductive success between dispersing and non-dispersing individuals are not fixed but can be modulated by the environment (Chapters IV and V). Considering that reaction norms rather than fixed differences often shape traits associated to dispersal might help improve our understanding of dispersal-related life-history syndromes.

The oxidative balance of breeding collared flycatchers, including both the levels of pro-oxidants and antioxidant and their correlation, was determined by a complex interaction of intrinsic (dispersal status) and extrinsic (breeding density, year, experimental treatments) factors. Interestingly, and contrarily to the idea that antioxidants are mainly driven by their availability in the environment, antioxidant capacity was repeatable, whereas ROM concentration showed a high within-individual variability. Our results confirmed the important impact of energy demands and dietary antioxidant acquisition on the maintenance of the oxidative balance, but also showed individual differences, here according to dispersal status, in the management of these constraints when the maintenance of the oxidative balance is competing with reproductive success. Data on other markers would help complete

this picture of oxidative balance and confirm the generality of these results. Further investigation of the long-term fitness consequences of oxidative balance management will be necessary to conclude on the selective pressures acting upon each strategy.

References


equal to zero and then solve
instead, the "equilibrium" de
Conversely, if e > i, the
stochastically, the
points (Hanski

Adamík, P. & Bureš, S. (2007) Experimental evidence for species-specific habitat preferences in two flycatcher species in their hybrid zone. *Naturwissenschaften*, **94**, 859–863.

Adelman, R., Saul, R.L. & Ames, B.N. (1988) Oxidative damage to DNA: relation to species metabolic rate and life span. *Proceedings of the National Academy of Sciences*, **85**, 2706–2708.

Adriaensen, F., Chardon, J.P., De Blust, G., Swinnen, E., Villalba, S., Gulinck, H. & Matthysen, E. (2003) The application of "least-cost" modelling as a functional landscape model. *Landscape and Urban Planning*, **64**, 233–247.

Agarwal, A., Saleh, R.A. & Bedaiwy, M.A. (2003) Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertility and Sterility*, **79**, 829–843.

Aguillon, S.M. & Duckworth, R.A. (2015) Kin aggression and resource availability influence phenotype-dependent dispersal in a passerine bird. *Behavioral Ecology and Sociobiology*, **69**, 625–633.

Alberti, A., Bolognini, L., Macciantelli, D. & Caratelli, M. (2000) The radical cation of N,N-diethyl-para-phenylendiamine: A possible indicator of oxidative stress in biological samples. *Research on Chemical Intermediates*, **26**, 253–267.

Albina, E., Kwiatek, O., Minet, C., Lancelot, R., Servan de Almeida, R. & Libeau, G. (2013) Peste des petits ruminants, the next eradicated animal disease? *Veterinary Microbiology*, **165**, 38–44.

Aloise King, E.D., Garratt, M. & Brooks, R.C. (2013) Manipulating reproductive effort leads to changes in female reproductive scheduling but not oxidative stress. *Ecology and Evolution*, **3**, 4161–4171.

Alonso-Alvarez, C., Bertrand, S., Devevey, G.L., Prost, J., Faivre, B., Chastel, O. & Sorci, G. (2006) An experimental manipulation of life-history trajectories and resistance to oxidative stress. *Evolution*, **60**, 1913–1924.

Andersson, M.S. & Gustafsson, L. (1995) Glycosylated haemoglobin: a new measure of condition in birds. *Proceedings of the Royal Society B: Biological Sciences*, **260**, 299–303.

Angelier, F., Vleck, C.M., Holberton, R.L. & Marra, P.P. (2013) Telomere length, non-breeding habitat and return rate in male American redstarts. *Functional Ecology*, **27**, 342–350.

Ardia, D.R. (2006) Glycated hemoglobin and albumin reflect nestling growth and condition in American kestrels. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, **143**, 62–66.

Ardia, D.R. & Clotfelter, E.D. (2007) Individual quality and age affect responses to an energetic constraint in a cavity-nesting bird. *Behavioral Ecology*, **18**, 259–266.

Armanios, M. & Blackburn, E.H. (2012) The telomere syndromes. *Nature reviews. Genetics*, **13**, 693–704.

Armitage, K.B., Van Vuren, D.H., Ozgul, A. & Oli, M.K. (2011) Proximate causes of natal dispersal in female yellow-bellied marmots, *Marmota flaviventris*. *Ecology*, **92**, 218–227.

Arnold, K.E., Herborn, K.A., Adam, A. & Alexander, L. (2015) Individual variation in the oxidative costs of personality traits. *Functional Ecology*, **29**, 522–530.

Arsenijevic, D., Onuma, H., Pecqueur, C., Raimbault, S., Manning, B.S., Miroux, B., Couplan, E., Alves-Guerra, M.-C., Goubern, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S. & Ricquier, D. (2000) Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nature Genetics*, **26**, 435–439.

Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H. & Bensch, S. (2015) Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science*, **347**, 436–438.

Aydinonat, D., Penn, D.J., Smith, S., Moodley, Y., Hoelzl, F., Knauer, F. & Schwarzenberger, F. (2014) Social isolation shortens telomeres in African Grey Parrots (*Psittacus erithacus erithacus*). *PloS one*, **9**, e93839.

Babin, A., Saciat, C., Gaillard, M., Troussard, J., Motreuil, S., Moreau, J. & Moret, Y. (2014) Limiting immunopathology: Interaction between carotenoids and enzymatic antioxidant defences. *Developmental and Comparative Immunology*, 10–13.

Baines, C.B., McCauley, S.J. & Rowe, L. (2014) The interactive effects of competition and predation risk on dispersal in an insect. *Biology Letters*, **10**, 3236–3244.

Balaban, R.S., Nemoto, S. & Finkel, T. (2005) Mitochondria, oxidants, and aging. *Cell*, **120**, 483–495.

Balat, F. (1971) Cluch size and mortality among the young of the Collared Flycatcher (*Ficedula albicollis* Temm.) in southern and central Moravia. *Zoologické Listy*, **20**, 161–175.

Baltazar, M.T., Dinis-Oliveira, R.J., Bastos, M. de L. & Carvalho, F. (2015) Paraquat: Molecular mechanisms of neurotoxicity and its relation with autophagy. *Toxicity and Autophagy in Neurodegenerative Disorders* Current Topics in Neurotoxicity. (ed J.M. Fuentes), pp. 159–170. Springer International Publishing, Switzerland.

Banks, R., Speakman, J.R. & Selman, C. (2010) Vitamin E supplementation and mammalian lifespan. *Molecular Nutrition and Food Research*, **54**, 719–725.

Barbraud, C., Johnson, A.R. & Bertault, G. (2003) Phenotypic correlates of post-fledging dispersal in a population of greater flamingos: the importance of body condition. *Journal of Animal Ecology*, **72**, 246–257.

Barrett, E.L.B., Burke, T.A., Hammers, M., Komdeur, J. & Richardson, D.S. (2013) Telomere length and dynamics predict mortality in a wild longitudinal study. *Molecular Ecology*, **22**, 249–259.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2014) *lme4: Linear Mixed-Effects Models Using Eigen and S4*. R package version 1.1-7.

Bauch, C., Becker, P.H. & Verhulst, S. (2013) Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 201221.

Bauch, C., Becker, P.H. & Verhulst, S. (2014) Within the genome, long telomeres are more informative than short telomeres with respect to fitness components in a long-lived seabird. *Molecular Ecology*, **23**, 300–310.

Beamonte-Barrientos, R. & Verhulst, S. (2013) Plasma reactive oxygen metabolites and non-enzymatic antioxidant capacity are not affected by an acute increase of metabolic rate in zebra finches. *Journal of Comparative Physiology B*, **183**, 675–683.

Beaulieu, M., Geiger, R.E., Reim, E., Zielke, L. & Fischer, K. (2015) Reproduction alters oxidative status when it is traded-off against longevity. *Evolution*, **69**, 1786–1796.

Beaulieu, M., Reichert, S., Le Maho, Y., Ancel, A. & Criscuolo, F. (2011) Oxidative status and telomere length in a long-lived bird facing a costly reproductive event. *Functional Ecology*, **25**, 577–585.

Beaulieu, M. & Schaefer, H.M. (2014) The proper time for antioxidant consumption. *Physiology & Behavior*, **128C**, 54–59.

Beckman, K.B. & Ames, B.N. (1998) The free radical theory of aging matures. *Physiological Reviews*, **78**, 547–81.

Bégin, M. & Roff, D.A. (2002) The common quantitative genetic basis of wing morphology and diapause occurrence in the cricket *Gryllus veletis*. *Heredity*, **89**, 473–479.

Bélichon, S., Clobert, J. & Massot, M. (1996) Are there differences in fitness components between philopatric and dispersing individuals? *Acta Oecologica*, **17**, 503–517.

Benard, M.F. & McCauley, S.J. (2008) Integrating across life-history stages: Consequences of natal habitat effects on dispersal. *The American Naturalist*, **171**, 553–567.

Bestion, E., Clobert, J. & Cote, J. (2015) Dispersal response to climate change: scaling down to intraspecific variation. *Ecology Letters*, Early view.

Bestion, E., Teyssier, A., Aubret, F., Clobert, J. & Cote, J. (2014) Maternal exposure to predator scents: offspring phenotypic adjustment and dispersal. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20140701.

Bicudo, J.E.P.W., Buttemer, W.A., Chappell, M.A., Pearson, J.T. & Bech, C. (2010) *Ecological and Environmental Physiology of Birds*. Oxford University Press, Oxford.

Biro, P.A. & Stamps, J.A. (2010) Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution*, **25**, 653–659.

Bitume, E. V., Bonte, D., Ronce, O., Olivieri, I. & Nieberding, C.M. (2014) Dispersal distance is influenced by parental and grand-parental density. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20141061.

Bize, P., Criscuolo, F., Metcalfe, N.B., Nasir, L. & Monaghan, P. (2009) Telomere dynamics rather than age predict life expectancy in the wild. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 1679–1683.

Bize, P., Devevey, G.L., Monaghan, P., Doligez, B. & Christe, P. (2008) Fecundity and survival in relation to resistance to oxidative stress in a free-living bird. *Ecology*, **89**, 2584–2593.

Blanc, S., Colman, R., Kemnitz, J., Weindruch, R., Baum, S., Ramsey, J. & Schoeller, D.A. (2005) Assessment of nutritional status in rhesus monkeys: comparison of dual-energy X-ray absorptiometry and stable isotope dilution. *Journal of Medical Primatology*, **34**, 130–138.

Blander, G., De Oliveira, R.M., Conboy, C.M., Haigis, M. & Guarente, L. (2003) Superoxide dismutase 1 knock-down induces senescence in human fibroblasts. *Journal of Biological Chemistry*, **278**, 38966–38969.

Blount, J.D. (2004) Carotenoids and life-history evolution in animals. *Archives of Biochemistry and Biophysics*, **430**, 10–15.

Blount, J.D., Moller, A.P. & Houston, D.C. (2001) Antioxidants, showy males and sperm quality. *Ecology Letters*, **4**, 393–396.

Blount, J.D., Vitikainen, E.I.K., Stott, I. & Cant, M.A. (2015) Oxidative shielding and the cost of reproduction. *Biological Reviews*, **44**, Early view.

Blumstein, D.T., Wey, T.W. & Tang, K. (2009) A test of the social cohesion hypothesis: interactive female marmots remain at home. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 3007–3012.

Bock, C.E. & Jones, Z.F. (2004) Avian habitat evaluation: should counting birds count? *Frontiers in Ecology and the Environment*, **2**, 403–410.

Bonawitz, N.D., Chatenay-Lapointe, M., Pan, Y. & Shadel, G.S. (2007) Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression. *Cell Metabolism*, **5**, 265–277.

Bonte, D. (2013) Cost–benefit balance of dispersal and the evolution of conditional dispersal strategies in spiders. *Spider Ecophysiology* (ed W. Nentwig), pp. 67–78. Springer Berlin Heidelberg.

Bonte, D., De Clercq, N., Zwertvaegher, I. & Lens, L. (2009) Repeatability of dispersal behaviour in a common dwarf spider: Evidence for different mechanisms behind short- and long-distance dispersal. *Ecological Entomology*, **34**, 271–276.

Bonte, D., Van Dyck, H., Bullock, J.M., Coulon, A., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K., Saastamoinen, M., Schtickzelle, N., Stevens, V.M., Vandewoestijne, S., Baguette, M., Barton, K., Benton, T.G., Chaput-Bardy, A., Clobert, J., Dytham, C., Hovestadt, T., Meier, C.M., Palmer, S.C.F., Turlure, C. & Travis, J.M.J. (2012) Costs of dispersal. *Biological Reviews*, **87**, 290–312.

Bonte, D. & De La Peña, E. (2009) Evolution of body condition-dependent dispersal in metapopulations. *Journal of Evolutionary Biology*, **22**, 1242–1251.

Bonte, D., De Roissart, A., Wybouw, N. & Van Leeuwen, T. (2014) Fitness maximization by dispersal: evidence from an invasion experiment. *Ecology*, **95**, 3104–3111.

Boonekamp, J.J., Mulder, G.A., Salomons, H.M., Dijkstra, C. & Verhulst, S. (2014) Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20133287.

Boonekamp, J.J., Simons, M.J.P., Hemerik, L. & Verhulst, S. (2013) Telomere length behaves as biomarker of somatic redundancy rather than biological age. *Aging Cell*, **12**, 330–332.

Booth, D.T., Clayton, D.H. & Block, B.A. (1993) Experimental demonstration of the energetic cost of parasitism in free-ranging hosts. *Proceedings of the Royal Society B: Biological Sciences*, **253**, 125–129.

Bouwhuis, S., Charmantier, A., Verhulst, S. & Sheldon, B.C. (2010) Individual variation in rates of senescence: natal origin effects and disposable soma in a wild bird population. *Journal of Animal Ecology*, **79**, 1251–1261.

Bowler, D.E. & Benton, T.G. (2005) Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews of the Cambridge Philosophical Society*, **80**, 205–225.

Brand, M.D. (2000) Uncoupling to survive? The role of mitochondrial inefficiency in ageing. *Experimental Gerontology*, **35**, 811–820.

Braun, E.J. & Sweazea, K.L. (2008) Glucose regulation in birds. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology*, **151**, 1–9.

Breuker, C.J., Brakefield, P.M. & Gibbs, M. (2007) The association between wing morphology and dispersal is sex-specific in the glanville fritillary butterfly *Melitaea cinxia* (Lepidoptera: Nymphalidae). *European Journal of Entomology*, **104**, 445–452.

Brommer, J.E., Karell, P., Ahola, K. & Karstinen, T. (2014) Residual correlations, and not individual properties, determine a nest defense boldness syndrome. *Behavioral Ecology*, **25**, 802–812.

Brommer, J.E., Wilson, A.J. & Gustafsson, L. (2007) Exploring the genetics of aging in a wild passerine bird. *The American Naturalist*, **170**, 643–650.

Brown, C.R. & Bomberger Brown, M. (1992) Ectoparasitism as a cause of natal dispersal in Cliff Swallows. *Ecology*, **73**, 1718–1723.

Bryant, D.M. & Tatner, P. (1991) Intraspecies variation in avian energy expenditure: correlates and constraints. *Ibis*, **133**, 236–245.

Burgess, S.C., Baskett, M.L., Grosberg, R.K., Morgan, S.G. & Strathmann, R.R. (2015) When is dispersal for dispersal? Unifying marine and terrestrial perspectives. *Biological Reviews*, Early view.

Cadenas, E. & Davies, K.J.A. (2000) Mitochondrial free radical generation, oxidative stress, and aging. *Free Radical Biology and Medicine*, **29**, 222–230.

Cain, M.L., Damman, H. & Muir, A. (1998) Seed dispersal and the Holocene migration of woodland herbs. *Ecological Monographs*, **68**, 325–347.

Calabrese, E.J., Bachmann, K. a., Bailer, a. J., Bolger, P.M., Borak, J., Cai, L., Cedergreen, N., Cherian, M.G., Chiueh, C.C., Clarkson, T.W., Cook, R.R., Diamond, D.M., Doolittle, D.J., Dorato, M. a., Duke, S.O., Feinendegen, L., Gardner, D.E., Hart, R.W., Hastings, K.L., Hayes, a. W., Hoffmann, G.R., Ives, J. a., Jaworowski, Z., Johnson, T.E., Jonas, W.B., Kaminski, N.E., Keller, J.G., Klaunig, J.E., Knudsen, T.B., Kozumbo, W.J., Lettieri, T., Liu, S.Z., Maisseu, A., Maynard, K.I., Masoro, E.J., McClellan, R.O., Mehendale, H.M., Mothersill, C., Newlin, D.B., Nigg, H.N., Oehme, F.W., Phalen, R.F., Philbert, M. a., Rattan, S.I.S., Riviere, J.E., Rodricks, J., Sapolsky, R.M., Scott, B.R., Seymour, C., Sinclair, D. a., Smith-Sonneborn, J., Snow, E.T., Spear, L., Stevenson, D.E., Thomas, Y., Tubiana, M., Williams, G.M. & Mattson, M.P. (2007) Biological stress response terminology: Integrating the concepts of adaptive response and preconditioning stress within a hormetic dose-response framework. *Toxicology and Applied Pharmacology*, **222**, 122–128.

Camacho, C., Canal, D. & Potti, J. (2013) Nonrandom dispersal drives phenotypic divergence within a bird population. *Ecology and Evolution*, **3**, 4841–4848.

Campa, D., Mergarten, B., De Vivo, I., Boutron-Ruault, M.-C., Racine, A., Severi, G., Nieters, A., Katzke, V.A., Trichopoulou, A., Yiannakouris, N., Trichopoulos, D., Boeing, H., Quiros, J.R., Duell, E.J., Molina-Montes, E., Huerta, J.M., Ardanaz, E., Dorronsoro, M., Khaw, K.-T., Wareham, N., Travis, R.C., Palli, D., Pala, V., Tumino, R., Naccarati, A., Panico, S., Vineis, P., Riboli, E., Siddiq, A., Bueno-de-Mesquita, H.B., Peeters, P.H., Nilsson, P.M., Sund, M., Ye, W., Lund, E., Jareid, M., Weiderpass, E., Duarte-Salles, T., Kong, S.Y., Stepien, M., Canzian, F. & Kaaks, R. (2014) Leukocyte telomere length in relation to pancreatic cancer risk: A prospective study. *Cancer Epidemiology Biomarkers & Prevention*, **23**, 2447–2454.

Caprioli, M., Romano, M., Romano, A., Rubolini, D., Motta, R., Folini, M. & Saino, N. (2013) Nestling telomere length does not predict longevity, but covaries with adult body size in wild barn swallows. *Biology letters*, **9**, 20130340.

Careau, V., Réale, D., Humphries, M.M. & Thomas, D.W. (2010) The pace of life under artificial selection: Personality, energy expenditure, and longevity are correlated in domestic dogs. *The American Naturalist*, **175**, 753–758.

Carrascal, L.M. & Polo, V. (2006) Effects of wing area reduction on winter body mass and foraging behaviour in coal tits: field and aviary experiments. *Animal Behaviour*, **72**, 663–672.

Catoni, C., Peters, A. & Martin Schaefer, H. (2008) Life history trade-offs are influenced by the diversity, availability and interactions of dietary antioxidants. *Animal Behaviour*, **76**, 1107–1119.

Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A. & Kerber, R.A. (2003) Association between telomere length in blood and mortality in people aged 60 years or older. *The Lancet*, **361**, 393–395.

Chai, P. (1997) Hummingbird hovering energetics during moult of primary flight feathers. *Journal of Experimental Biology*, **200**, 1527–1536.

Chalfoun, A.D. & Schmidt, K.A. (2012) Adaptive breeding-habitat selection: Is it for the birds? *The Auk*, **129**, 589–599.

Chan, S.R.W.L. & Blackburn, E.H. (2004) Telomeres and telomerase. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **359**, 109–121.

Cheptou, P.-O., Carrue, O., Rouifed, S. & Cantarel, A. (2008) Rapid evolution of seed dispersal in an urban environment in the weed *Crepis sancta*. *Proceedings of the National Academy of Sciences*, **105**, 3796–3799.

Choi, S.H., Jee, B.Y., Lee, S.J., Cho, M.Y., Lee, S.J., Kim, J.W., Jeong, H. Do & Kim, K.H. (2013) Effects of RNA interference-mediated knock-down of hypoxia-inducible factor-α on respiratory burst activity of the Pacific oyster *Crassostrea gigas* hemocytes. *Fish & Shellfish Immunology*, **35**, 476–479.

Chouchani, E.T., Pell, V.R., Gaude, E., Aksentijević, D., Sundier, S.Y., Robb, E.L., Logan, A., Nadtochiy, S.M., Ord, E.N.J., Smith, A.C., Eyassu, F., Shirley, R., Hu, C., Dare, A.J., James, A.M., Rogatti, S., Hartley, R.C., Eaton, S., Costa, A.S.H., Brookes, P.S., Davidson, S.M., Duchen, M.R., Saeb-Parsy, K., Shattock, M.J., Robinson, A.J., Work, L.M., Frezza, C., Krieg, T. & Murphy, M.P. (2014) Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature*, **515**, 431–435.

Christe, P., Glaizot, O., Strepparava, N., Devevey, G. & Fumagalli, L. (2012) Twofold cost of reproduction: an increase in parental effort leads to higher malarial parasitaemia and to a decrease in resistance to oxidative stress. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1142–1149.

Clobert, J., Baguette, M., Benton, T.G., Bullock, J.M. & Ducatez, S. (eds). (2012) *Dispersal Ecology and Evolution*. Oxford University Press, New York.

Clobert, J., Danchin, E., Dhondt, A.A. & Nichols, J.D. (2001) *Dispersal*. Oxford University Press, New York.

Clobert, J., De Fraipont, M. & Danchin, É. (2008) Evolution of dispersal. *Behavioural Ecology* (eds É. Danchin, L.-A. Giraldeau & F. Cézilly), pp. 323–359. Oxford University Press, Oxford.

Clobert, J., Le Galliard, J.-F., Cote, J., Meylan, S. & Massot, M. (2009) Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology letters*, **12**, 197–209.

Clobert, J., Perrins, C.M., McCleery, R.H. & Gosler, A.G. (1988) Survival rate in the Great Tit Parus major in relation to sex, age, and immigration status. *Journal of Animal Ecology*, **57**, 287–306.

Clutton-Brock, T.H. & Lukas, D. (2012) The evolution of social philopatry and dispersal in female mammals. *Molecular Ecology*, **21**, 472–492.

Cohen, A.A., Hau, M. & Wikelski, M.C. (2008) Stress, metabolism, and antioxidants in two wild passerine bird species. *Physiological and Biochemical Zoology*, **81**, 463–472.

Cohen, A.A. & McGraw, K.J. (2009) No simple measures for antioxidant status in birds: Complexity in inter- and intraspecific correlations among circulating antioxidant types. *Functional Ecology*, **23**, 310–320.

Cohen, A.A., Milot, E., Yong, J., Seplaki, C.L., Fülöp, T., Bandeen-Roche, K. & Fried, L.P. (2013) A novel statistical approach shows evidence for multi-system physiological dysregulation during aging. *Mechanisms of Ageing and Development*, **134**, 110–117.

Costantini, D. (2011) On the measurement of circulating antioxidant capacity and the nightmare of uric acid. *Methods in Ecology and Evolution*, **2**, 321–325.

Costantini, D., Casasole, G. & Eens, M. (2014a) Does reproduction protect against oxidative stress? *Journal of Experimental Biology*, **217**, 4237–4243.

Costantini, D., Coluzza, C., Fanfani, A. & Dell'Omo, G. (2007) Effects of carotenoid supplementation on colour expression, oxidative stress and body mass in rehabilitated captive adult kestrels (*Falco tinnunculus*). *Journal of Comparative Physiology B*, **177**, 723–731.

Costantini, D., Goutte, A., Barbraud, C., Faivre, B., Sorci, G., Weimerskirch, H., Delord, K. & Chastel, O. (2015) Demographic responses to oxidative stress and inflammation in the wandering albatross (*Diomedea exulans*). *Plos One*, **10**, e0133967.

Costantini, D., Meillère, A., Carravieri, A., Lecomte, V., Sorci, G., Faivre, B., Weimerskirch, H., Bustamante, P., Labadie, P., Budzinski, H. & Chastel, O. (2014b) Oxidative stress in relation to reproduction, contaminants, gender and age in a long-lived seabird. *Oecologia*, **175**, 1107–1116.

Costantini, D., Rowe, M., Butler, M.W. & McGraw, K.J. (2010) From molecules to living systems: historical and contemporary issues in oxidative stress and antioxidant ecology. *Functional Ecology*, **24**, 950–959.

Costantini, D. & Verhulst, S. (2009) Does high antioxidant capacity indicate low oxidative stress? *Functional Ecology*, **23**, 506–509.

Cote, J. & Clobert, J. (2007) Social personalities influence natal dispersal in a lizard. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 383–390.

Cote, J., Fogarty, S., Tymen, B., Sih, A. & Brodin, T. (2013) Personality-dependent dispersal cancelled under predation risk. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 20132349.

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T. & Sih, A. (2010) Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1571–1579.

Cotto, O., Kubisch, A. & Ronce, O. (2014) Optimal life-history strategy differs between philopatric and dispersing individuals in a metapopulation. *The American Naturalist*, **183**, 384–393.

Cram, D.L., Blount, J.D., York, J.E. & Young, A.J. (2015a) Immune response in a wild bird is predicted by oxidative status, but does not cause oxidative stress. *Plos One*, **10**, e0122421.

Cram, D.L., Blount, J.D. & Young, A.J. (2015b) Oxidative status and social dominance in a wild cooperative breeder. *Functional Ecology*, **29**, 229–238.

Criscuolo, F., Bize, P., Nasir, L., Metcalfe, N.B., Foote, C.G., Griffiths, K., Gault, E.A. & Monaghan, P. (2009) Real-time quantitative PCR assay for measurement of avian telomeres. *Journal of Avian Biology*, **40**, 342–347.

Criscuolo, F., Gonzalez-Barroso, M. d. M., Maho, Y.L., Ricquier, D. & Bouillaud, F. (2005) Avian uncoupling protein expressed in yeast mitochondria prevents endogenous free radical damage. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 803–810.

Criscuolo, F., Monaghan, P., Nasir, L. & Metcalfe, N.B. (2008) Early nutrition and phenotypic development: "catch-up" growth leads to elevated metabolic rate in adulthood. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 1565–1570.

Van de Crommenacker, J., Komdeur, J., Burke, T. & Richardson, D.S. (2011) Spatio-temporal variation in territory quality and oxidative status: a natural experiment in the Seychelles warbler (*Acrocephalus sechellensis*). *Journal of Animal Ecology*, **80**, 668–680.

Van de Crommenacker, J., Richardson, D.S., Koltz, a. M., Hutchings, K. & Komdeur, J. (2012) Parasitic infection and oxidative status are associated and vary with breeding activity in the Seychelles warbler. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1466–1476.

Daniali, L., Benetos, A., Susser, E., Kark, J.D., Labat, C., Kimura, M., Desai, K., Granick, M. & Aviv, A. (2013) Telomeres shorten at equivalent rates in somatic tissues of adults. *Nature Communications*, **4**, 1597.

Davidson, B.L. & McCray, P.B. (2011) Current prospects for RNA interference-based therapies. *Nature Reviews Genetics*, **12**, 329–340.

Davis, J.M. & Stamps, J.A. (2004) The effect of natal experience on habitat preferences. *Trends in Ecology & Evolution*, **19**, 411–416.

Debeffe, L., Morellet, N., Bonnot, N., Gaillard, J.-M., Cargnelutti, B., Verheyden-Tixier, H., Vanpé, C., Coulon, A., Clobert, J., Bon, R. & Hewison, A.J.M. (2014) The link between

behavioural type and natal dispersal propensity reveals a dispersal syndrome in a large herbivore. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20140873.

Debeffe, L., Morellet, N., Cargnelutti, B., Lourtet, B., Coulon, A., Gaillard, J.-M., Bon, R. & Hewison, A.J.M. (2013) Exploration as a key component of natal dispersal: dispersers explore more than philopatric individuals in roe deer. *Animal Behaviour*, **86**, 143–151.

Decker, M.L., Chavez, E., Vulto, I. & Lansdorp, P.M. (2009) Telomere length in Hutchinson-Gilford progeria syndrome. *Mechanisms of ageing and development*, **130**, 377–383.

Delany, M.E., Daniels, L.M., Swanberg, S.E. & Taylor, H.A. (2003) Telomeres in the chicken: Genome stability and chromosome ends. *Poultry Science*, **82**, 917–926.

Descamps, S., Boutin, S., Berteaux, D., McAdam, A.G. & Gaillard, J.-M. (2008) Cohort effects in red squirrels: the influence of density, food abundance and temperature on future survival and reproductive success. *Journal of Animal Ecology*, **77**, 305–314.

Dickinson, J.L., Ferree, E.D., Stern, C. a., Swift, R. & Zuckerberg, B. (2014) Delayed dispersal in western bluebirds: Teasing apart the importance of resources and parents. *Behavioral Ecology*, **25**, 843–851.

Diekmann, M. (1994) *Deciduous Forest Vegetation in Boreo-Nemoral Scandinavia*. University of Uppsala, PhD thesis.

Dingemanse, N.J., Both, C., van Noordwijk, A.J., Rutten, A.L. & Drent, P.J. (2003) Natal dispersal and personalities in great tits (*Parus major*). *Proceedings of the Royal Society B: Biological Sciences*, **270**, 741–747.

Dingemanse, N.J. & Dochtermann, N.A. (2014) Individual behaviour: behavioural ecology meets quantitative genetics. *Quantitative Genetics in the Wild* (eds A. Charmantier, D. Garant & L.E.B. Kruuk), pp. 54–67. Oxford University Press, Oxford.

Dingemanse, N.J., Kazem, A.J.N., Réale, D. & Wright, J. (2010) Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution*, **25**, 81–89.

Dingle, H. & Drake, V.A. (2007) What is migration? *BioScience*, **57**, 113–121.

Doligez, B. & Clobert, J. (2003) Clutch size reduction as a response to increased nest predation rate in the Collared Flycatcher. *Ecology*, **84**, 2582–2588.

Doligez, B., Danchin, E. & Clobert, J. (2002) Public information and breeding habitat selection in a wild bird population. *Science*, **297**, 1168–1170.

Doligez, B., Danchin, E., Clobert, J. & Gustafsson, L. (1999) The use of conspecific reproductive success for breeding habitat selection in a non-colonial, hole-nesting species, the collared flycatcher. *Journal of Animal Ecology*, **68**, 1193–1206.

Doligez, B., Daniel, G., Warin, P., Pärt, T., Gustafsson, L. & Réale, D. (2012) Estimation and comparison of heritability and parent-offspring resemblance in dispersal probability from capture-recapture data using different methods: The Collared Flycatcher as a case study. *Journal of Ornithology*, **152**, 539–554.

Doligez, B., Gustafsson, L. & Pärt, T. (2009) "Heritability" of dispersal propensity in a patchy population. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 2829–2836.

Doligez, B. & Pärt, T. (2008) Estimating fitness consequences of dispersal: a road to "know-where"? Non-random dispersal and the underestimation of dispersers' fitness. *Journal of Animal Ecology*, **77**, 1199–1211.

Doligez, B., Pärt, T., Danchin, E., Clobert, J. & Gustafsson, L. (2004) Availability and use of public information and conspecific density for settlement decisions in the collared flycatcher. *Journal of Animal Ecology*, **73**, 75–87.

Doncaster, C.P., Clobert, J., Doligez, B., Gustafsson, L. & Danchin, E. (1997) Balanced dispersal between spatially varying local populations: an alternative to the source-sink model. *The American Naturalist*, **150**, 425–445.

Donohue, K., Polisetty, C.R. & Wender, N.J. (2005) Genetic basis and consequences of niche construction: plasticity-induced genetic constraints on the evolution of seed dispersal in *Arabidopsis thaliana*. *The American Naturalist*, **165**, 537–550.

Dotan, Y., Lichtenberg, D. & Pinchuk, I. (2004) Lipid peroxidation cannot be used as a universal criterion of oxidative stress. *Progress in Lipid Research*, **43**, 200–227.

Dowling, D.K. & Simmons, L.W. (2009) Reactive oxygen species as universal constraints in life-history evolution. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 1737–1745.

Dray, S. & Dufour, A.B. (2007) The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, **22**, 1–20.

Dreiss, a. N., Antoniazza, S., Burri, R., Fumagalli, L., Sonnay, C., Frey, C., Goudet, J. & Roulin, a. (2012) Local adaptation and matching habitat choice in female barn owls with respect to melanic coloration. *Journal of Evolutionary Biology*, **25**, 103–114.

Dubois, F. & Giraldeau, L.-A. (2014) How the cascading effects of a single behavioral trait can generate personality. *Ecology and Evolution*, **4**, 3038–3045.

Ducatez, S., Humeau, A., Congretel, M., Fréville, H. & Baguette, M. (2014) Butterfly species differing in mobility show different structures of dispersal-related syndromes in the same fragmented landscape. *Ecography*, **37**, 378–389.

Duckworth, R.A. (2006) Behavioral correlations across breeding contexts provide a mechanism for a cost of aggression. *Behavioral Ecology*, **17**, 1011–1019.

Duckworth, R.A. (2008) Adaptive dispersal strategies and the dynamics of a range expansion. *The American Naturalist*, **172**, S4–S17.

Duckworth, R.A. (2012) Evolution of genetically integrated dispersal strategies. *Dispersal* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 81–94. Oxford University Press, Oxford.

Duckworth, R.A. & Badyaev, A. V. (2007) Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. *Proceedings of the National Academy of Sciences*, **104**, 15017–15022.

Duckworth, R.A., Belloni, V. & Anderson, S.R. (2015) Cycles of species replacement emerge from locally induced maternal effects on offspring behavior in a passerine bird. *Science*, **347**, 875–877.

Duckworth, R.A. & Kruuk, L.E.B. (2009) Evolution of genetic integration between dispersal and colonization ability in a bird. *Evolution*, **63**, 968–977.

Dufty Jr, A.M. & Belthoff, J.R. (2001) Proximate mechanisms of natal dispersal: the role of body condition and hormones. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 217–229. Oxford University Press, Oxford.

Duputié, A. & Massol, F. (2013) An empiricist's guide to theoretical predictions on the evolution of dispersal. *Interface Focus*, **3**, 20130028.

Dytham, C. & Travis, J.M.J. (2006) Evolving dispersal and age at death. *Oikos*, **113**, 530–538.

Edelaar, P. & Bolnick, D.I. (2012) Non-random gene flow: An underappreciated force in evolution and ecology. *Trends in Ecology & Evolution*, **27**, 659–665.

Edelaar, P., Siepielski, A.M. & Clobert, J. (2008) Matching habitat choice causes directed gene flow: A neglected dimension in evolution and ecology. *Evolution*, **62**, 2462–2472.

Edelsparre, A.H., Vesterberg, A., Lim, J.H., Anwari, M. & Fitzpatrick, M.J. (2014) Alleles underlying larval foraging behaviour influence adult dispersal in nature. *Ecology Letters*, **17**, 333–339.

Ehrlenbach, S., Willeit, P., Kiechl, S., Willeit, J., Reindl, M., Schanda, K., Kronenberg, F. & Brandstätter, A. (2009) Influences on the reduction of relative telomere length over 10 years in the population-based Bruneck Study: introduction of a well-controlled high-throughput assay. *International Journal of Epidemiology*, **38**, 1725–1734.

Ekman, J., Bylin, A. & Tegelström, H. (2000) Parental nepotism enhances survival of retained offspring in the Siberian jay. *Behavioral Ecology*, **11**, 416–420.

Elchuri, S., Oberley, T.D., Qi, W., Eisenstein, R.S., Jackson Roberts, L., Van Remmen, H., Epstein, C.J. & Huang, T.-T. (2005) CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene*, **24**, 367–380.

Entringer, S., Epel, E.S., Kumsta, R., Lin, J., Hellhammer, D.H., Blackburn, E.H., Wüst, S. & Wadhwa, P.D. (2011) Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. *Proceedings of the National Academy of Sciences*, **108**, E513–E518.

Epel, E.S., Blackburn, E.H., Lin, J., Dhabhar, F.S., Adler, N.E., Morrow, J.D. & Cawthon, R.M. (2004) Accelerated telomere shortening in response to life stress. *Proceedings of the National Academy of Sciences*, **101**, 17312–17315.

Epps, C.W., Wehausen, J.D., Bleich, V.C., Torres, S.G. & Brashares, J.S. (2007) Optimizing dispersal and corridor models using landscape genetics. *Journal of Applied Ecology*, **44**, 714–724.

Esteban, R., Moran, J.F., Becerril, J.M. & García-Plazaola, J.I. (2015) Versatility of carotenoids: An integrated view on diversity, evolution, functional roles and environmental interactions. *Environmental and Experimental Botany*, **119**, 63–75.

Finkel, T. (2003) Oxidant signals and oxidative stress. *Current Opinion in Cell Biology*, **15**, 247–254.

Finkel, T. & Holbrook, N.J. (2000) Oxidants, oxidative stress and the biology of ageing. *Nature*, **408**, 239–247.

Fjerdingstad, E.J., Schtickzelle, N., Manhes, P., Gutierrez, A. & Clobert, J. (2007) Evolution of dispersal and life history strategies – *Tetrahymena* ciliates. *BMC Evolutionary Biology*, **7**, 133.

Fletcher, Q.E., Selman, C., Boutin, S., McAdam, A.G., Woods, S.B., Seo, A.Y., Leeuwenburgh, C., Speakman, J.R. & Humphries, M.M. (2013) Oxidative damage increases with reproductive energy expenditure and is reduced by food-supplementation. *Evolution; international journal of organic evolution*, **67**, 1527–1536.

Fogarty, S., Cote, J. & Sih, A. (2011) Social personality polymorphism and the spread of invasive species: a model. *The American Naturalist*, **177**, 273–287.

Foote, C.G., Daunt, F., Gonzalez-Solis, J., Nasir, L., Phillips, R.A. & Monaghan, P. (2010) Individual state and survival prospects: age, sex, and telomere length in a long-lived seabird. *Behavioral Ecology*, **22**, 156–161.

Foote, C.G., Vleck, D. & Vleck, C.M. (2013) Extent and variability of interstitial telomeric sequences and their effects on estimates of telomere length. *Molecular Ecology Ressources*, **13**, 417–428.

Forsyth, N.R., Wright, W.E. & Shay, J.W. (2002) Telomerase and differentiation in multicellular organisms: turn it off, turn it on, and turn it off again. *Differentiation*, **69**, 188–197.

Fox, J. & Weisberg, S. (2011) *An {R} Companion to Applied Regression*. Second Edition. Sage, Thousand Oaks CA.

Fry, D.M. (1995) Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environmental Health Perspectives*, **103**, 165.

Le Galliard, J.-F., Rémy, A., Ims, R.A. & Lambin, X. (2012) Patterns and processes of dispersal behaviour in arvicoline rodents. *Molecular Ecology*, **21**, 505–523.

Gandon, S. & Michalakis, Y. (2001) Multiple causes of the evolution of dispersal. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 153–167. Oxford University Press, Oxford.

Garant, D., Kruuk, L.E.B., Wilkin, T.A., McCleery, R.H. & Sheldon, B.C. (2005) Evolution driven by differential dispersal within a wild bird population. *Nature*, **433**, 60–65.

García-Navas, V., Ferrer, E.S., Sanz, J.J. & Ortego, J. (2014) The role of immigration and local adaptation on fine-scale genotypic and phenotypic population divergence in a less mobile passerine. *Journal of Evolutionary Biology*, **27**, 1590–1603.

Gaston, K.J. (2009) Geographic range limits: achieving synthesis. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 1395–1406.

Geiger, S., Le Vaillant, M., Lebard, T., Reichert, S., Stier, A., Le Maho, Y. & Criscuolo, F. (2012) Catching-up but telomere loss: half-opening the black box of growth and ageing trade-off in wild king penguin chicks. *Molecular Ecology*, **21**, 1500–1510.

Gems, D. & Partridge, L. (2013) Genetics of longevity in model organisms: debates and paradigm shifts. *Annual review of physiology*, **75**, 621–644.

Georgiev, A. V, Thompson, M.E., Mandalaywala, T.M. & Maestripieri, D. (2015) Oxidative stress as an indicator of the costs of reproduction among free-ranging rhesus macaques. *Journal of Experimental Biology*, **218**, 1981–1985.

Germain, M. (2014) *The Links between Dispersal and Individual Fitness: Correlation or Causality?* Université Claude Bernard Lyon 1 and University of Uppsala, PhD thesis.

Gienapp, P. & Merilä, J. (2011) Sex-specific fitness consequences of dispersal in Siberian jays. *Behavioral Ecology and Sociobiology*, **65**, 131–140.

Gilroy, J.J. & Sutherland, W.J. (2007) Beyond ecological traps: perceptual errors and undervalued resources. *Trends in Ecology & Evolution*, **22**, 351–356.

Giordano, M., Costantini, D., Pick, J.L. & Tschirren, B. (2015) Female oxidative status, egg antioxidant protection and eggshell pigmentation: a supplemental feeding experiment in great tits. *Behavioral Ecology and Sociobiology*, **69**, 777–785.

Giraudeau, M., Chavez, A., Toomey, M.B. & McGraw, K.J. (2015) Effects of carotenoid supplementation and oxidative challenges on physiological parameters and carotenoid-based coloration in an urbanization context. *Behavioral Ecology and Sociobiology*, **69**, 957–970.

Glazier, D.S. (2015) Is metabolic rate a universal "pacemaker" for biological processes? *Biological Reviews*, **90**, 377–407.

Głowaciński, Z. (1973) Phenology and breeding success in a population of collared flycatcher, *Ficedula albicollis* (Temm.) in the Niepołomice Forest (southern Poland). *Ekologia Polska*, **21**, 219–228.

Greenwood, P.J. & Harvey, P.H. (1982) The natal and breeding dispersal of birds. *Annual Review of Ecology and Systematics*, **13**, 1–21.

Greenwood, P.J., Harvey, P.H. & Perrins, C.M. (1979) The role of dispersal in the Great Tit (*Parus major*): The causes, consequences and heritability of natal dispersal. *Journal of Animal Ecology*, **48**, 123–142.

Grieco, F. (2002) Time constraint on food choice in provisioning blue tits, *Parus caeruleus*: the relationship between feeding rate and prey size. *Animal Behaviour*, **64**, 517–526.

Grinnell, J. (1922) The role of the "accidental". *The Auk*, **39**, 373–380.

Grundel, R. (1987) Determinants of nestling feeding rates and parental investment in the Mountain Chickadee. *The Condor*, **89**, 319–328.

Gu, H. & Danthanarayana, W. (1992) Quantitative genetic analysis of dispersal in Epiphyas postvittana. II. Genetic covariations between flight capacity and life-history traits. *Heredity*, **68**, 61–69.

Gueijman, A., Ayali, A., Ram, Y. & Hadany, L. (2013) Dispersing away from bad genotypes: the evolution of Fitness-Associated Dispersal (FAD) in homogeneous environments. *BMC Evolutionary Biology*, **13**, 125.

Gustafsson, L. (1985) *Fitness Factors in the Collared Flycatcher Ficedula Albicollis Temm.* University of Uppsala, PhD dissertation.

Gustafsson, L. (1986) Lifetime reproductive success and heritability: Empirical support for Fisher's fundamental theorem. *The American Naturalist*, **128**, 761–764.

Hadfield, J.D. (2010) MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software*, **33**, 1–22.

Hajek, V. (1968) Pffletová data a nëkteré poznatky ze zivota lejska bélokrkého (*Ficedula albicollis*) na jizni Morave. *Zprávy MOS*, **1968**, 41–45.

Hakkarainen, H. & Korpimaki, E. (1994) Nest defense of Tengmalms owls reflects offspring survival prospects under fluctuating food conditions. *Animal Behaviour*, **48**, 843–849.

Halliwell, B. & Gutteridge, J.M.C. (2007) *Free Radicals in Biology and Medicine*, 4th Ed. Oxford University Press.

Hamilton, W.D. & May, R.M. (1977) Dispersal in stable habitats. *Nature*, **269**, 578–581.

Hanski, I. (2001) Population dynamics consequences of dispersal in local populations and in metapopulations. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 283–298. Oxford University Press, Oxford.

Hanski, I. (2012) Dispersal and eco-evolutionary dynamics in the Glanville fritillary butterfly. *Dispersal Ecology and Evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 290–303. Oxford University Press, Oxford.

Hanski, I., Saastamoinen, M. & Ovaskainen, O. (2006) Dispersal-related life-history trade-offs in a butterfly metapopulation. *Journal of Animal Ecology*, **75**, 91–100.

Hanski, I. & Saccheri, I.J. (2006) Molecular-level variation affects population growth in a butterfly metapopulation. *PLoS biology*, **4**, e129.

Hanssen, S.A., Bustnes, J.O., Schnug, L., Bourgeon, S., Johnsen, T.V., Ballesteros, M., Sonne, C., Herzke, D., Eulaers, I., Jaspers, V.L.B., Covaci, A., Eens, M., Halley, D.J., Moum, T., Ims, R.A. & Erikstad, K.E. (2013) Antiparasite treatments reduce humoral immunity and impact oxidative status in raptor nestlings. *Ecology and Evolution*, **3**, 5157–5166.

Hansson, B., Bensch, S. & Hasselquist, D. (2003) Heritability of dispersal in the great reed warbler. *Ecology Letters*, **6**, 290–294.

Hansson, B., Bensch, S. & Hasselquist, D. (2004) Lifetime fitness of short- and long-distance dispersing great reed warblers. *Evolution*, **58**, 2546–2557.

Harding, A.M.A., Kitaysky, A.S., Hall, M.E., Welcker, J., Karnovsky, N.J., Talbot, S.L., Hamer, K.C. & Grémillet, D. (2009) Flexibility in the parental effort of an Arctic-breeding seabird. *Functional Ecology*, **23**, 348–358.

Hardouin, L.A., Nevoux, M., Robert, A., Gimenez, O., Lacroix, F. & Hingrat, Y. (2012) Determinants and costs of natal dispersal in a lekking species. *Oikos*, **121**, 804–812.

Harman, D. (1956) Aging: a theory based on free radical and radiation chemistry. *Journal of Gerontology*, **11**, 298–300.

Harrison, X.A., Blount, J.D., Inger, R., Norris, D.R. & Bearhop, S. (2011) Carry-over effects as drivers of fitness differences in animals. *Journal of Animal Ecology*, **80**, 4–18.

Harshman, L.G. & Zera, A.J. (2007) The cost of reproduction: the devil in the details. *Trends in Ecology & Evolution*, **22**, 80–86.

Harwood, H.J., Listrani, P. & Wagner, J.D. (2012) Nonhuman primates and other animal models in diabetes research. *Journal of Diabetes Science and Technology*, **6**, 503–514.

Hau, M., Haussmann, M.F., Greives, T.J., Matlack, C., Costantini, D., Quetting, M., Adelman, J.S., Miranda, A.C. & Partecke, J. (2015) Repeated stressors in adulthood increase the rate of biological ageing. *Frontiers in Zoology*, **12**, 1–10.

Haughland, D.L. & Larsen, K.W. (2004) Exploration correlates with settlement: red squirrel dispersal in contrasting habitats. *Journal of Animal Ecology*, **73**, 1024–1034.

Haussmann, M.F., Longenecker, A.S., Marchetto, N.M., Juliano, S.A. & Bowden, R.M. (2012) Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1447–1456.

Haussmann, M.F. & Marchetto, N.M. (2010) Telomeres: Linking stress and survival, ecology and evolution. *Current Zoology*, **56**, 714–727.

Haussmann, M.F., Winkler, D.W., O'Reilly, K.M., Huntington, C.E., Nisbet, I.C.T. & Vleck, C.M. (2003) Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 1387–1392.

Haussmann, M.F., Winkler, D.W. & Vleck, C.M. (2005) Longer telomeres associated with higher survival in birds. *Biology letters*, **1**, 212–214.

Hawlena, H., Khokhlova, I.S., Abramsky, Z. & Krasnov, B.R. (2006) Age, intensity of infestation by flea parasites and body mass loss in a rodent host. *Parasitology*, **133**, 187–193.

Haynes, K.J., Dillemuth, F.P., Anderson, B.J., Hakes, A.S., Jackson, H.B., Jackson, S.E. & Cronin, J.T. (2007) Landscape context outweighs local habitat quality in its effects on herbivore dispersal and distribution. *Oecologia*, **151**, 431–441.

Hayward, A.D., Nussey, D.H., Wilson, A.J., Berenos, C., Pilkington, J.G., Watt, K.A., Pemberton, J.M. & Graham, A.L. (2014) Natural selection on individual variation in tolerance of gastrointestinal nematode infection. *PLoS Biology*, **12**, e1001917.

Heath, G., Childs, D., Docker, M.F., McCauley, D.W. & Whyard, S. (2014) RNA interference technology to control pest sea lampreys - A proof-of-concept. *PLoS ONE*, **9**, e88387.

Heidinger, B.J., Blount, J.D., Boner, W., Griffiths, K., Metcalfe, N.B. & Monaghan, P. (2012) Telomere length in early life predicts lifespan. *Proceedings of the National Academy of Sciences*, **109**, 1743–1748.

Heiss, R.S. & Schoech, S.J. (2012) Oxidative cost of reproduction is sex specific and correlated with reproductive effort in a cooperatively breeding bird, the Florida scrub jay. *Physiological and Biochemical Zoology*, **85**, 499–503.

Hemborg, C. & Lundberg, A. (1998) Costs of overlapping reproduction and moult in passerine birds: an experiment with the pied flycatcher. *Behavioral Ecology and Sociobiology*, **43**, 19–23.

Hénaux, V., Bregnballe, T. & Lebreton, J.-D. (2007) Dispersal and recruitment during population growth in a colonial bird, the great cormorant *Phalacrocorax carbo sinensis*. *Journal of Avian Biology*, **38**, 44–57.

Herborn, K.A., Coffey, J., Larcombe, S.D., Alexander, L. & Arnold, K.E. (2011) Oxidative profile varies with personality in European greenfinches. *Journal of Experimental Biology*, **214**, 1732–1739.

Herborn, K.A., Daunt, F., Heidinger, B.J., Granroth-Wilding, H.M. V., Burthe, S.J., Newell, M. a. & Monaghan, P. (2015) Age, oxidative stress exposure and fitness in a long-lived seabird. *Functional Ecology*, Early view.

Ho, Y.-S., Gargano, M., Cao, J., Bronson, R.T., Heimler, I. & Hutz, R.J. (1998) Reduced fertility in female mice lacking copper-zinc superoxide dismutase. *Journal of Biological Chemistry*, **273**, 7765–7769.

Hoeijmakers, J.H.J. (2001) Genome maintenance mechanisms for preventing cancer. *Nature*, **411**, 366–374.

Holland, S.M. (2010) Chronic granulomatous disease. *Clinical Reviews in Allergy & Immunology*, **38**, 3–10.

Van Horne, B. (1983) Density as a misleading indicator of habitat quality. *The Journal of Wildlife Management*, **47**, 893–901.

Hoset, K.S., Ferchaud, A.-L., Dufour, F., Mersch, D., Cote, J. & Le Galliard, J.-F. (2010) Natal dispersal correlates with behavioral traits that are not consistent across early life stages. *Behavioral Ecology*, **22**, 176–183.

Houben, J.M.J., Moonen, H.J.J., van Schooten, F.J. & Hageman, G.J. (2008) Telomere length assessment: biomarker of chronic oxidative stress? *Free Radical Biology and Medicine*, **44**, 235–246.

Hughes, C.L., Dytham, C. & Hill, J.K. (2007) Modelling and analysing evolution of dispersal in populations at expanding range boundaries. *Ecological Entomology*, **32**, 437–445.

Hulbert, A.J., Pamplona, R., Buffenstein, R. & Buttemer, W.A. (2007) Life and death: Metabolic rate, membrane composition, and life span of animals. *Physiological Reviews*, **87**, 1175–1213.

Ilmonen, P., Kotrschal, A. & Penn, D.J. (2008) Telomere attrition due to infection. *PloS one*, **3**, e2143.

Ims, R.A. (1990) Determinants of natal dispersal and space use in grey-sided voles, *Clethrionomys rufocanus*: A combined field and laboratory experiment. *Oikos*, **57**, 106–113.

Ims, R.A. & Hjermann, D.O. (2001) Condition-dependent dispersal. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 203–216. Oxford University Press, Oxford.

Isaksson, C. (2013) Opposing effects on glutathione and reactive oxygen metabolites of sex, habitat, and spring date, but no effect of increased breeding density in great tits (*Parus major*). *Ecology and Evolution*, **3**, 2730–2738.

Isaksson, C. & Andersson, S. (2008) Oxidative stress does not influence carotenoid mobilization and plumage pigmentation. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 309–314.

Isaksson, C., Sheldon, B.C. & Uller, T. (2011) The challenges of integrating oxidative stress into life-history biology. *BioScience*, **61**, 194–202.

Jacquin, L., Récapet, C., Bouche, P., Leboucher, G. & Gasparini, J. (2012) Melanin-based coloration reflects alternative strategies to cope with food limitation in pigeons. *Behavioral Ecology*, **23**, 907–915.

Jennings, B.J., Ozanne, S.E., Dorling, M.W. & Hales, C.N. (1999) Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. *FEBS Letters*, **448**, 4–8.

Joeng, K.S., Song, E.J., Lee, K.-J. & Lee, J. (2004) Long lifespan in worms with long telomeric DNA. *Nature genetics*, **36**, 607–611.

Julliard, R., Perret, P. & Blondel, J. (1996) Reproductive strategies of philopatric and immigrant blue tits. *Acta Oecologica*, **17**, 487–501.

Kamata, H. & Hirata, H. (1999) Redox regulation of cellular signalling. *Cellular Signalling*, **11**, 1–14.

Kanazawa, K. & Ashida, H. (1998) Dietary hydroperoxides of linoleic acid decompose to aldehydes in stomach before being absorbed into the body. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, **1393**, 349–361.

Kim, S.Y., Velando, A., Sorci, G. & Alonso-Alvarez, C. (2010) Genetic correlation between resistance to oxidative stress and reproductive life span in a bird species. *Evolution*, **64**, 852–857.

Kisdi, É. (2004) Conditional dispersal under kin competition: extension of the Hamilton–May model to brood size-dependent dispersal. *Theoretical Population Biology*, **66**, 369–380.

Kisdi, E., Utz, M. & Gyllenberg, M. (2012) Evolution of condition-dependent dispersal. *Dispersal Ecology and Evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 139–151. Oxford University Press, Oxford.

Kivelä, S.M., Seppänen, J.-T., Ovaskainen, O., Doligez, B., Gustafsson, L., Mönkkönen, M. & Forsman, J.T. (2014) The past and the present in decision-making: the use of conspecific and heterospecific cues in nest site selection. *Ecology*, **95**, 3428–3439.

Kokko, H. & Lopez-Sepulcre, A. (2006) From individual dispersal to species ranges: Perspectives for a changing world. *Science*, **313**, 789–791.

Kontiainen, P., Pietiäinen, H., Huttunen, K., Karell, P., Kolunen, H. & Brommer, J.E. (2009) Aggressive ural owl mothers recruit more offspring. *Behavioral Ecology*, **20**, 789–796.

Korshunov, S.S., Skulachev, V.P. & Starkov, A.A. (1997) High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Letters*, **416**, 15–18.

Korsten, P., van Overveld, T., Adriaensen, F. & Matthysen, E. (2013) Genetic integration of local dispersal and exploratory behaviour in a wild bird. *Nature communications*, **4**, 2362.

Kotrschal, A., Ilmonen, P. & Penn, D.J. (2007) Stress impacts telomere dynamics. *Biology Letters*, **3**, 128–130.

Krackow, S. (2003) Motivational and heritable determinants of dispersal latency in wild male house mice (*Mus musculus musculus*). *Ethology*, **109**, 671–689.

Kralj, J., Ćiković, D., Dumbović, V., Dolenec, Z. & Tutiš, V. (2009) Habitat preferences of the Collared Flycatcher, *Ficedula albicollis* (Temm.) in mountains of continental Croatia. *Polish Journal of Ecology*, **57**, 537–545.

Kruuk, L.E.B., Charmantier, A. & Garant, D. (2014) The study of quantitative genetics in wild populations. *Quantitative Genetics in the Wild* (eds A. Charmantier, D. Garant & L.E.B. Kruuk), pp. 1–15. Oxford University Press, Oxford.

Kruuk, L.E.B., Sheldon, B.C. & Merilä, J. (2002) Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*). *Proceedings of the Royal Society B: Biological Sciences*, **269**, 1581–1589.

Kurhalyuk, N., Hetmański, T., Antonowicz, J. & Tkachenko, H. (2009) Oxidative stress and protein oxidation affected by toxic metals in feral pigeons (*Columba livia*) from northern Poland. *Baltic Coastal Zone*, 187–197.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2013) *lmerTest: Tests for Random and Fixed Effects for Linear Mixed Effect Models (lmer Objects of lme4 Package)*. R package version 1.2-0.

Lack, D.L. (1954) *The Natural Regulation of Animal Numbers*. Clarendon Press, Oxford.

Leclaire, S., Bourret, V., Blanchard, P., de Franceschi, C., Merkling, T., Hatch, S.A. & Danchin, É. (2015) Carotenoids increase immunity and sex specifically affect color and redox homeostasis in a monochromatic seabird. *Behavioral Ecology and Sociobiology*, **69**, 1097–1111.

Legrand, D., Trochet, A., Moulherat, S., Calvez, O., Stevens, V.M., Ducatez, S., Clobert, J. & Baguette, M. (2015) Ranking the ecological causes of dispersal in a butterfly. *Ecography*, **38**, 822–831.

Lemaître, J.-F., Berger, V., Bonenfant, C., Douhard, M., Gamelon, M., Piard, F. & Gaillard, J.-M. (2015) Early-late life trade-offs and the evolution of ageing in the wild. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 20150209.

Lemel, J.-Y., Bélichon, S., Clobert, J. & Hochberg, M.E. (1997) The evolution of dispersal in a two-patch system: Some consequences of differences between migratns and residents. *Evolutionary Ecology*, **11**, 613–629.

Lewin, N., Treidel, L.A., Holekamp, K.E., Place, N.J. & Haussmann, M.F. (2015) Socioecological variables predict telomere length in wild spotted hyenas. *Biology Letters*, **11**, 20140991.

Li, J. & Margolies, D.C. (1993) Quantitative genetics of aerial dispersal behaviour and life-history traits in *Tetranychus urticae*. *Heredity*, **70**, 544–552.

Lichtfouse, E., Schwarzbauer, J. & Robert, D. (2013) *Pollutant Diseases, Remediation and Recycling* (eds E Lichtfouse, J Schwarzbauer, and D Robert). Springer International Publishing, Cham.

Lifson, N. & McClintock, R. (1966) Theory of use of the turnover rates of body water for measuring energy and material balance. *Journal of Theoretical Biology*, **12**, 46–74.

Limón-Pacheco, J. & Gonsebatt, M.E. (2009) The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, **674**, 137–147.

Lind, J. & Jakobsson, S. (2001) Body building and concurrent mass loss: flight adaptations in tree sparrows. *Proceedings of the Royal Society B: Biological Sciences*, **268**, 1915–1919.

Lindén, M., Gustafsson, L. & Pärt, T. (1992) Selection on fledging mass in the Collared Flycatcher and the Great Tit. *Ecology*, **73**, 336–343.

Lindschinger, M., Nadlinger, K., Adelwöhrer, N., Holweg, K., Wögerbauer, M., Birkmayer, J., Smolle, K.-H. & Wonisch, W. (2004) Oxidative stress: potential of distinct peroxide determination systems. *Clinical Chemistry and Laboratory Medicine*, **42**, 907–914.

Löhrl, H. (1957) Populationsökologische Untersuchungen beim Halsbandschnäpper (*Ficedula albicollis*). *Bonner zoologische Beiträge*, **5**, 130–177.

López-Arrabé, J., Cantarero, A., Pérez-Rodríguez, L., Palma, A., Alonso-Alvarez, C., González-Braojos, S. & Moreno, J. (2015) Nest-dwelling ectoparasites reduce antioxidant defences in females and nestlings of a passerine: a field experiment. *Oecologia*, **179**, 29–41.

López-Arrabé, J., Cantarero, A., Pérez-Rodríguez, L., Palma, A. & Moreno, J. (2014) Plumage ornaments and reproductive investment in relation to oxidative status in the Iberian Pied Flycatcher (*Ficedula hypoleuca iberiae*). *Canadian Journal of Zoology*, **92**, 1019–1027.

López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M. & Kroemer, G. (2013) The hallmarks of aging. *Cell*, **153**, 1194–1217.

Losdat, S., Helfenstein, F., Blount, J.D., Marri, V., Maronde, L. & Richner, H. (2013) Nestling erythrocyte resistance to oxidative stress predicts fledging success but not local recruitment in a wild bird. *Biology letters*, **9**, 20120888.

Lowe, F. (2014) Biomarkers of oxidative stress. *Systems Biology of Free Radicals and Antioxidants* (ed I. Laher), pp. 65–87. Springer-Verlag, Berlin Heidelberg.

Lundberg, A. & Alatalo, R. V. (2010) *The Pied Flycatcher*. A&C Black.

Lurz, P.W.W., Garson, P.J. & Wauters, L.A. (1997) Effects of temporal and spatial variation in habitat quality on red squirrel dispersal behaviour. *Animal Behaviour*, **54**, 427–435.

Maccoll, A.D.C. & Hatchwell, B.J. (2004) Determinants of lifetime fitness in a cooperative breeder, the Long-tailed Tit *Aegithalos caudatus*. *Journal of Animal Ecology*, **73**, 1137–1148.

Maestre, R., Douglass, J.D., Kodukula, S., Medina, I. & Storch, J. (2013) Alterations in the intestinal assimilation of oxidized PUFAs are ameliorated by a polyphenol-rich grape seed extract in an in vitro model and Caco-2 cells. *Journal of Nutrition*, **143**, 295–301.

Markó, G., Costantini, D., Michl, G. & Török, J. (2011) Oxidative damage and plasma antioxidant capacity in relation to body size, age, male sexual traits and female reproductive performance in the collared flycatcher (*Ficedula albicollis*). *Journal of Comparative Physiology B*, **181**, 73–81.

Marler, C.A., Walsberg, G., White, M.L. & Moore, M. (1995) Increased energy expenditure due to increased territorial defense in male lizards after phenotypic manipulation. *Behavioral Ecology and Sociobiology*, **37**, 225–231.

Marr, A.B., Keller, L.F. & Arcese, P. (2002) Heterosis and outbreeding depression in descendants of natural immigrants to an inbred population of song sparrows (*Melospiza melodia*). *Evolution*, **56**, 131–142.

Marri, V. & Richner, H. (2014) Immune response, oxidative stress and dietary antioxidants in great tit nestlings. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*, **179**, 192–196.

Martin, J.G.A., Nussey, D.H., Wilson, A.J. & Réale, D. (2011) Measuring individual differences in reaction norms in field and experimental studies: A power analysis of random regression models. *Methods in Ecology and Evolution*, **2**, 362–374.

Mateos, R. & Bravo, L. (2007) Chromatographic and electrophoretic methods for the analysis of biomarkers of oxidative damage to macromolecules (DNA, lipids, and proteins). *Journal of Separation Science*, **30**, 175–191.

Matthysen, E. (2005) Density-dependent dispersal in birds and mammals. *Ecography*, **28**, 403–416.

Matthysen, E. (2012) Multicausality of dispersal: a review. *Dispersal Ecology and Evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 3–28. Oxford University Press, Oxford.

McCauley, S.J. (2010) Body size and social dominance influence breeding dispersal in male *Pachydiplax longipennis* (Odonata). *Ecological Entomology*, **35**, 377–385.

Meitern, R., Sild, E., Kilk, K., Porosk, R. & Horak, P. (2013) On the methodological limitations of detecting oxidative stress: effects of paraquat on measures of oxidative status in greenfinches. *Journal of Experimental Biology*, **216**, 2713–2721.

Mestre, L. & Bonte, D. (2012) Food stress during juvenile and maternal development shapes natal and breeding dispersal in a spider. *Behavioral Ecology*, **23**, 759–764.

Metcalfe, N.B. & Alonso-Alvarez, C. (2010) Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology*, **24**, 984–996.

Metcalfe, N.B. & Monaghan, P. (2001) Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution*, **16**, 254–260.

Metcalfe, N.B. & Monaghan, P. (2013) Does reproduction cause oxidative stress? An open question. *Trends in Ecology & Evolution*, **28**, 347–350.

Meylan, S., De Fraipont, M., Aragon, P., Vercken, E. & Clobert, J. (2009) Are dispersal-dependent behavioral traits produced by phenotypic plasticity? *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, **311**, 377–388.

Meyne, J., Ratliff, R.L. & Moyzis, R.K. (1989) Conservation of the human telomere sequence (TTAGGG)n among vertebrates. *Proceedings of the National Academy of Sciences*, **86**, 7049–7053.

Milot, E., Cohen, A.A., Vézina, F., Buehler, D.M., Matson, K.D. & Piersma, T. (2014) A novel integrative method for measuring body condition in ecological studies based on physiological dysregulation. *Methods in Ecology and Evolution*, **5**, 146–155.

Mitchell, P. (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature*, **191**, 144–148.

Monaghan, P. (2014) Organismal stress, telomeres and life histories. *Journal of Experimental Biology*, **217**, 57–66.

Monaghan, P. & Costantini, D. (2014) Free radicals - An evolutionary perspective. *Systems Biology of Free Radicals and Antioxidants* (ed I. Laher), pp. 39–64. Springer-Verlag, Berlin Heidelberg.

Monaghan, P., Metcalfe, N.B. & Torres, R. (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecology Letters*, **12**, 75–92.

Moreno, J., Merino, S., Potti, J., de Leon, A. & Rodriguez, R. (1999) Maternal energy expenditure does not change with flight costs or food availability in the pied flycatcher (*Ficedula hypoleuca*): costs and benefits for nestlings. *Behavioral Ecology and Sociobiology*, **46**, 244–251.

Mougeot, F., Galván, I. & Alonso-Alvarez, C. (2012) Contrasted effects of an oxidative challenge and α-melanocyte-stimulating hormone on cellular immune responsiveness: an experiment with red-legged partridges *Alectoris rufa*. *Oecologia*, **169**, 385–394.

Moyzis, R.K., Buckingham, J.M., Cram, L.S., Dani, M., Deaven, L.L., Jones, M.D., Meyne, J., Ratliff, R.L. & Wu, J.-R. (1988) A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. *Proceedings of the National Academy of Sciences*, **85**, 6622–6626.

Murphy, M.P. (2009) How mitochondria produce reactive oxygen species. *Biochemical Journal*, **417**, 1–13.

Murray, B.G. (1967) Dispersal in vertebrates. *Ecology*, **48**, 975–978.

Myers, J.H. & Krebs, C.J. (1971) Genetic, behavioral, and reproductive attributes of dispersing field voles *Microtus pennsylvanicus* and *Microtus ochrogaster*. *Ecological Entomology*, **41**, 53–78.

Naef-Daenzer, L., Naef-Daenzer, B. & Nager, R.G. (2000) Prey selection and foraging performance of breeding Great Tits *Parus major* in relation to food availability. *Journal of Avian Biology*, **31**, 206–214.

Nettle, D., Monaghan, P., Boner, W., Gillespie, R. & Bateson, M. (2013) Bottom of the heap: Having heavier competitors accelerates early-life telomere loss in the European Starling, *Sturnus vulgaris*. *PloS one*, **8**, e83617.

Nettle, D., Monaghan, P., Gillespie, R., Brilot, B., Bedford, T. & Bateson, M. (2015) An experimental demonstration that early-life competitive disadvantage accelerates telomere loss. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 20141610.

Neuhaus, P. (2003) Parasite removal and its impact on litter size and body condition in Columbian ground squirrels (*Spermophilus columbianus*). *Proceedings of the Royal Society B: Biological Sciences*, **270**, S213–S215.

Nevoux, M., Arlt, D., Nicoll, M.A.C., Jones, C.G. & Norris, K. (2013) The short- and long-term fitness consequences of natal dispersal in a wild bird population. *Ecology letters*, **16**, 438–445.

Newton, I. (1994) The role of nest sites in limiting the numbers of hole-nesting birds: A review. *Biological Conservation*, **70**, 265–276.

Nicholls, D.G. & Ferguson, S.J. (2003) Respiratory chains. *Bioenergetics* pp. 89–155. Elsevier, 3rd ed.

Niitepõld, K., Mattila, A.L.K., Harrison, P.J. & Hanski, I. (2011) Flight metabolic rate has contrasting effects on dispersal in the two sexes of the Glanville fritillary butterfly. *Oecologia*, **165**, 847–854.

Niki, E. (2010) Assessment of antioxidant capacity in vitro and in vivo. *Free Radical Biology and Medicine*, **49**, 503–515.

Nikolaidis, M.G., Jamurtas, A.Z., Paschalis, V., Fatouros, I.G., Koutedakis, Y. & Kouretas, D. (2008) The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress: Magnitude and time-course considerations. *Sports Medicine*, **38**, 579–606.

Nilsson, J.-Å. (1989) Causes and consequences of natal dispersal in the Marsh Tit, *Parus palustris*. *Journal of Animal Ecology*, **58**, 619–636.

Nilsson, J.-Å. (2002) Metabolic consequences of hard work. *Proceedings of the Royal Society B: Biological Sciences*, **269**, 1735–1739.

Nisar, R., Hanson, P.S., He, L., Taylor, R.W., Blain, P.G. & Morris, C.M. (2015) Diquat causes caspase-independent cell death in SH-SY5Y cells by production of ROS independently of mitochondria. *Archives of Toxicology*, **89**, 1811–1825.

Van Noordwijk, A.J. & de Jong, G. (1986) Acquisition and allocation of resources: Their influence on variation in life history tactics. *The American Naturalist*, **128**, 137–142.

Nour, N., Currie, D., Matthysen, E., Van Damme, R. & Dhondt, A. a. (1998) Effects of habitat fragmentation on provisioning rates, diet and breeding success in two species of tit (great tit and blue tit). *Oecologia*, **114**, 522–530.

Nussey, D.H., Coulson, T., Festa-Bianchet, M. & Gaillard, J.-M. (2008) Measuring senescence in wild animal populations: towards a longitudinal approach. *Functional Ecology*, **22**, 393–406.

Nussey, D.H., Wilson, A.J. & Brommer, J.E. (2007) The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of Evolutionary Biology*, **20**, 831–844.

O'Connor, C.M., Norris, D.R., Crossin, G.T. & Cooke, S.J. (2014) Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere*, **5**, art28.

O'Riain, M.J., Jarvis, J.U.M. & Faulkes, C.G. (1996) A dispersive morph in the naked mole-rat. *Nature*, **380**, 619–621.

Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T., Miller, E. & Blomqvist, D. (2011) Sexual differences in telomere selection in the wild. *Molecular Ecology*, **20**, 2085–2099.

Orell, M., Lahti, K., Koivula, K., Rytkönen, S. & Welling, P. (1999) Immigration and gene flow in a northern willow tit (*Parus montanus*) population. *Journal of Evolutionary Biology*, **12**, 283–295.

Oropesa, A.-L., Gravato, C., Guilhermino, L. & Soler, F. (2013) Antioxidant defences and lipid peroxidation in wild White Storks, *Ciconia ciconia*, from Spain. *Journal of Ornithology*, **154**, 971–976.

Pallauf, K., Bendall, J.K., Scheiermann, C., Watschinger, K., Hoffmann, J., Roeder, T. & Rimbach, G. (2013) Vitamin C and lifespan in model organisms. *Food and Chemical Toxicology*, **58**, 255–263.

Pärn, H., Jensen, H., Ringsby, T.H. & Sæther, B.E. (2009) Sex-specific fitness correlates of dispersal in a house sparrow metapopulation. *Journal of Animal Ecology*, **78**, 1216–1225.

Pärt, T. (1990) Natal dispersal in the Collared flycatcher: Possible causes and reproductive consequences. *Ornis Scandinavica*, **21**, 83–88.

Pärt, T. (1991) Philopatry pays: A comparison between Collared Flycatcher sisters. *The American Naturalist*, **138**, 790–796.

Pärt, T. (1994) Male philopatry confers a mating advantage in the migratory collared flycatcher, *Ficedula albicollis*. *Animal Behaviour*, **48**, 401–409.

Pärt, T. (1995) The importance of local familiarity and search costs for age- and sex-biased philopatry in the collared flycatcher. *Animal Behaviour*, **49**, 1029–1038.

Pärt, T. & Doligez, B. (2003) Gathering public information for habitat selection: prospecting birds cue on parental activity. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 1809–1813.

Pärt, T. & Gustafsson, L. (1989) Breeding dispersal in the collared flycatcher (*Ficedula albicollis*): possible causes and reproductive consequences. *Journal of Animal Ecology*, **58**, 305–320.

Pasinelli, G., Schiegg, K. & Walters, J.R. (2004) Genetic and environmental influences on natal dispersal distance in a resident bird species. *The American Naturalist*, **164**, 660–669.

Passos, J.F., Saretzki, G., Ahmed, S., Nelson, G., Richter, T., Peters, H., Wappler, I., Birket, M.J., Harold, G., Schaeuble, K., Birch-Machin, M.A., Kirkwood, T.B.L. & von Zglinicki, T. (2007) Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. *PLoS biology*, **5**, e110.

Pauliny, A., Wagner, R.H., Augustin, J., Szép, T. & Blomqvist, D. (2006) Age-independent telomere length predicts fitness in two bird species. *Molecular Ecology*, **15**, 1681–1687.

Peig, J. & Green, A.J. (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos*, **118**, 1883–1891.

Pennekamp, F., Mitchell, K. a., Chaine, A. & Schtickzelle, N. (2014) Dispersal propensity in *tetrahymena thermophila* ciliates-a reaction norm perspective. *Evolution*, **68**, 2319–2330.

Pérez, V.I., Bokov, A., Van Remmen, H., Mele, J., Ran, Q., Ikeno, Y. & Richardson, A. (2009) Is the oxidative stress theory of aging dead? *Biochimica et Biophysica Acta*, **1790**, 1005–1014.

Pérez-Rodríguez, L., Romero-Haro, A.A., Sternalski, A., Muriel, J., Mougeot, F., Gil, D. & Alonso-Alvarez, C. (2015) Measuring oxidative stress: The confounding effect of lipid concentration in measures of lipid peroxidation. *Physiological and Biochemical Zoology*, **88**, 345–351.

Péron, G., Crochet, P.-A., Choquet, R., Pradel, R., Lebreton, J.-D. & Gimenez, O. (2010) Capture–recapture models with heterogeneity to study survival senescence in the wild. *Simulation*, **119**, 524–532.

Peterson, C.M., Carrell, D.T., Varner, M., Stanford, J., Croughan, M. & Louis, G.B. (2010) The environment and reproduction: Endocrine disruption, reproductive impairment, and epigenetics. *Reproductive Endocrinology and Infertility* (eds D.T. Carrell & C.M. Peterson), pp. 781–803. Springer New York, New York, NY.

Peterson, D.A., Hilborn, R. & Hauser, L. (2014) Local adaptation limits lifetime reproductive success of dispersers in a wild salmon metapopulation. *Nature Communications*, **5**, 3696.

Pfaffl, M.W. (2004) Relative quantification. *Real-time PCR* (ed M.T. Dorak), pp. 63–82. International University Line, San Diego, CA.

Phillips, B.L., Brown, G.P., Webb, J.K. & Shine, R. (2006) Invasion and the evolution of speed in toads. *Nature*, **439**, 803.

Plot, V., Criscuolo, F., Zahn, S. & Georges, J.-Y. (2012) Telomeres, age and reproduction in a long-lived reptile. *PloS one*, **7**, e40855.

Poethke, H.J., Pfenning, B. & Hovestadt, T. (2007) The relative contribution of individual and kin selection to the evolution of density-dependent dispersal rates. *Evolutionary Ecology Research*, **9**, 41–50.

Van de Pol, M. & Wright, J. (2009) A simple method for distinguishing within- versus between-subject effects using mixed models. *Animal Behaviour*, **77**, 753–758.

Price, T., Kirkpatrick, M. & Arnold, S.J. (1988) Directional selection and the evolution of breeding date in birds. *Science*, **240**, 798–799.

Pusey, A.E. (1987) Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology & Evolution*, **2**, 295–299.

Puterman, E., Lin, J., Krauss, J., Blackburn, E.H. & Epel, E.S. (2015) Determinants of telomere attrition over 1 year in healthy older women: stress and health behaviors matter. *Molecular Psychiatry*, **20**, 529–535.

Quinn, J.L., Cole, E.F., Patrick, S.C. & Sheldon, B.C. (2011) Scale and state dependence of the relationship between personality and dispersal in a great tit population. *Journal of Animal Ecology*, **80**, 918–928.

R Core Team. (2014) R: A language and environment for statistical computing.

Van Raamsdonk, J.M. & Hekimi, S. (2012) Superoxide dismutase is dispensable for normal animal lifespan. *Proceedings of the National Academy of Sciences*, **109**, 5785–5790.

Raha, S. & Robinson, B.H. (2000) Mitochondria, oxygen free radicals, disease and ageing. *Trends in Biochemical Sciences*, **25**, 502–508.

Ramakers, C., Ruijter, J.M., Deprez, R.H.L. & Moorman, A.F.. (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neuroscience Letters*, **339**, 62–66.

Ravigné, V., Dieckmann, U. & Olivieri, I. (2009) Live where you thrive: joint evolution of habitat choice and local adaptation facilitates specialization and promotes diversity. *The American Naturalist*, **174**, E141–E169.

Réale, D. & Festa-Bianchet, M. (2003) Predator-induced natural selection on temperament in bighorn ewes. *Animal Behaviour*, **65**, 463–470.

Reed, J.M., Boulinier, T., Danchin, E. & Oring, L.W. (1999) Informed Dispersal. *Current Ornithology*, **15**, 189–259.

Reichert, S., Criscuolo, F., Verinaud, E., Zahn, S. & Massemin, S. (2013) Telomere length correlations among somatic tissues in adult zebra finches. *PLoS ONE*, **8**, 1–7.

Reichert, S., Criscuolo, F., Zahn, S., Arrivé, M., Bize, P. & Massemin, S. (2015) Immediate and delayed effects of growth conditions on ageing parameters in nestling zebra finches. *Journal of Experimental Biology*, **218**, 491–499.

Reichert, S., Stier, A., Zahn, S., Arrivé, M., Bize, P., Massemin, S. & Criscuolo, F. (2014) Increased brood size leads to persistent eroded telomeres. *Frontiers in Ecology and Evolution*, **2**, 1–11.

Rey, B., Pélisson, P.-F., Bel-Venner, M.-C., Voituron, Y. & Venner, S. (2015) Revisiting the link between breeding effort and oxidative balance through field evaluation of two sympatric sibling insect species. *Evolution*, **69**, 815–822.

Reznick, D., Nunney, L. & Tessier, A. (2000) Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology & Evolution*, **15**, 421–425.

Richter, T. & von Zglinicki, T. (2007) A continuous correlation between oxidative stress and telomere shortening in fibroblasts. *Experimental gerontology*, **42**, 1039–1042.

Ricklefs, R.E. (2008) The evolution of senescence from a comparative perspective. *Functional Ecology*, **22**, 379–392.

Ricklefs, R. & Schew, W. (1994) Foraging stochasticity and lipid accumulation by nestling petrels. *Functional Ecology*, **8**, 159–170.

Rietjens, I.M.C.M., Boersma, M.G., Haan, L. De, Spenkelink, B., Awad, H.M., Cnubben, N.H.P., van Zanden, J.J., Woude, H. Van Der, Alink, G.M. & Koeman, J.H. (2002) The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. *Environmental Toxicology and Pharmacology*, **11**, 321–333.

Ristow, M. & Zarse, K. (2010) How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). *Experimental Gerontology*, **45**, 410–418.

Roff, D.A. & Fairbairn, D.J. (2001) The genetic basis of dispersal and migration and its consequences for the evolution of correlated traits. *Dispersal* (eds J. Clobert, É. Danchin & A.A. Dhondt), pp. 191–202. Oxford University Press, New York.

Roff, D.A., Tucker, J., Stirling, G. & Fairbairn, D.J. (1999) The evolution of threshold traits: Effects of selection on fecundity and correlated response in wing dimorphism in the sand cricket. *Journal of Evolutionary Biology*, **12**, 535–546.

Romero-Haro, A.A. & Alonso-Alvarez, C. (2014) Covariation in oxidative stress markers in the blood of nestling and adult birds. *Physiological and Biochemical Zoology*, **87**, 353–362.

Ronce, O. (2007) How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 231–253.

Ronce, O. & Clobert, J. (2012) Dispersal syndromes. *Dispersal Ecology and Evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 119–138. Oxford University Press, Oxford.

Ruijter, J.M., Pfaffl, M.W., Zhao, S., Spiess, A.N., Boggy, G., Blom, J., Rutledge, R.G., Sisti, D., Lievens, A., De Preter, K., Derveaux, S., Hellemans, J. & Vandesompele, J. (2013) Evaluation of qPCR curve analysis methods for reliable biomarker discovery: bias, resolution, precision, and implications. *Methods*, **59**, 32–46.

Rytkönen, S. (2002) Nest defence in great tits *Parus major*: support for parental investment theory. *Behavioral Ecology and Sociobiology*, **52**, 379–384.

Rytter, E., Vessby, B., Åsgård, R., Johansson, C., Sjödin, A., Abramsson-Zetterberg, L., Möller, L. & Basu, S. (2009) Glycaemic status in relation to oxidative stress and inflammation in well-controlled type 2 diabetes subjects. *British Journal of Nutrition*, **101**, 1423–1426.

Sahin, E., Colla, S., Liesa, M., Moslehi, J., Müller, F.L., Guo, M., Cooper, M., Kotton, D., Fabian, A.J., Walkey, C., Maser, R.S., Tonon, G., Foerster, F., Xiong, R., Wang, Y.A., Shukla, S.A., Jaskelioff, M., Martin, E.S., Heffernan, T.P., Protopopov, A., Ivanova, E., Mahoney, J.E., Kost-Alimova, M., Perry, S.R., Bronson, R., Liao, R., Mulligan, R., Shirihai, O.S., Chin, L. & DePinho, R.A. (2011) Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature*, **470**, 359–365.

Saino, N., Caprioli, M., Romano, M., Boncoraglio, G., Rubolini, D., Ambrosini, R., Bonisoli-Alquati, A. & Romano, A. (2011) Antioxidant defenses predict long-term survival in a passerine bird. *PloS one*, **6**, e19593.

Salin, K., Auer, S.K., Rey, B., Selman, C. & Metcalfe, N.B. (2015a) Variation in the link between oxygen consumption and ATP production, and its relevance for animal performance. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 20151028.

Salin, K., Auer, S.K., Rudolf, A.M., Anderson, G.J., Cairns, A.G., Mullen, W., Hartley, R.C., Selman, C. & Metcalfe, N.B. (2015b) Individuals with higher metabolic rates have lower levels of reactive oxygen species in vivo. *Biology Letters*, **11**, 20150538.

Salomons, H.M., Mulder, G.A., van de Zande, L., Haussmann, M.F., Linskens, M.H.K. & Verhulst, S. (2009) Telomere shortening and survival in free-living corvids. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 3157–3165.

Sanz, J.J., Kranenbarg, S. & Tinbergen, J.M. (2000) Differential response by males and females to manipulation of partner contribution in the great tit (*Parus major*). *Journal of Animal Ecology*, **69**, 74–84.

Sanz, A., Pamplona, R. & Barja, G. (2006) Is the mitochondrial free radical theory of aging intact? *Antioxidants & Redox Signaling*, **8**, 582–599.

Scandolara, C., Lardelli, R., Sgarbi, G., Caprioli, M., Ambrosini, R., Rubolini, D. & Saino, N. (2014) Context-, phenotype-, and kin-dependent natal dispersal of barn swallows (*Hirundo rustica*). *Behavioral Ecology*, **25**, 180–190.

Schäfer, G., Purschke, W. & Schmidt, C.L. (1996) On the origin of respiration: electron transport proteins from archaea to man. *FEMS Microbiology Reviews*, **18**, 173–188.

Schubert, K.A., Vaanholt, L.M., Stavasius, F., Demas, G.E., Daan, S. & Visser, G.H. (2008) Female mice respond differently to costly foraging versus food restriction. *Journal of Experimental Biology*, **211**, 2214–2223.

Schulte-Hostedde, A.I., Zinner, B., Millar, J.S. & Hickling, G.J. (2005) Restitution of mass-size residuals: Validating body condition indices. *Ecology*, **86**, 155–163.

Schultner, J., Moe, B., Chastel, O., Bech, C. & Kitaysky, A.S. (2014) Migration and stress during reproduction govern telomere dynamics in a seabird. *Biology Letters*, **10**, 20130889.

Schürch, R. & Heg, D. (2010) Life history and behavioral type in the highly social cichlid *Neolamprologus pulcher*. *Behavioral Ecology*, **21**, 588–598.

Schwagmeyer, P.L. & Mock, D.W. (2008) Parental provisioning and offspring fitness: size matters. *Animal Behaviour*, **75**, 291–298.

Seifried, H.E., Anderson, D.E., Fisher, E.I. & Milner, J.A. (2007) A review of the interaction among dietary antioxidants and reactive oxygen species. *The Journal of Nutritional Biochemistry*, **18**, 567–579.

Selman, C., Blount, J.D., Nussey, D.H. & Speakman, J.R. (2012) Oxidative damage, ageing, and life-history evolution: where now? *Trends in Ecology & Evolution*, **27**, 570–577.

Selman, C., McLaren, J.S., Collins, A.R., Duthie, G.G. & Speakman, J.R. (2013) Deleterious consequences of antioxidant supplementation on lifespan in a wild-derived mammal. *Biology Letters*, **9**, 20130432.

Sena, L.A. & Chandel, N.S. (2012) Physiological roles of mitochondrial reactive oxygen species. *Molecular Cell*, **48**, 158–166.

Senar, J.C., Domenech, J. & Uribe, F. (2002) Great tits (*Parus major*) reduce body mass in response to wing area reduction: a field experiment. *Behavioral Ecology*, **13**, 725–727.

Sepp, T., Sild, E., Blount, J.D., Männiste, M., Karu, U. & Hõrak, P. (2012) Individual consistency and covariation of measures of oxidative status in greenfinches. *Physiological and Biochemical Zoology*, **85**, 299–307.

Serrano, D. & Tella, J.L. (2012) Lifetime fitness correlates of natal dispersal distance in a colonial bird. *Journal of Animal Ecology*, **81**, 97–107.

Sexton, J.P., McIntyre, P.J., Angert, A.L. & Rice, K.J. (2009) Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 415–436.

Shafer, A.B.A., Poissant, J., Cote, S.D. & Coltman, D.W. (2011) Does reduced heterozygosity influence dispersal? A test using spatially structured populations in an alpine ungulate. *Biology Letters*, **7**, 433–435.

Sharick, J.T., Vazquez-Medina, J.P., Ortiz, R.M. & Crocker, D.E. (2015) Oxidative stress is a potential cost of breeding in male and female northern elephant seals. *Functional Ecology*, **29**, 367–376.

Shaw, M.J.P. (1970) Effects of population density on alienicolae of *Aphis fabae* Scop. *Annals of Applied Biology*, **65**, 197–203.

Sies, H. (2007) Total antioxidant capacity: Appraisal of a concept. *Journal of Nutrition*, **137**, 1493–1495.

Siikamäki, P. (1998) Limitation of reproductive success by food availability and breeding time in Pied Flycatchers. *Ecology*, **79**, 1789–1796.

Sinervo, B., Chaine, A., Clobert, J., Calsbeek, R., Hazard, L., Lancaster, L., McAdam, A.G., Alonzo, S., Corrigan, G. & Hochberg, M.E. (2006) Self-recognition, color signals, and cycles of greenbeard mutualism and altruism. *Proceedings of the National Academy of Sciences*, **103**, 7372–7377.

Sluse, F.E. (2012) Uncoupling proteins: Molecular, functional, regulatory, physiological and pathological aspects. *Advances in Mitochondrial Medecine, Advances in Experimental Medicine and Biology Series* (eds R. Scatena, P. Bottoni & B. Giardina), pp. 137–156. Springer Netherlands.

Snoeijs, T., Van de Casteele, T., Adriaensen, F., Matthysen, E. & Eens, M. (2004) A strong association between immune responsiveness and natal dispersal in a songbird. *Proceedings of the Royal Society B: Biological Sciences*, **271 Suppl**, S199–201.

Solmsen, N., Johannesen, J. & Schradin, C. (2011) Highly asymmetric fine-scale genetic structure between sexes of African striped mice and indication for condition dependent alternative male dispersal tactics. *Molecular Ecology*, **20**, 1624–1634.

Sorci, G. & Faivre, B. (2009) Inflammation and oxidative stress in vertebrate host-parasite systems. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **364**, 71–83.

Speakman, J.R. (1997) *Doubly Labelled Water: Theory and Practice*. Chapman & Hall, London.

Speakman, J.R. & Garratt, M. (2014) Oxidative stress as a cost of reproduction: Beyond the simplistic trade-off model. *Bioessays*, **36**, 93–106.

Speakman, J.R. & Król, E. (2005) Comparison of different approaches for the calculation of energy expenditure using doubly labeled water in a small mammal. *Physiological and Biochemical Zoology*, **78**, 650–667.

Speakman, J.R. & Król, E. (2010) The heat dissipation limit theory and evolution of life histories in endotherms--time to dispose of the disposable soma theory? *Integrative and Comparative Biology*, **50**, 793–807.

Speakman, J.R. & Selman, C. (2011) The free-radical damage theory: Accumulating evidence against a simple link of oxidative stress to ageing and lifespan. *Bioessays*, **33**, 255–259.

Speakman, J.R., Talbot, D.A., Selman, C., Snart, S., McLaren, J.S., Redman, P., Krol, E., Jackson, D.M., Johnson, M.S. & Brand, M.D. (2004) Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. *Aging Cell*, **3**, 87–95.

Spear, L.B., Pyle, P. & Nur, N. (1998) Natal dispersal in the western gull: Proximal factors and fitness consequences. *Journal of Animal Ecology*, **67**, 165–179.

Stamps, J. a. (2006) The silver spoon effect and habitat selection by natal dispersers. *Ecology Letters*, **9**, 1179–1185.

Starrfelt, J. & Kokko, H. (2012) The theory of dispersal under multiple influences. *Dispersal Ecology and Evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 19–28. Oxford University Press, Oxford.

Stearns, S.C. (1989) Trade-offs in life-history evolution. *Functional Ecology*, **3**, 259–268.

Stevens, V.M., Trochet, A., Blanchet, S., Moulherat, S., Clobert, J. & Baguette, M. (2013) Dispersal syndromes and the use of life-histories to predict dispersal. *Evolutionary Applications*, **6**, 630–42.

Stevens, V.M., Whitmee, S., Le Galliard, J.-F., Clobert, J., Böhning-Gaese, K., Bonte, D., Brändle, M., Dehling, D.M., Hof, C., Trochet, A. & Baguette, M. (2014) A comparative analysis of dispersal syndromes in terrestrial and semi-terrestrial animals. *Ecology Letters*, **17**, 1039–1052.

Stier, A., Bize, P., Habold, C., Bouillaud, F., Massemin, S. & Criscuolo, F. (2014a) Mitochondrial uncoupling prevents cold-induced oxidative stress: a case study using UCP1 knockout mice. *Journal of Experimental Biology*, **217**, 624–630.

Stier, A., Bize, P., Roussel, D., Schull, Q., Massemin, S. & Criscuolo, F. (2014b) Mitochondrial uncoupling as a regulator of life-history trajectories in birds: an experimental study in the zebra finch. *Journal of Experimental Biology*, **217**, 3579–3589.

Stier, A., Bize, P., Schull, Q., Zoll, J., Singh, F., Geny, B., Gros, F., Royer, C., Massemin, S. & Criscuolo, F. (2013) Avian erythrocytes have functional mitochondria, opening novel

perspectives for birds as animal models in the study of ageing. *Frontiers in Zoology*, **10**, 33.

Stier, A., Massemin, S. & Criscuolo, F. (2014c) Chronic mitochondrial uncoupling treatment prevents acute cold-induced oxidative stress in birds. *Journal of Comparative Physiology B*, **184**, 1021–1029.

Stier, A., Massemin, S., Zahn, S., Tissier, M.L. & Criscuolo, F. (2015a) Starting with a handicap: effects of asynchronous hatching on growth rate, oxidative stress and telomere dynamics in free-living great tits. *Oecologia*, Early view.

Stier, A., Reichert, S., Criscuolo, F. & Bize, P. (2015b) Red blood cells open promising avenues for longitudinal studies of ageing in laboratory, non-model and wild animals. *Experimental Gerontology*, Early view.

Stier, A., Reichert, S., Massemin, S., Bize, P. & Criscuolo, F. (2012) Constraint and cost of oxidative stress on reproduction: correlative evidence in laboratory mice and review of the literature. *Frontiers in Zoology*, **9**, 37.

Stier, A., Viblanc, V.A., Massemin-Challet, S., Handrich, Y., Zahn, S., Rojas, E.R., Saraux, C., Le Vaillant, M., Prud'homme, O., Grosbellet, E., Robin, J.-P., Bize, P. & Criscuolo, F. (2014d) Starting with a handicap: phenotypic differences between early- and late-born king penguin chicks and their survival correlates. *Functional Ecology*, **28**, 601–611.

Svensson, L. (1992) *Identification Guide to European Passerines*. British Trust for Ornithology, London.

Swaddle, J.P. & Witter, M.S. (1997) The effects of molt on the flight performance, body mass, and behavior of European starlings (*Sturnus vulgaris*): an experimental approach. *Canadian Journal of Zoology*, **75**, 1135– 1146.

Talegawkar, S. a, Beretta, G., Yeum, K.-J., Johnson, E.J., Carithers, T.C., Taylor, H. a, Russell, R.M. & Tucker, K.L. (2009) Total antioxidant performance is associated with diet and serum antioxidants in participants of the diet and physical activity substudy of the Jackson Heart Study. *Journal of Nutrition*, **139**, 1964–1971.

Tarwater, C.E. (2012) Influence of phenotypic and social traits on dispersal in a family living, tropical bird. *Behavioral Ecology*, **23**, 1242–1249.

Taylor, H.A. & Delany, M.E. (2000) Ontogeny of telomerase in chicken: Impact of downregulation on pre- and postnatal telomere length in vivo. *Development Growth and Differentiation*, **42**, 613–621.

Teplitsky, C., Robinson, M.R. & Merilä, J. (2014) Evolutionary potential and constraints in wild populations. *Quantitative Genetics in the Wild* (eds A. Charmantier, D. Garant & L.E.B. Kruuk), pp. 190–208. Oxford University Press, Oxford.

Tinbergen, J.M. & Dietz, W. (1994) Parental energy expenditure during brood rearing in the Great Tit (*Parus major*) in relation to body mass, temperature, food availability and clutch size. *Functional Ecology*, **8**, 563–572.

Tinner, T. & G, M. (1951) Über das Brüten des Halsbandfliegenschnäppers Ficedula albicollis Temm. im Tessin. *Ornithologische Beobachtungen*, **48**, 161–165.

Tolmasoff, J.M., Ono, T. & Cutler, R.G. (1980) Superoxide dismutase: correlation with life-span and specific metabolic rate in primate species. *Proceedings of the National Academy of Sciences*, **77**, 2777–2781.

Tschirren, B., Fitze, P.S. & Richner, H. (2007) Maternal modulation of natal dispersal in a passerine bird: an adaptive strategy to cope with parasitism? *The American Naturalist*, **169**, 87–93.

Turrens, J.F. (2003) Mitochondrial formation of reactive oxygen species. *The Journal of Physiology*, **552**, 335–344.

Ubuka, T., Mizuno, T., Fukuda, Y., Bentley, G.E., Wingfield, J.C. & Tsutsui, K. (2013) RNA interference of gonadotropin-inhibitory hormone gene induces aggressive and sexual behaviors in birds. *General and Comparative Endocrinology*, **181**, 179–186.

Ujvari, B. & Madsen, T. (2009) Short telomeres in hatchling snakes: erythrocyte telomere dynamics and longevity in tropical pythons. *PloS one*, **4**, e7493.

De V. Weir, J.B. (1949) New methods for calculating metabolic rate with special reference to protein metabolism. *The Journal of Physiology*, **109**, 1–9.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M. & Telser, J. (2007) Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, **39**, 44–84.

Vanfleteren, J.R. & Braeckman, B.P. (1999) Mechanisms of life span determination in *Caenorhabditis elegans*. *Neurobiology of Ageing*, **20**, 487–502.

Vaugoyeau, M., Decenciere, B., Perret, S., Karadas, F., Meylan, S. & Biard, C. (2015) Is oxidative status influenced by dietary carotenoid and physical activity after moult in the great tit (*Parus major*)? *Journal of Experimental Biology*, **218**, 2106–2115.

Vera, E., Bernardes de Jesus, B., Foronda, M., Flores, J.M. & Blasco, M.A. (2012) The rate of increase of short telomeres predicts longevity in mammals. *Cell reports*, **2**, 732–737.

Vercken, E., Sinervo, B. & Clobert, J. (2012) The importance of a good neighborhood: Dispersal decisions in juvenile common lizards are based on social environment. *Behavioral Ecology*, **23**, 1059–1067.

Verhulst, S. (1994) Supplementary food in the nestling phase affects reproductive success in Pied Flycatchers (*Ficedula hypoleuca*). *The Auk*, **111**, 714–716.

Verhulst, S., Perrins, C.M. & Riddington, R. (1997) Natal dispersal of great tits in a patchy environment. *Ecology*, **78**, 864–872.

Verspoor, J.J., Love, O.P., Rowland, E., Chin, E.H. & Williams, T.D. (2007) Sex-specific development of avian flight performance under experimentally altered rearing conditions. *Behavioral Ecology*, **18**, 967–973.

Veskoukis, A.S., Nikolaidis, M.G., Kyparos, A. & Kouretas, D. (2009) Blood reflects tissue oxidative stress depending on biomarker and tissue studied. *Free Radical Biology and Medicine*, **47**, 1371–1374.

Visser, M.E. & Lessells, C.M. (2001) The costs of egg production and incubation in great tits (*Parus major*). *Proceedings of the Royal Society B: Biological Sciences*, **268**, 1271–1277.

Voillemot, M., Hine, K., Zahn, S., Criscuolo, F., Gustafsson, L., Doligez, B. & Bize, P. (2012) Effects of brood size manipulation and common origin on phenotype and telomere length in nestling collared flycatchers. *BMC Ecology*, **12**, 17.

Waldeck, P., Andersson, M., Kilpi, M. & Öst, M. (2008) Spatial relatedness and brood parasitism in a female-philopatric bird population. *Behavioral Ecology*, **19**, 67–73.

Wang, Y., Yang, M., Lee, S.-G., Davis, C.G., Kenny, A., Koo, S.I. & Chun, O.K. (2012a) Plasma total antioxidant capacity is associated with dietary intake and plasma level of antioxidants in postmenopausal women. *The Journal of Nutritional Biochemistry*, **23**, 1725–1731.

Wang, Y., Yang, M., Lee, S.G., Davis, C.G., Koo, S.I. & Chun, O.K. (2012b) Dietary total antioxidant capacity is associated with diet and plasma antioxidant status in healthy young adults. *Journal of the Academy of Nutrition and Dietetics*, **112**, 1626–1635.

Waser, P.M. (1985) Does competition drive dispersal? *Ecology*, **66**, 1170–1175.

Waser, P.M., Nichols, K.M. & Hadfield, J.D. (2013) Fitness consequences of dispersal: Is leaving home the best of a bad lot? *Ecology*, **94**, 1287–1295.

Wauters, L., Matthysen, E. & Dhondt, A.A. (1994) Survival and lifetime reproductive success in dispersing and resident red squirrels. *Behavioral Ecology and Sociobiology*, **34**, 197–201.

Wegmann, M., Voegeli, B. & Richner, H. (2015a) Physiological responses to increased brood size and ectoparasite infestation: Adult great tits favour self-maintenance. *Physiology & Behavior*, **141**, 127–134.

Wegmann, M., Voegeli, B. & Richner, H. (2015b) Oxidative status and reproductive effort of great tits in a handicapping experiment. *Behavioral Ecology*, **26**, 747–754.

Van der Werf, E. (1992) Lack's clutch size hypothesis: An examination of the evidence using meta-analysis. *Ecology*, **73**, 1699–1705.

Wey, T.W., Spiegel, O., Montiglio, P. & Mabry, K.E. (2015) Natal dispersal in a social landscape: Considering individual behavioral phenotypes and social environment in dispersal ecology. *Current Zoology*, **61**, 543–556.

Wheelwright, N.T. & Mauck, R.A. (1998) Philopatry, natal dispersal, and inbreeding avoidance in an island population of Savannah Sparrows. *Ecology*, **79**, 755–767.

Whitlock, M.C. (2001) Dispersal and the genetic properties of metapopulations. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 273–282. Oxford University Press, Oxford.

Wiens, J.A. (2001) The landscape context of dispersal. *Dispersal* (eds J. Clobert, E. Danchin, A.A. Dhondt & J.D. Nichols), pp. 96–109. Oxford University Press, Oxford.

Williams, J.B. (1987) Field metabolism and food consumption of Savannah Sparrows during the breeding season. *The Auk*, **104**, 277–289.

Wilson, A.J. & Nussey, D.H. (2009) What is individual quality? An evolutionary perspective. *Trends in Ecology & Evolution*, **25**, 207–214.

Winkler, D.W. (1987) A general model for parental care. *The American Naturalist*, **130**, 526–543.

Winkler, D.W. & Allen, P.E. (1995) Effects of handicapping on female condition and reproduction in Tree Swallows (*Tachycineta bicolor*). *The Auk*, **112**, 737–747.

Xu, Y.-C., Yang, D.-B., Speakman, J.R. & Wang, D.-H. (2014) Oxidative stress in response to natural and experimentally elevated reproductive effort is tissue dependent. *Functional Ecology*, **28**, 402–410.

Yang, W. & Omaye, S.T. (2009) Air pollutants, oxidative stress and human health. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, **674**, 45–54.

Yang, D.-B., Xu, Y.-C., Wang, D.-H. & Speakman, J.R. (2013) Effects of reproduction on immuno-suppression and oxidative damage, and hence support or otherwise for their roles as mechanisms underpinning life history trade-offs, are tissue and assay dependent. *Journal of Experimental Biology*, **216**, 4242–4250.

Yki-Järvinen, H. (1992) Glucose toxicity. *Endocrine Reviews*, **13**, 415–431.

Yoder, J.M., Marschall, E. a. & Swanson, D. a. (2004) The cost of dispersal: Predation as a function of movement and site familiarity in ruffed grouse. *Behavioral Ecology*, **15**, 469–476.

Young, R.C., Kitaysky, A.S., Haussmann, M.F., Descamps, S., Orben, R.A., Elliott, K.H. & Gaston, A.J. (2013) Age, sex, and telomere dynamics in a long-lived seabird with male-biased parental care. *PloS one*, **8**, e74931.

Zera, A.J. & Brisson, J.A. (2012) Quantitative, physiological, and molecular genetics of dispersal/migration. *Dispersal Ecology and Evolution* (eds J. Clobert, M. Baguette, T.G. Benton & J.M. Bullock), pp. 63–82. Oxford University Press, Oxford.

Zera, A.J. & Denno, R.F. (1997) Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology*, **42**, 207–230.

Von Zglinicki, T. (2002) Oxidative stress shortens telomeres. *Trends in Biochemical Sciences*, **27**, 339–344.

Zimmer, C. & Spencer, K.A. (2015) Reduced resistance to oxidative stress during reproduction as a cost of early-life stress. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*, **183**, 9–13.

APPENDIX I: VARIATION OF MITOCHONDRIAL ATTRIBUTES IS RELATED TO BREEDING STAGE AND SEX, BUT NOT ACCESS TO FOOD OR AGE, IN FREE LIVING ADULT COLLARED FLYCATCHERS

Luiza C. Duarte, Charlotte Récapet, Antoine Stier, Blandine Doligez, Pierre Bize

In preparation

Keywords: life history, mitochondria, ROS, bird, erythrocyte, ATP, oxidative stress, Ficedula albicollis

Abbreviations

ATP: Adenosine Tri-Phosphate

NAO: nonyl acridine orange

ROS: Reactive Oxygen Species

HBSS: Hunk's Buffered Salt Solution

1. INTRODUCTION

Life history theories point out that free-living organisms compete for limited pool of energy resources, and that resources allocated to one trait are no more available for other traits. Hence, it is frequently proposed that variation in energy acquisition, transformation and allocation underlie the variation in life history trajectories of individuals, populations or species (Stearns, 1992; Ricklefs and Wikelski, 2002; Williams et al., 2010). Because energy flux at the cellular level takes mostly place in the mitochondria, there is currently growing interest on the role of these organelles in shaping animal life history diversities (e.g. Passos et al., 2007; Williams et al., 2010; Salin et al., 2012d; Hill, 2014).

Mitochondria enclose the protein complexes from the electron transport chain that are responsible for ATP synthesis through oxidative phosphorylation (OXPHOS). ATP is the primary cellular energy resources for cell functioning, and because most of its synthesis takes place in the mitochondria, mitochondria are vital to support whole organism growth, maturation, reproduction and survival. Mitochondria use electrons harvested from oxidizable substrates and O_2 as a final electron acceptor to build up a proton motive force by pumping protons from the mitochondrial matrix into the intermembrane space (Mitchell, 1961). The subsequent backflow of protons to the matrix across the protein complex 'ATP synthase' drives the synthesis of ATP (Mitchell, 1961). The coupling of substrate oxidation to ATP synthesis is however variable and not all the electrons harvested during respiration and equivalent protons pumped into the intermembrane space are used for ATP synthesis. Two factors are contributing to this variability. Firstly, some electrons are known to escape the mitochondrial electron transport chain and to react directly with molecular oxygen. It leads to the production of harmful reactive oxygen species (ROS) (Halliwell and Gutteridge, 2007), and damage caused by ROS during respiration has been proposed to act as an inevitable and universal constraint in life history evolution (Balaban et al., 2005; Dowling and Simmons, 2009; Metcalfe and Alonso-Alvarez, 2010). Secondly, protons can also flow back to the matrix across other protein complexes than the ATP synthase, which leads to the decoupling of substrate oxidation from ATP synthesis (Divakaruni and Brand, 2011). Although

mitochondrial uncoupling may be viewed as costly by reducing ATP synthesis, it can also be beneficial by lowering ROS production (Mookerjee *et al*. 2010, Stier *et al*. 2014) and enabling heat production in endotherms (Golozoubova et al., 2006). Hence, a first hypothesis is that, to maximize fitness, selection should select for optimal mitochondrial ATP synthesis and ROS production per amount of oxidized substrates (Salin et al., 2012d; Salin et al., 2012a). Interestingly, the number of mitochondria per cell (hereafter referred to as mitochondrial density) may also respond to variation in the efficiency of mitochondrial energy transduction and be related to animal life histories (Passos et al., 2007). Accordingly, interspecific comparisons suggest that longer lived mammalian species have lower densities of mitochondria in their liver compared to shorter lived mammalian species (Passos et al., 2007), whereas intraspecific studies are suggesting that beneficial effects of aerobic exercise and caloric restriction animal health and lifespan might be related to increased mitochondrial biogenesis in responses to these stimuli (Viña et al., 2009). Hence, a second non-mutually hypothesis is that, to maximise fitness, selection may also act on optimal adjustment of densities of functional mitochondria per cell (Passos et al., 2007). The relationships between mitochondrial density, ATP synthesis, ROS production, oxygen consumption and life history trajectories are however complex, and most remains to be done on this topic (Passos et al., 2007; Salin et al., 2012d).

So far, studies on mitochondrial attributes, like ATP synthesis, ROS production, oxygen consumption and mitochondrial densities, have mostly been performed on cell cultures, laboratory animals or humans, and we still know very little information on mitochondrial variation and life trajectories in free living animals (but see Melvin and Ballard, 2006; Zheng et al., 2008; Robert and Bronikowski, 2010; Glanville et al., 2012; Salin et al., 2012a; White et al., 2012). The weak number of studies in free-living animal populations is most likely attributed to methodological issues. Indeed, insights on mitochondrial attributes involve a terminal sampling to obtain fresh tissues used to extract and measure mitochondria (*e.g.* Melvin and Ballard, 2006; Zheng et al., 2008; Robert and Bronikowski, 2010; Glanville et al., 2012; Salin et al., 2012a; White et al., 2012), which is difficult to reconcile

with the need of long-term observation to gather information on an individual life history, and access to laboratory equipment to quickly process the samples.

In the present study we investigated the use of blood samples to gain insight on mitochondrial attributes in a natural population of collared flycatchers (*Ficedula albicollis*). It has been recently demonstrated that birds, unlike mammals, have functional mitochondria in their erythrocytes, both in terms of respiratory activities and ROS production (Stier et al., 2013), thus making feasible to gather information on mitochondrial functioning with minimally invasive sampling procedure. We measured variation in mitochondrial densities, total reactive species and ATP concentration and ROS production in erythrocytes of adult birds sampled at incubation and during chick rearing. We investigated variation in mitochondrial attributes in relation to breeding stage, sex and to a food supplementation experiment. As mentioned before, availability of resources is viewed as a prime life history constraint in free-living animals (Prevedello et al., 2013; Ruffino et al., 2014), and thus we predicted that food supplementation should lead to an increase in reproductive success and adult body condition. Moreover, if mitochondria play a central role in energy transduction from environmental resources to reproductive success, we expected that mitochondrial attributes should change in response to our food supplementation experiment. There is however little information on the relationships between mitochondrial attributes and environmental factors, and thus we were not able to make *a priori* prediction on the mitochondrial attributes that should respond to our food supplementation experiment and the direction of the responses. Similarly, because the energetic needs may differ between the reproductive stages and between the sexes (Monaghan and Nager, 1997), we predicted that mitochondrial attributes may differ between reproductive stages and between the sexes, but we made no further prediction on the mitochondrial attributes involved and the direction of the changes.

2. Material and Methods

Study species and field data collection

The collared flycatcher is a ca. 13g migratory passerine bird that breeds in natural cavities and readily accepts nest boxes (Pärt and Gustafsson, 1989). Most males are monogamous, and females lay one clutch per year of 5-7 eggs that they incubate alone during 13-14 days. Both parents feed the young, mainly with caterpillars, until the young leave the nest between 15 and 18 days after hatching. The abundance of caterpillars can greatly vary between- and within-years depending on weather conditions, and this can have dramatic effects on reproductive success of collared flycatchers (Torok and Toth, 1988). Adult collared flycatchers show moderate dimorphism in body size, with females having slightly longer tarsi and being heavier than males during incubation (Merilä, 1997).

Data were collected from May to June 2014 in a population of collared flycatchers breeding in the southern part of the Swedish island of Gotland (57°10'N, 18°20'E), where artificial nest-boxes have been provided to the birds in discrete deciduous forest patches. This population is part of a long-term monitoring (> 35 years), and more information on the study site can be found in (Pärt and Gustafsson, 1989) and (Doligez et al., 1999). Each year, nests are monitored throughout the breeding season to assess occupancy, egg laying date, clutch size, hatching date, brood size at hatching and fledgling. In 2014, mothers were caught a first time inside the nest box while incubating eggs at 7.2 ± 0.11 days (mean ± SE) after initiating egg incubation. Mothers were trapped a second time inside the nest box while provisioning food to their brood at 9.3 ± 0.11 days after hatching. Fathers were trapped once inside the nest box while provisioning the brood at 9.5 ± 0.12 days after hatching. If adults had not been ringed before, they were individually identified with numbered metal ring at their first capture. Adult tarsus length was measured to the nearest 0.1mm with a digital calliper, and adults were weighted to the nearest 0.1g on an electronic scale. Bird ringing was carried out under licence from the Bird Ringing Centre, Swedish Museum of Natural History (licence number 471:M009 to CR).

Food supplementation experiment

Food availability was manipulated at the nest level by providing additional food. When chicks were 2 days old, two 60mL transparent plastic containers were attached to the front side of the nest box, below the nest hole. For supplemented nests, 15g live maggots were placed in each container (i.e. 30g per nest in total) once a day between 7:00 and 19:00 until chicks were 13 days old. When food had not been supplied one particular day for technical constraints, the nest was supplemented twice on the following day, once in the morning and once in the afternoon. Each experimental nest therefore received a total of 360g maggots over the whole chick phase. Control nests received no food, but were visited daily to control for disturbance linked with human presence. Nests were assigned either to the control or supplemented group alternatively in space, so as to distribute treatments homogenously in space both within and between study plots. Nests that did not survive till day 2 or that were affected by accidental events were excluded. The final sample sizes were of 69 nests in the control group and 77 nests in the supplemented group.

Body condition estimates

We estimated body condition of an individual i using the "scaled mass index" proposed by Peig and Green (2009). This method uses a standard major axis (SMA) regression of body mass (M) on a linear body measurement (L); tarsus length is used here as a linear body measurement. Hence, M_i and L_i are the body mass and tarsus length of individual i respectively, L_0 is the arithmetic mean value of tarsus length for the study population, and b_{SMA} is the scaling exponent estimated by the SMA regression of M on L. We computed b_{SMA} indirectly by dividing the slope from an ordinary least square regression (b) of M on L by the Pearson's correlation coefficient (r) between M and L. This body condition estimate is viewed as a reliable indicator of the relative size of energy reserves accumulated in the body as a result of feeding (Peig and Green, 2009). We estimated body condition separately for females at incubation (N = 164), females at chick-rearing stage (N = 146) and males (N = 121).

Blood collection and sample preparation

Blood samples were collected in adult females and males from the brachial vein using a heparin-lithium coated Microvette® (CB300 LH, Sarstedt). Immediately after collection, 15μL of whole blood were transferred into a 500 μL of 1× Hunk's Buffered Salt Solution (HBSS) and gently homogenized. This solution and the remaining blood in the Microvette® were stored at 5°C in the field before being process at the end of the day in a field laboratory. Microvettes were centrifuged for 5 min to separate plasma from the red cells. Plasma and red blood cells were then stored at -80°C before being shipped on dry ice to Aberdeen, UK, for further laboratory analysis. Red cells in HBSS solution were washed at their arrival in the field laboratory by centrifuging samples for 5 min to pellet cells, discarding the supernatant, and re-suspending cells in 785μL fresh 1× HBSS. Concentration of red cells in each sample was determined using a handheld automated cell counter (*Scepter™ 2.0 Cell Counter*, Merck Millipore, Germany), and all the samples were further diluted in HBSS to reach a final stock concentration of 4.5×10^7 cells/mL. This stock concentration of red cells was used for measurements of ATP concentration, ROS production and mitochondrial densities using a plate reader Fluostar OMEGA (BMG Labtech Ltd. UK) in the sequence as described below.

ATP concentration

ATP concentration of red cells was measured using the ATP Bioluminescence Assay HS II Kit (Roche Applied Science, Mannheim, Germany, cat# 699709001) according to the manufacturer's protocol. This assay uses a standard luciferase reaction that allows quantifying intracellular ATP concentration in whole cell lysate (Fan and Wood, 2007). ATP was extracted from 100 μl of cell suspension at 4.5×10^7 cells/mL by adding the cell lysis reagent provided in the kit and incubating samples for 5 min at room temperature. Samples were then centrifuged for 10 min, and 50 μl of supernatant was loaded in duplicates in a white 96 well plate. We then added 50 μl of luciferase per well before bioluminescence reading at room temperature with 1 sec time delay and 1sec integration time. A standard curve was produced in every plate by 5 serial dilutions of known ATP concentration (20.625×10^{-6}mM to 1.289×10^{-6}mM) run in duplicate. Measurement of luciferase luminescence

using a plate reader is associated with a short time delay between two consecutive readings, and preliminary analyses showed a significant constant decline in reading values along rows (b = -0.157 ± xx0.011, $t_{1,1325}$ =- 13.97, P= 0.000) but not columns (b = 0.013 ± xx0.011, $t_{1,1325}$ = 1.15, P = 0.25). Plate reading was carried out sequentially row by row. Thus, we corrected our initial values to account for row position of a sample on the plate using the slope of linear regression of mean row luminescence on row number. In total, we measured ATP concentration in red cells of 156 females at incubation, 134 females at chick-rearing and 117 males at chick rearing. The mean ± SE time interval between blood sampling in the field and ATP measurement in the laboratory was 10.1 ± 0.2 hours. ATP concentration did not significantly vary with time interval (b = 0.02 ± xx0.014, $t_{1, 406}$ = 1.34, P = 0.18). Repeatability (mean ± SE of CV) was 4.26 ± 0.16.

Total reactive species and superoxide production

Total reactive species and superoxide production were measured using the commercial *Total ROS/Superoxide detection kit* (Enzo Life Sciences AG, Lausen, Switzerland, cat# Enz-51001) that allows real-time detection of reactive oxygen species and also distinguishes between different types of reactive species in live cells via the combination of two specific fluorescent dyes. The Oxidative Stress Detection Reagent (green fluorescence) reacts with a wide range of reactive oxygen species (ROS) and/or nitrogen species (RNS), such as hydrogen peroxide, peroxynitrite and hydroxyl radicals, thus yielding a measure of total reactive species, whereas the Superoxide Detection Reagent (orange fluorescence) reacts specifically with superoxide. The kit was used according to the manufacturer's instructions, with some adjustments. We made a total reactive species/superoxide detection mix by diluting 2µl of reconstitute Oxidative Stress Detection Reagent and 2µl Superoxide Detection Reagent to 10 ml of 1× HBSS Buffer. 160 µl of total reactive species/superoxide detection mix was added to 65 µl the cell suspension at 4.5 × 10^7 cells/mL in an "eppendorf" tube to reach a final concentration of 13 × 10^6 cells/mL. 100µl of each sample was loaded in a black 96 well plate (3.97 x 10^5 cells/well).

Plates were incubated in the dark for 30 minutes at 40°C, which is close to bird body temperature, before adding 0.9 µl of the reactive species inducer pyocyanin per well (final inducer concentration: 450 µM). Pyocyanin, a toxin produced by human pathogen *Pseudomonas aeruginosa that* cause cells premature senescence by inducing oxidative stress and decrease anti-oxidant activity (Muller 2002, 2009), was used to compare the maximum signal of total reactive species and superoxide among individuals. Plates were then read for one hour at 40°C in kinetic mode in two concomitant measurements, one at 485/520nm excitation/emission (orange fluorescence) and the second at 544/610nm excitation/emission (green fluorescence). A negative control was run on every plate by adding 3 µl of N-acetyl–L–cysteine (NAC) reactive species inhibitor in a supplementary randomly chosen sample. NAC acts as an antioxidant, leading to a lack of fluorescence detection in live cells (Zafarullah et al., 2003). We measured data of total reactive species production in red cells of 152 females at incubation, 119 females at chick-rearing and 95 males at chick-rearing, and superoxide production in red cells of 125 females at incubation, 102 females at chick-rearing and 73 males at chick-rearing. This discrepancy between the two probes was due to the fact that, to improve the reliability of the data, we excluded measurements with values of CV higher than 15%, and superoxide probe showed more readings above this limit. Time interval between blood sampling in the field and the measurements of total reactive species/superoxide (10.94 hours ± 0.18) had a significant and positive effect on both total reactive species (b = 0.045 ± xx0.015, $t_{1,365}$ = 3.04, P = 0.003) and superoxide (b = 0.050 ±0.016 xx, $t_{1,299}$ = 3.07, P = 0.002). Therefore, the residuals of a linear regression of total reactive species and superoxide on time interval were extracted and used in the subsequent statistical analyses. Repeatability (mean ± SE of CVs) was 2.70 ± 0.11 for total reactive species and 6.13 ± 0.24 for superoxide production.

Index of mitochondrial density

As an index of mitochondrial density, we used the specific fluorescent probe nonyl acridine orange (NAO, cat# A1372, Life Technologies). NAO is specifically binding to the polyunsaturated acidic phospholipid cardiolipin that is only found in the inner

mitochondrial membrane. Although some controversy regarding its dependence upon membrane potential (Jacobson et al., 2002), NAO is a well-established dye to estimate mitochondria density for either in living cells (Dykens et al., 2002; Widlansky et al., 2010). For this assay, three μL of 2 mM NAO stock solution were diluted in 897μL of 1 × HBSS. Then 100 μL of cell suspension was added to get 6 µM NAO working solution and cell concentration of 4.5 x 10^6 cells/mL. Samples were incubated in the dark at 40°C for 30 minutes, and cells were then washed by centrifuging samples at room temperature for 10 minutes, discarding the supernatant and re-suspending cells in 400 μL of 1 × HBSS. Samples were then incubated a second time in the dark at 4°C for 40 minutes before being measured. We did a second incubation after washing the cells because we observed that NAO fluorescence required time to reach a stable intensity. 100µl of cell samples were loaded in duplicates in a black 96 well plate and read at 485nm/538nm excitation/emission. An internal calibrator was added in duplicates on every plate. This calibrator was made of a pool of red cells from different individuals that we made at the beginning of the field season, divided in aliquots kept at -80° C until being used in the analysis. Mean fluorescence value of the calibrator remained constant throughout the season (CV±SE = 4.04 ±0.63). We measured mitochondrial densities in red cells of 160 females at incubation, 145 females at chick-rearing and 120 males at chick-rearing. The mean ± SE time interval between blood sampling in the field and NAO measurement was 22.3 hours ± 0.2, and NAO fluorescence intensity did not vary with time interval (b = -0.011 ± xx0.013, $t_{1,424}$ = -0.87, P = 0.39). Repeatability of measurements (mean ± SE of CVs) was 3.51 ± 0.13.

Oxidative stress and anti-oxidant capacity measurements on plasma

The concentration of Reactive Oxygen Metabolites and the antioxidant barrier in the plasma were measured using the d-ROMs and OXY-Adsorbent tests respectively (*Diacron* International, Italy). D-ROMs test mostly measures hydroperoxydes as a marker of global early oxidative damage (mainly on lipids and proteins). Briefly, 4μL of plasma were mixed with 198μL acidic buffer and 2μL chromogenic substrate (N, N- diethylparaphenilendiamine) and left to incubate for 75min at 37°C, before measuring optical density (OD) at 550nm twice

in a row. To control for the natural opacity of some samples, OD at 800nm was measured and those measurements with OD800 > 0.100 were excluded from the analysis. The average of valid d-ROM measurements for a sample was used in the analysis. All measurements were run in duplicates and repeatability was high (mean ± s.e. CVs). OXY adsorbent test estimates the capacity of plasma to oppose the oxidative action of the hypochlorous acid HClO, Briefly, each plasma sample was diluted at 1/100 in ultra-pure water; 5μL diluted sample were incubated 10 min at 37°C with 200μL HClO solution. 2μL chromogenic substrate (N, N- diethylparaphenilendiamine) were then added and OD at 550nm was measured twice in a row to quantify HClO excess and averaged. Repeatability was low but similar to previous analyses (mean ± s.e. CVs): the inter-plate CVs were 8% and 13% on 11 duplicates for the d-ROMS and OXY tests respectively and the intra-plate CVs were both 6% on 11 duplicates. We measured OXY and d-ROMs, respectively, in the plasma of 147 and 146 females at incubation, 141 and 135 females at chick-rearing, and 117 and 116 males at chick-rearing.

Statistical Analysis

Our datasets were not balanced for females and males, having samples for females at the incubation stage and chick-rearing stage, and of males at the rearing stage only, we performed two different sets of statistical analyses. Firstly, we used data for females and males at chick-rearing to test for effects of food supplementation and sex on red cells mitochondrial parameters and plasmatic oxidative stress markers. We ran mixed model analyses where food supplementation, sex and their interaction were entered as fixed factors. In the same models, we entered sampling date and age of chicks as covariates to control for possible seasonal effects and effects of chick development on parent reproductive effort. Finally, we entered the nest-box as a random factor to control for the fact that males and females from the same nest-box are not independent observations. Secondly, we used data for females at incubation and chick-rearing to test for effects of reproductive stage on red cells mitochondrial parameters and plasmatic oxidative stress markers. Here, we entered reproductive stage, food supplementation and their interaction as fixed factors, sampling

date and incubation/chick age as covariates to control for timing effects, and female identity as random factor to control for pseudo-replication. Note that fluorimeter measurements of mitochondrial densities, total reactive species and superoxide production have an arbitrary unit (AU). Hence, to facilitate their interpretation and the direct comparison of our different statistical analyses, we normalised our different values (i.e. mitochondrial densities, total reactive species and superoxide production, but also ATP concentration, d-ROM and OXY) by subtracting population mean and dividing it by the standard deviation before using them in our statistical analyses. The different mixed models were performed in *JMP 11.0* using the restricted maximum likelihood (REML) method. As recommended by Forstmeier and Schielzeth (2011), throughout the manuscript we are reporting the full models to avoid type I errors (Forstmeier and Schielzeth, 2011). Model selections based on *P*-values are however leading to similar conclusions.

Age of parents and body condition was also added as covariate in a separate model in both data set.

3. Results

Food supplementation on reproductive success

There was a significantly positive effect of food supplementation on offspring survival at day 13th (ANOVA $F_{1,143}$=28.46, p=0.000; Figure 1A)

Adult males and females at chick-rearing stage

There was a significant effect of sex on body condition, mitochondrial density, ATP concentration, total reactive species and d-ROMS, with females presenting higher values for body condition and d-ROM, but lower values for mitochondrial density, ATP and total reactive species in comparison to the males; no significant effect of sex was found on superoxide or OXY (Table 1; Figure 2). Food supplementation had a marginally significant

effect on body condition, but no significant effect on any mitochondrial attributes, d-ROM or OXY.

No significant interaction between sex and food supplementation was found for any variable, apart from a marginally significant effect for OXY (Table 1). Age of chicks had a positive effect on ATP levels and superoxide production, but a negative effect on mitochondrial density, and no significant effect on body condition or total reactive species (Table 1). Sampling date had a significant effect only on ATP concentration, with late breeders having lower levels of ATP compared to early breeders (Table 1). No significant effects were found for interaction between sex and age of chicks (data not shown).

Adding body condition as a covariate and age of parents as fixed effect did not change most of the results, apart from the analysis on total reactive species, where the effect of sex became non-significant and sampling date became significant; for superoxide age of chicks is no more significant and sampling date became significant (data not shown).

Incubation and chick rearing stages in adult females

Reproductive stage had a significant effect on body condition, NAO fluorescence intensity, ATP concentration, total reactive species, and d-ROMS, but not on superoxide or OXY (Table 2). Body condition and mitochondrial density were higher during incubation, while ATP, total reactive species, d-ROM and OXY were higher during chick rearing (Table 2; Figure 3). Age of chicks (for chick-rearing) and time from incubation (for incubating) females had a significant effect on body condition and mitochondrial density, with lower values during chick rearing; but for ATP level was lower during incubation (Table 2). Sampling date had a significant effect on total reactive species and ATP, with late breeders having lower levels of ATP compared to early breeders, but here also reflect the fact that chick-rearing females has lower ATP level than incubating females.

Again, no effect of food supplementation was found on any mitochondrial attributes, OXY or d-ROM as well as no significant interaction between reproductive stage and food supplementation, apart from OXY (Table 2). Adding body condition as a covariate did not

change the results (data not shown). When age of parents was added to the model the significant effect of age of chick on NAO and ATP disappeared.

Since there was an effect of supplementation on fledges and survival, we did analyses again using only controlling nests. The lack of effect on both fledge and survival persists for OXY and all mitochondrial attributes and body condition (results not shown). Only d-ROM there was a marginally significant negative effect on fledge ($T_{1,62}$ = -1.75, P=0.084, R^2=0.05), and survival ($T_{1,60}$ = -1.82, P=0.073, R^2=0.05).

Relationships between mitochondrial attributes, OXY, d-ROMS and primary reproductive investment

Because the previous analyses revealed significant effect of sex and reproductive stage on most of the mitochondrial attributes (Table 1 and 2), the seeking for relationship between mitochondrial attributes (ATP, mitochondrial density, total reactive species, superoxide), d-ROMS, OXY and the primary reproductive investment traits (laying date, clutch size, brood size at hatching), was performed in the three groups separately (incubating females, chick-rearing females and chick-rearing males (Table 3).

Regarding the comparisons among mitochondrial attributes, the correlation values were significant and positive between ATP concentration and total reactive species for the three groups. Also significant correlation was found between mitochondrial density and Superoxide for the three groups, being positive for incubating females and negative for both chick-rearing males and females (Table 3). Mitochondrial density and total reactive species were not significant for any comparisons. ATP concentration and mitochondrial density had significant negative correlation for males and females at chick rearing, but not for females at incubation.

For the comparisons between mitochondrial attributes and reproductive investment traits, laying date was significant and positively correlated with superoxide for chick-rearing males and incubating females, but not for chick-rearing females. The oxidation measured on the plasma by d-ROMS was significantly correlated with ATP in all cases, and was negative

only for females on incubation. Body condition was significantly correlated with superoxide for males.

4. DISCUSSION

Hence, we used a food supplementation experiment during the chick-rearing phase to address the links between resources availability, body condition, mitochondrial attributes and plasma damage/protection in adult collared flycatchers. We predicted that food supplementation should lead to an increase in reproductive success and adult body condition. However and as said previously, relationships between life history traits and mitochondrial attributes are complex. We had thus no *a priori* predictions on the direction of changes in mitochondrial attributes in response to food supplementation.

Birds have two well-defined reproductive stages, egg incubation and chick rearing, with different contributions of parents at each stage depending on the species. Incubation and chick-rearing stages are both costly, and how resources are allocated within a breeding event is still not clear (Dobbs *et al*. 2006), especially for uniparental incubating birds, where parents do not contribute equally in both reproductive phases. In the collared flycatcher, females are incubating alone the clutch but both parents provide food to the chicks. Hence, we investigated changes in adult female body condition and mitochondrial attributes between reproductive stages and in relation to the food supplementation experiment. Furthermore, because male and female parents do often differ in their optimum levels of reproductive investment at the chick rearing stage (review in Santos and Nakagawa 2012), we tested for differences between male and female parents regarding body condition mitochondrial attributes and plasma damage/protection in interaction with our food supplement experiment.

Finally, given that we still know very little on the relationships between life history traits and mitochondrial attributes, we explored how our different measurements of mitochondrial attributes were correlated among each other's and with reproductive life history traits.

Effect of food supplementation

Although the relevance of food supply on population ecology in mammal species, (Prevedello et al. 2013) as well as on reproductive parameters in birds (Ruffino *et al*. 2014), the extent to which food supplementation affect the different phases of reproduction in birds is not unequivocal. Previous studies showed that receiving high protein on supplementary food during incubation did not affect current reproductive success, in terms of hatching success on captive kept zebra finch (Gorman and Nager 2003). As well as food-supplemented owls during nestling period (Broomer *et al*. 2004) or house wrens during incubation (Lothery 2014) showed no effect on reproductive output. All contrasting with our results, which show a positive effect of food supplementation during chick rearing on fledge and survival. More important, Broomer *et al*. detected a positive effect of food supplementation on body mass during chick-rearing stage and an effect on reproduction timing in the following year. This is related to the fact that chick rearing is synchronized with peak of food availability; hence extra-food supply in a given breeding season will result in early breeding the following year. Although we do not have data for the subsequent season, our lack of effect on body condition, mitochondrial attributes and d-ROM/OXY for the parents suggests that flycatchers may invest all resources available in the current reproduction, *i.e.* in the offspring growth. In the absence of data on chicks, the positive effect on nestling survival suggests that this may well be the case. This conclusion is supported by the results from Santos and Nakagawa (2012), who showed in a meta-analysis of clutch/brood size manipulation that, differently from males, females do not seem to undergo survival cost from increasing parental effort, but rather transfer the cost to the offspring. It is possible, however, that such a strategy may change year to year, according to the habitat conditions. In poor environmental conditions, parents struggled to feed their chicken will relocate all the supplementary energy to the offspring in order to protect the next generation as much as they can.

Effect of gender

Sexual dimorphism in the expression of mitochondria-related genes has already been shown in heart of rats, where in old animals most of the genes involved in the oxidative phosphorylation have significantly higher expression for females than males, and apoptotic genes have lower expression for females (Vijay *et al*. 2015). This matches our results regarding total reactive species – with lower values for females in comparison to males, but was in the opposite direction regarding d-ROMs, mitochondrial density and ATP concentration. In our case, because there was no change in the results after adding age as covariate, we can suggest that reproductive stage may play a main role. Especially due to the fact that in birds, reproduction physiology is mainly controlled by neuroendocrine system (Ubuka and Bentley 2010), being stress hormones also confounded by parental care type (Silverin 1986, Wada 2006, Bókony *et al*. 2009). There is some evidence that parental care has a negative relationship with gender differences in corticosteroid hormones: the more sex care tends to have a lower baseline corticosteroid hormone level (Bókony *et al*. 2009). However, a previous study found no difference of plasma corticosteroid between sexes during chick rearing for pied flycatcher (*Ficedula hypoleuca*, Silverin 1986), in which, as well as his congeneric collared flycatcher, both parents feed the chicks. This hence would prevent us to ascribe differences in mitochondrial attributes to stress hormones variation. However, we can speculate that, as an only-female incubating species, after spending the whole incubation period, females will have metabolic traits slow down when the partner is there to assist feeding the chicks.

Effect of reproductive stage for females

Loss of body mass from incubation to chick-rearing stage has already been reported as an adjustment to reduce wing-loading during chick rearing stage in passerines (Cavitt & Thompson 1997) and in the black-legged kittiwakes (Bech *et al*.2002, Rønning 2008). In our study, a decrease in body condition from incubation to chick-rearing stage is followed by a decrease in mitochondrial density as well as an increase in ATP concentration and total reactive species production. Because reproduction and survival requires a suitable amount of oxygen and ATP, and being the ATP consumption fix per cycle for the most of the

biochemical processes, the ratio ATP/O will dictate the necessary oxygen supply (Brand 2005). Therefore, a degree of plasticity on mitochondrial attributes would be expected to cope with variation in energetic demand during life cycle, such as during different reproductive stages. Reproduction is known to be affected by thyroid hormones (Ubuka and Bentley 2011), which are important for mitochondria energy transfer (Soboll 1993), and therefore considered the major endocrine controller of energy expenditure. This involves changes in ATP synthesis through oxidative phosphorylation in mitochondria (Harper and Seifert 2008), being mitochondria in turn regarded as the subcellular target for the thyroid hormones (Goglia *et al*. 2002). Thriiodothyronine (T3), the metabolically active form of thyroid hormone, has a profound impact on mitochondrial biogenesis (Weitzel *et al* 2003). In birds T3 hormone has high turnover (Chastel *et al*. 2003), is positively associated with metabolic rate (Chastel *et al*. 2003, Rønning *et al*. 2008), and has its level increased on incubating females in comparison with chick-rearing.

Thus, we can suggest that the higher mitochondrial density found during incubation could be due to the high affinity binding site for T3 on mitochondrial inner membrane and the ability of thyroid hormone to stimulate synthesis of cardiolipin (Goglia 2002). The lower total reactive species production during incubation goes in the opposite direction, but with no measurement on damage or anti-oxidant protection we cannot hold a conclusive outcome in this regard.

The uncoupling property of thyroid hormone may also play a role in this case. Indeed, there are evidences that metabolic effects of thyroid hormone include mild uncoupling (Skulachev 1996), thus preventing large increase of proton electrochemical gradient in lack of ADP and reducing ROS production (Venditti and Di Meo 2006). Incubating females with higher mitochondrial density and lower ATP concentration, in comparison to chick rearing, produce lower total reactive species – all at the same magnitude (estimate value Table 2).

It is unfortunate that we were not able to measure mitochondrial respiration at all, neither measure the mitochondrial attributes before reproductive period. However our data on mitochondrial density during both reproductive stages suggest that mitochondria

biogenesis might take place during incubation, and uncoupling could play a role, with an unexpected lower ATP concentration accompanied by a lower total reactive species production.

Acknowledgments

We are grateful to Jukka Forsman and the landowners on Gotland for access to the field site; to Lars Gustafsson for field logistics; to Myriane Chalopin, Arthur Colliot, Uéline Courcoux-Caro, Justine Le Vaillant, Pierre-Lou Marion, Joëlle Taroni, and Murielle Vergniol for their help on the field. This study was financially supported by travel and research grants from the Carnegie Trust, the Royal Society and the British Ecological Society to P.B., from the French Ministry of Research, the Région Rhône-Alpes (CMIRA Explora'Doc), the Rectors' Conference of the Swiss Universities, the Fondation pour l'Université de Lausanne and the University of Aberdeen to C.R. and by a research fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes: 11084-13-8), Brazil to L.C.D.

References

Balaban, R. S., Nemoto, S. and Finkel, T. (2005). Mitochondria, oxidants, and aging. *Cell* 120, 483-495.

Divakaruni, A. S. and Brand, M. D. (2011). The regulation and physiology of mitochondrial proton leak. *Physiology* 26, 192-205.

Doligez, B., Danchin, E., Clobert, J. and Gustafsson, L. (1999). The use of conspecific reproductive success for breeding habitat selection in a non-colonial, hole-nesting species, the collared flycatcher. *J. Anim. Ecol.* 68, 1193-1206.

Dowling, D. K. and Simmons, L. W. (2009). Reactive oxygen species as universal constraints in life-history evolution. *Proceedings of The Royal Society B-Biological Sciences* 276, 1737-1745.

Dykens, J. A., Fleck, B., Ghosh, S., Lewis, M., Velicelebi, G. and Ward, M. W. (2002). High-throughput assessment of mitochondrial membrane potential in situ using fluorescence resonance energy transfer. *Mitochondrion* 1, 461-473.

Fan, F. and Wood, K. V. (2007). Bioluminescent assays for high-throughput screening. *Assay Drug Dev. Technol.* 5, 127-136.

Forstmeier, W. and Schielzeth, H. (2011). Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. *Behav. Ecol. Sociobiol.* 65, 47-55.

Glanville, E. J., Murray, S. A. and Seebacher, F. (2012). Thermal adaptation in endotherms: climate and phylogeny interact to determine population-level responses in a wild rat. *Funct. Ecol.* 26, 390-398.

Golozoubova, V., Cannon, B. and Nedergaard, J. (2006). UCP1 is essential for adaptive

adrenergic nonshivering thermogenesis *American Journal of Physiology, Endocrinology and Metabolism* 291, E350-E357.

Halliwell, B. and Gutteridge, J. (2007). Free radicals in biology and medicine: Oxford: Oxford University Press.

Hill, G. E. (2014). Cellular Respiration: The Nexus of Stress, Condition, and Ornamentation. *Integr. Comp. Biol.* 54, 645-657.

Jacobson, J., Duchen, M. R. and Heales, S. J. R. (2002). Intracellular distribution of the fluorescent dye nonyl acridine orange responds to the mitochondrial membrane potential: implications for assays of cardiolipin and mitochondrial mass. *J. Neurochem.* 82, 224-233.

Melvin, R. G. and Ballard, J. W. O. (2006). Intraspecific variation in survival and mitochondrial oxidative phosphorylation in wild-caught Drosophila simulans. *Aging Cell* 5, 225-233.

Merilä, J. (1997). Expression of genetic variation in body size of the collared flycatcher under different environmental conditions. *Evolution* 51, 526-536.

Metcalfe, N. B. and Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Funct. Ecol.* 24, 984-996.

Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* 191, 144-148.

Monaghan, P. and Nager, R. G. (1997). Why don't birds lay more eggs? *Trends Ecol. Evol.* 12, 270-274.

Pärt, T. and Gustafsson, L. (1989). Breeding dispersal in the collared flycatcher (*Ficedula albicollis*): Possible causes and reproductive consequences. *J. Anim. Ecol.* 58, 305-320.

Passos, J. F., von Zglinicki, T. and Kirkwood, T. B. (2007). Mitochondria and ageing: winning and losing in the numbers game. *Bioessays* 29, 908-917.

Peig, J. and Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* 118, 1883-1891.

Prevedello, J. A., Dickman, C. R., Vieira, M. V. and Vieira, E. M. (2013). Population responses of small mammals to food supply and predators: a global meta-analysis. *J. Anim. Ecol.* 82, 927-936.

Ricklefs, R. E. and Wikelski, M. (2002). The physiology/life-history nexus. *Trends Ecol. Evol.* 17, 462-468.

Robert, K. A. and Bronikowski, A. M. (2010). Evolution of senescence in nature: Physiological evolution in populations of garter snake with divergent life histories. *The American Naturalist* 175, 147-159.

Ruffino, L., Salo, P., Koivisto, E., Banks, P. and Korpimaki, E. (2014). Reproductive responses of birds to experimental food supplementation: a meta-analysis. *Frontiers in Zoology* 11, 80.

Salin, K., Roussel, D., Rey, B. and Voituron, Y. (2012a). David and Goliath: A Mitochondrial Coupling Problem? *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* 317, 283-293.

Salin, K., Luquet, E., Rey, B., Roussel, D. and Voituron, Y. (2012d). Alteration of mitochondrial efficiency affects oxidative balance, development and growth in frog (Rana temporaria) tadpoles. *The Journal of Experimental Biology* 215, 863-869.

Stearns, S. C. (1992). The evolution of life histories: Oxford University Press.

Stier, A., Bize, P., Schull, Q., Zoll, J., Singh, F., Geny, B., Gros, F., Royer, C., Massemin, S. and Criscuolo, F. (2013). Avian erythrocytes have functional mitochondria, opening novel perspectives for birds as animal models in the study of ageing. *Frontiers in Zoology* 10, 33.

Torok, J. and Toth, L. (1988). Density dependence in reproduction of the collared flycatcher *Ficedula albicollis* at high population levels. *J. Anim. Ecol.* 57, 251-258.

Viña, J., Gomez-Cabrera, M. C., Borras, C., Froio, T., Sanchis-Gomar, F., Martinez-Bello, V. E. and Pallardo, F. V. (2009). Mitochondrial biogenesis in exercise and in ageing. *Adv. Drug Del. Rev.* 61, 1369-1374.

White, C. R., Alton, L. A. and Frappell, P. B. (2012). Metabolic cold adaptation in fishes occurs at the level of whole animal, mitochondria and enzyme. *Proceedings of the Royal Society B: Biological Sciences* 279, 1740-1747.

Widlansky, M. E., Wang, J., Shenouda, S. M., Hagen, T. M., Smith, A. R., Kizhakekuttu, T. J., Kluge, M. A., Weihrauch, D., Gutterman, D. D. and Vita, J. A. (2010). Altered mitochondrial membrane potential, mass, and morphology in the mononuclear cells of humans with type 2 diabetes. *Translational Research* 156, 15-25.

Williams, J. B., Miller, R. A., Harper, J. M. and Wiersma, P. (2010). Functional linkages for the pace of life, life-history, and environment in birds. *Integr. Comp. Biol.* 50, 855-868.

Zafarullah, M., Li, W. Q., Sylvester, J. and Ahmad, M. (2003). Molecular mechanisms of N-acetylcysteine actions. *CMLS, Cell. Mol. Life Sci.* 60, 6-20.

Zheng, W.-H., Li, M., Liu, J.-S. and Shao, S.-L. (2008). Seasonal acclimatization of metabolism in Eurasian tree sparrows (Passer montanus). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 151, 519-525.

Table 1 : Females and Males chick-rearing

	Term		Estimate	SE	Dfden	*t*	*P*
Body	Intercept		-0,091008	0,56226	154,1	-0,16	0,8716
Rsq=-0.08	Sex[F]		0,1414573	0,040822	143,9	3,47	0.0007*
	supplementation[no]		-0,071503	0,038182	139,5	-1,87	0,0632
	Sex[F]*supplementation[no]		0,0057668	0,040697	143,8	0,14	0,8875
	Sampling date 1=May		-0,004665	0,012289	147,9	-0,38	0,7048
	Age of chicks/Days from Incub		-0,030627	0,030452	141,8	-1,01	0,3163
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**95%H**	**%**
	Area Box	-0,068165	-0,029775	0,036638	-0,101584	0,0420342	0
	Residual		0,4368055	0,0544518	0,3470297	0,5667596	100
	Term		**Estimate**	**SE**	**Dfden**	***t***	***P***
NAO	Intercept		-0,073227	0,707595	152,5	-0,1	0,9177
	Sex[F]		-0,184637	0,041333	134,7	-4,47	<.0001*
	supplementation[no]		-0,018761	0,048758	139,5	-0,38	0,701
	supplementation[no]*Sex[F]		0,0387166	0,041209	134,8	0,94	0,3491
	Sampling date 1=May		0,018068	0,015543	145,8	1,16	0,247
	Age of chicks		-0,128406	0,038679	141,9	-3,32	0.0011*
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**95%H**	**%**
	Nest box	0,2296133	0,0989484	0,0499993	0,0009514	0,1969453	18,674
	Residual		0,4309347	0,0556882	0,3396644	0,5648725	81,326
	Term		**Estimate**	**SE**	**Dfden**	***t***	***P***
ATP	Intercept		1,6461838	1,112161	142,7	1,48	0,141
	Sex[F]		-0,197366	0,035621	114,5	-5,54	<.0001*
	supplementation[no]		-0,023274	0,076575	132,5	-0,3	0,7617
	Sex[F]*supplementation[no]		-0,031341	0,035573	114,3	-0,88	0,3801
	Sampling date 1=May		-0,060761	0,024508	135	-2,48	0.0144*
	Age of chicks		0,1529942	0,059118	148,3	2,59	0.0106*
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**95%H**	**%**
	Area Box	2,1692673	0,632455	0,1021617	0,4322217	0,8326883	68,447
	Residual		0,2915524	0,0402578	0,2263225	0,3898535	31,553
	Term		**Estimate**	**SE**	**Dfden**	***t***	***P***
	Intercept		-0,154282	1,090821	121,9	-0,14	0,8878
Total ROS	Sex[F]		-0,083023	0,035917	93,3	-2,31	0.0230*
(Residual)	supplementation[no]		0,0168063	0,079229	116,3	0,21	0,8324
	Sex[F]*supplementation[no]		0,0183415	0,035891	93,9	0,51	0,6105
	Sampling date 1=May		-0,011587	0,023693	117,8	-0,49	0,6257
	Age of chicks/Days from Incub		0,0881972	0,064142	118,2	1,38	0,1717
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**95%H**	**%**
	Nest Box	2,5084108	0,6075715	0,1022952	0,4070766	0,8080664	71,497
	Residual		0,2422137	0,0367266	0,1837276	0,3340188	28,503

	Term		Estimate	SE	Dfden	t	P
	Intercept		-2,926484	0,939813	104,3	-3,11	0.0024*
Superoxide	Sex[F]		-0,062698	0,053854	74,2	-1,16	0,2481
(Residual)	supplementation[no]		-0,021074	0,070363	93,56	-0,3	0,7652
	Sex[F]*supplementation[no]		-0,022492	0,053616	75,25	-0,42	0,676
	Sampling date 1=May		0,0355332	0,020627	103,4	1,72	0,0879
	Age of chicks		0,1352026	0,054648	92,07	2,47	0.0152*
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**95%H**	**%**
	Nest box	0,5381662	0,2338684	0,0957388	0,0462237	0,4215131	34,988
	Residual		0,4345654	0,0822718	0,3095723	0,6545078	65,012
	Term		**Estimate**	**SE**	**Dfden**	***t***	***P***
d-ROMs	Intercept		1,2641792	1,017844	162,2	1,24	0,216
Rsq=0.36	Sex[F]		0,2328129	0,05818	136	4	0.0001*
	supplementation[no]		-0,029138	0,067786	142	-0,43	0,668
	Sex[F]*supplementation[no]		0,0078961	0,057986	136,2	0,14	0,8919
	Sampling date 1=May		-0,032703	0,023094	164,1	-1,42	0,1587
	Age of chicks/Days from Incub		0,0504932	0,056968	164,8	0,89	0,3767
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**95%H**	**%**
	Area Box	0,223459	0,1796422	0,0919449	-0,000566	0,3598509	18,265
	Residual		0,8039158	0,1051929	0,6318723	1,0576193	81,735
OXY	**Term**		**Estimate**	**SE**	**Dfden**	***t***	***P***
Rsq = -0.14	Intercept		0,5796684	0,955374	147,9	0,61	0,545
	Sex[F]		-0,074836	0,068549	133,2	-1,09	0,2769
	supplementation[no]		-0,018567	0,063807	128,3	-0,29	0,7715
	Sex[F]*supplementation[no]		-0,128522	0,068253	133,1	-1,88	0,0619
	Sampling date 1=May		-0,005145	0,021713	149,6	-0,24	0,813
	Age of chicks/Days from Incub		-0,032843	0,055196	154,9	-0,6	0,5527
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**95%H**	**%**
	Area Box	-0,072612	-0,086372	0,1095956	-0,301176	0,1284314	0
	Residual		1,1895067	0,1573155	0,9326833	1,5698265	100

Table 2 : Females chick-rearing and incubation

Body condition	**Term**		**Estimate**	**SE**	**Dfden**	***t***	***P***
Rsq=0.94	Intercept		-0,917821	0,544066	175,1	-1,69	0,0934
	State[Feeding]		-0,912564	0,102055	187,4	-8,94	<.0001*
	supplementation[no]		-0,053137	0,050189	145,3	-1,06	0,2915
	Sampling date 1=May		0,0380376	0,017568	197,3	2,17	0.0316*
	Age of chicks/Days from Incub		-0,049112	0,032233	227,3	-1,52	0,129
	State[Feeding]*supplementation[no]		-0,012801	0,019133	129,9	-0,67	0,5046
	age.min		0,0255945	0,040709	146,8	0,63	0,5305
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**95%H**	**%**
	ID ring	3,3918456	0,3187885	0,0441938	0,2321703	0,4054067	77,231
	Residual		0,0939867	0,0118846	0,074438	0,1224365	22,769
	Term		**Estimate**	**SE**	**Dfden**	***t***	***P***
NAO	Intercept		0,404	0,504	171,5	0,80	0,424
	State[Feeding]		-0,665	0,101	237,2	-6,58	<.0001*
	supplementation[no]		-0,017	0,046	157,6	-0,38	0,705
	State[Feeding]*supplementation[no]		0,036	0,046	158,8	0,79	0,430
	Sampling date 1=May		0,016	0,016	191,4	0,98	0,330
	Age of chicks/Days from Incub		-0,116	0,044	296,7	-2,63	0.0091*
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**955%H**	**%**
	ID ring	0,0005631	0,0003536	0,0537023	-0,104901	0,1056082	0,056
	Residual		0,627871	0,0741677	0,5045401	0,8029415	99,944
	Term		**Estimate**	**SE**	**Dfden**	***t***	***P***
ATP	Intercept		1,290	0,594	183,9	2,17	0.0311*
	State[Feeding]		0,537	0,119	242,7	4,52	<.0001*
	supplementation[no]		-0,058	0,052	159,9	-1,11	0,267
	State[Feeding]*supplementation[no]		-0,005	0,055	164,1	-0,09	0,930
	Sampling date 1=May		-0,066	0,019	201,0	-3,49	0.0006*
	Age of chicks/Days from Incub		0,137	0,051	280,6	2,67	0.0081*
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**955%H**	**%**
	ID ring	-0,058655	-0,051095	0,071261	-0,190764	0,0885741	0
	Residual		0,8711106	0,1044844	0,6977806	1,1184974	100
	Term		**Estimate**	**SE**	**Dfden**	***t***	***P***
	Intercept		2,889	0,671	164,2	4,31	<.0001*
Total ROS	State[Feeding]		0,665	0,133	227,9	5,01	<.0001*
(Residual)	supplementation[no]		0,022	0,058	139,8	0,38	0,704
	State[Feeding]*supplementation[no]		0,015	0,061	144,9	0,25	0,806
	Sampling date 1=May		-0,084	0,021	195,1	-3,97	0.0001*
	Age of chicks/Days from Incub		0,040	0,058	255,2	0,69	0,490
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**955%H**	**%**
	ID ring	-0,047525	-0,046933	0,0989309	-0,240834	0,1469676	0
	Residual		0,9875443	0,1335261	0,7703895	1,3119868	100

	Term		Estimate	SE	Dfden	*t*	*P*
Superoxide (Residual)	Intercept		1,225	0,729	145,6	1,68	0,095
	State[Feeding]		0,144	0,145	201,9	0,99	0,322
	supplementation[no]		0,012	0,064	121,0	0,19	0,850
	State[Feeding]*supplementation[no]		-0,042	0,071	134,8	-0,60	0,550
	Sampling date 1=May		-0,030	0,023	183,7	-1,28	0,201
	Age of chicks/Days from Incub		-0,010	0,063	212,1	-0,15	0,879
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**955%H**	**%**
	ID ring	-0,12485	-0,144941	0,1288642	-0,39751	0,1076284	0
	Residual		1,1609222	0,1777818	0,8783517	1,6064944	100
	Term		**Estimate**	**SE**	**Dfden**	***t***	***P***
d-ROMS Rsq=0.22	Intercept		-0,456104	0,674061	156,3	-0,68	0,4996
	State[Feeding]		0,375204	0,136101	211,3	2,76	0.0063*
	supplementation[no]		-0,037929	0,058386	141,5	-0,65	0,517
	Sampling date 1=May		0,0092959	0,023205	179,2	0,4	0,6892
	Age of chicks/Days from Incub		0,0126573	0,064073	267	0,2	0,8436
	State[Feeding]*supplementation[no]		0,0112206	0,057391	142,2	0,2	0,8453
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**95%H**	**%**
	ID ring	0,019772	0,018094	0,0830965	-0,144772	0,1809601	1,939
	Residual		0,9151329	0,1126913	0,7289684	1,1833926	98,061
OXY Rsq=0.21	**Term**		**Estimate**	**SE**	**Dfden**	***t***	***P***
	Intercept		2,1452499	0,665114	156,7	3,23	0.0015*
	State[Feeding]		0,4093281	0,13314	207,7	3,07	0.0024*
	supplementation[no]		-0,020118	0,058699	147,7	-0,34	0,7323
	Sampling date 1=May		-0,071306	0,022678	180,6	-3,14	0.0019*
	Age of chicks/Days from Incub		0,073226	0,062726	278,1	1,17	0,244
	State[Feeding]*supplementation[no]		-0,124549	0,054088	147,8	-2,3	0.0227*
	REML Variance Component Estimates						
	Random Effect	**Var ratio**	**Var comp**	**SE**	**95% L**	**95%H**	**%**
	ID ring	0,0940656	0,0784604	0,0768473	-0,072158	0,2290783	8,598
	Residual		0,834103	0,0991646	0,6693702	1,0684776	91,402

Table 3: Correlations between traits. The p-values of the correlation tests are given in parentheses.

	NAO		ATP		Total ROS		Superox.		ROM	*P*	OXY	*P*	Repr Stage
NAO	xxx		-0.10	(0.22)	-0.02	(0.77)	**0.41**	**(0.00)**	0,01	0,950	-0,05	0,58	Females Incubation
ATP	xxx		xxx		**0.22**	**(0.008)**	0.13	(0.14)	**-0,20**	**0,017**	0,02	0,862	
Total ROS	xxx		xxx		xxx		**0.29**	**(0.001)**	-0,15	0,078	0,02	0,852	
Superoxide	xxx		xxx		xxx		xxx		0,05	0,603	-0,06	0,511	
d-ROMs	xxx		xxx		xxx		xxx		xxx	xxx	-0,05	0,53	
Laying date	-0.03	(0.70)	-0.07	(0.39)	**-0.47**	**(0.000)**	**-0.28**	**(0.001)**	0,12	0,161	-0,30	0,000	
Clutch size	-0.12	(0.14)	-0.03	(0.68)	0.06	(0.48)	0.05	(0.61)	-0,02	0,810	-0,04	0,596	
brood size	-0.00	(0.95)	-0.01	(0.89)	-0.02	(0.77)	0.07	(0.41)	0,03	0,769	-0,03	0,761	
Body Condition	0.04	(0.65)	0.03	(0.72)	-0.01	(0.87)	0.12	(0.18)	0,16	0,060	-0,1	0,252	
NAO	xxx		**-0.45**	**(0.000)**	-0.14	(0.143)	**-0.24**	**(0.017)**	**-0,22**	**0,009**	0.07	0.436	Females chick-rearing
ATP	xxx		xxx		**0.22**	**(0.024)**	**0.36**	**(0.001)**	**0,23**	**0,012**	-0.08	0.368	
Total ROS	xxx		xxx				0.02	(0.83)	0,08	0,384	**-0.05**	0.567	
Superoxide	xxx		xxx		xxx		xxx		-0,09	0.370	-0.07	0,507	
d-ROMs									xxx	xx	-0.03	0.751	
Laying date	0.12	(0.15)	**-0.21**	**(0.016)**	-0.03	(0.75)	-0.03	(0.80)	-0.06	0.496	**-0.18**	**0.037**	
Clutch size	**-0.18**	**(0.027)**	0.10	(0.27)	0.06	(0.53)	0.12	(0.21)	0.04	0.645	0.01	(0.863)	
brood size	-0.15	(0.065)	0.04	(0.63)	0.15	(0.09)	-0.05	(0.65)	0.06	0.526	-0.15	0.095	
Body Condition	0.06	(0.48)	-0.14	(0.11)	-0.09	(0.31)	-0.00	(0.99)	-0,15	0,082	0.13	0.125	
NAO	xxx		**-0.44**	**(0.000)**	-0.06	(0.58)	**-0.26**	**(0.026)**	-0.08	0.396	0.14	0.127	Males chick-rearing
ATP	xxx		xxx		**0.30**	**(0.004)**	0.22	(0.07)	**0.34**	**0.000**	-0.13	0.165	
Total ROS	xxx		xxx		xxx		0.12	(0.32)	0.15	0.168	0.15	0.168	
Superoxide	xxx		xxx		xxx		xxx		0.13	0.289	-0.13	0.289	
d-ROMs									xxx	xxx	0.13	0.178	
Laying date	0.06	(0.54)	**-0.22**	**(0.017)**	-0.09	(0.37)	0.31	(0.08)	-0,15	0,120	0.14	0.139	
Clutch size	-0.03	(0.74)	0.09	(0.36)	-0.06	(0.54)	-0.07	(0.53)	**0,20**	**0,034**	-0.05	0.597	
brood size	-0.05	(0.56)	-0.00	(0.99)	0.04	(0.70)	-0.00	(0.98)	0,08	0,370	0.09	0.31	
Body Condition	-0.02	(0.80)	0.13	(0.16)	0.03	(0.79)	**0.36**	**(0.002)**	0,16	0,079	-0,01	0,88	

Figure 1A

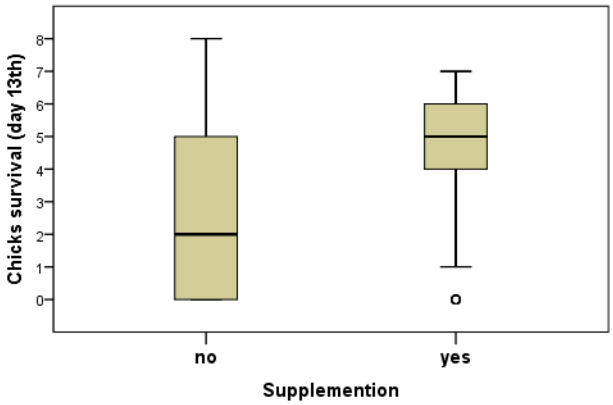


Figure 1B

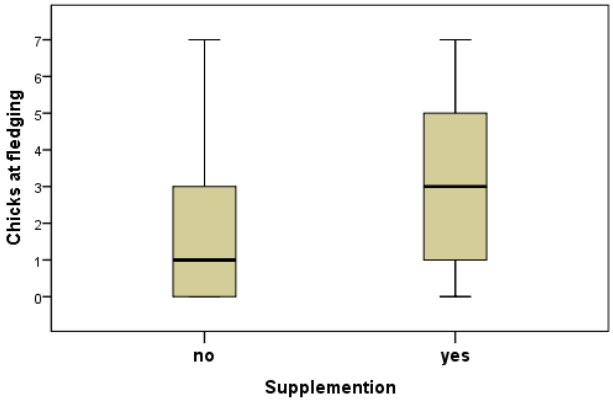


Figure 2

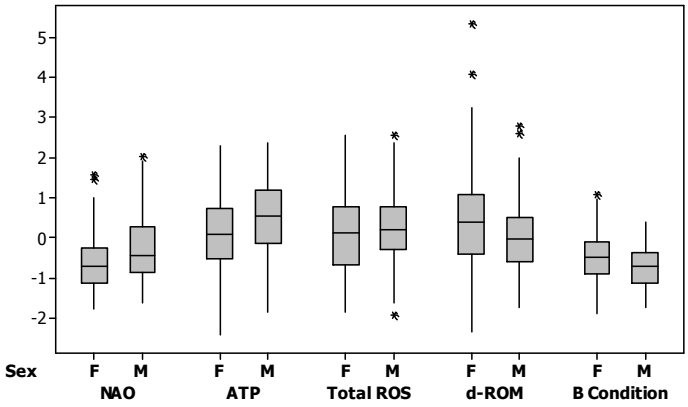
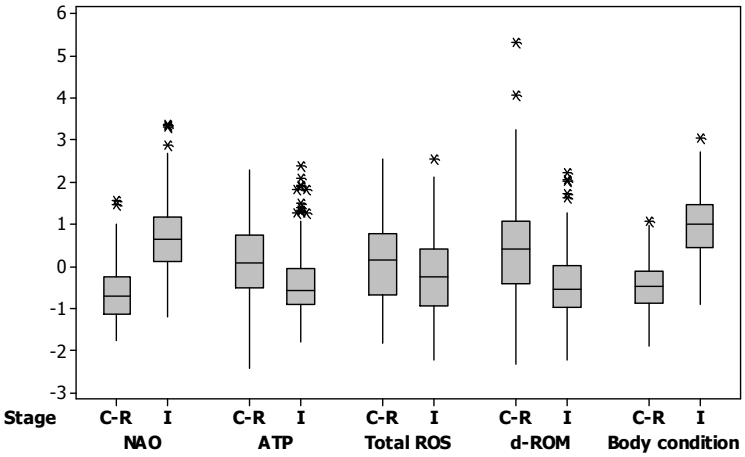


Figure 3



APPENDIX II: PUBLICATIONS AND COMMUNICATION OF PHD RESULTS

Publications

Submitted:

Récapet C., Zahariev A., Blanc S., Arrivé M., Criscuolo F., Bize P. and Doligez B. Differences in the oxidative balance of dispersing and non-dispersing individuals: an experimental approach in a passerine bird. Submitted to *Functional Ecology*. (Part IV)

In preparation:

Récapet C., Taroni J., Daniel G., Bize P. and Doligez B. *in prep.* Food supplementation mitigates differences in nest-defence behaviour between dispersing and non-dispersing individuals in a passerine bird. (Part V)

Récapet C., Daniel G., Bize P. and Doligez B. *in prep.* Food supplementation mitigates dispersal-dependent responses to habitat characteristics in collared flycatchers. (Part V)

Duarte L., **Récapet C.**, Stier A., Doligez B. and Bize P. *in prep.* Variation of mitochondrial attributes is related to breeding stage and sex, but not access to food or age, in free living adult collared flycatchers. (Appendix I)

Récapet C., Sibeaux A., Doligez B., and Bize P. *in prep.* Selective disappearance of individuals with high levels of glycated hemoglobin in the free living collared flycatcher. (adapted from Part VI)

Not presented in the thesis:

Gasparini J., Dauphin L., Favrelière J., Frantz A., Jacquin L., **Récapet C.**, Prévot A.-C. Effects of confinement on body mass and site fidelity of feral pigeons (Columbia livia) during the setting-up of pigeon houses for regulation purpose in Paris. Submitted to *Journal of Wildlife Management and Wildlife Monographs*.

Récapet C., Dauphin L., Jacquin L., Gasparini J. and Prévot-Julliard A.-C. (2013). Eumelanin-based colouration reflects local survival of juvenile feral pigeons in an urban pigeon house. *Journal of Avian Biology* 44(6):583-590.

Jacquin L., **Récapet C.**, Prévot-Julliard A.-C., Leboucher G., Lenouvel P., Erin N., Corbel H., Frantz A. and Gasparini J. (2013). A potential role for parasites in the maintenance of melanin-based coloration polymorphism in urban pigeons. *Oecologia* 173:1089-1099.

Jacquin L., **Récapet C.** , Bouche P. , Leboucher G. and Gasparini J. (2012). Melanin-based coloration reflects alternative strategies to cope with food limitation in pigeons. *Behavioral Ecology* 23(4):907-915.

Oral communications

Récapet C., Zahariev A., Chéry I., Arrivé M., Blanc S., Criscuolo F., Bize P. and Doligez B. (2014). *Habitat quality modulates dispersal-related differences in physiology in the collared flycatcher* Ficedula albicollis. Conference of the International Society for Behavioral Ecology, New York, U.S.A. Aug 2014.

Récapet C., Zahariev A., Chéry I., Blanc S., Criscuolo F., Bize P. and Doligez B. (2013). *Linking dispersal and oxidative status in the collared flycatcher* Ficedula albicollis. Symposium of Animal Physiological Ecology, Lyon, France. Nov 2013.

Récapet C. (2013). *Do dispersing and philopatric individuals exhibit similar responses to changes in their flight costs? An experiment in the collared flycatcher* Ficedula albicollis. 19th European Meeting of PhD Students in Evolutionary Biology, Penryn, United-Kingdom. Sept 2013.

Récapet C. (2013). *Reproductive and metabolic differences between dispersing and philopatric individuals in a bird species.* Biology13 conference, Bâle, Suisse. Feb 2013.

Posters

Récapet C., Doligez B. And Bize P. (2015). *Do telomeres differ according to dispersal? Implications for life-histories in the Collared flycatcher.* Conference of the European Society for Evolutionary Biology, Lausanne, Switzerland Aug 10-14 2015.

Récapet C., Arrivé M., Criscuolo F., Bize P. and Doligez B. (2013 & 2014). *Female Collared flycatchers make the best of a bad job: A trade-off between reproductive output and oxidative state.* EvoLyon, Lyon, France, 21 Nov. 2013, and Meeting of the students from the E2M2 doctoral school, Lyon, France, 19 March 2014.

APPENDIX III: AFFILIATIONS OF CO-AUTHORS

Arrivé, Mathilde
Institut de Biologie Moléculaire des Plantes
UPR 2357 CNRS-Université de Strasbourg, France

Bize, Pierre
School of Biological Sciences
University of Aberdeen, United-Kingdom

Blanc, Stéphane
Institut Pluridisciplinaire Hubert Curien (IPHC)
Départment d'Ecologie, Physiologie, Ethologie (DEPE)
UMR 7178 CNRS-Université de Strasbourg, France

Criscuolo, François
Institut Pluridisciplinaire Hubert Curien (IPHC)
Départment d'Ecologie, Physiologie, Ethologie (DEPE)
UMR 7178 CNRS-Université de Strasbourg, France

Daniel, Grégory
Laboratoire de Biométrie et Biologie Evolutive
UMR 5558 CNRS-Université de Lyon-Université Lyon 1, France

Doligez, Blandine
Laboratoire de Biométrie et Biologie Evolutive
UMR 5558 CNRS-Université de Lyon-Université Lyon 1, France

Duarte, Luiza C.
School of Biological Sciences
University of Aberdeen, United-Kingdom

Stier, Antoine
Institute of Biodiversity, Animal Health & Comparative Medicine
University of Glasgow, United-Kingdom

Taroni, Joëlle
Département d'Ecologie et d'Evolution (DEE)
Université de Lausanne, Switzerland

Zahariev, Alexandre
Institut Pluridisciplinaire Hubert Curien (IPHC)
Départment d'Ecologie, Physiologie, Ethologie (DEPE)
UMR 7178 CNRS-Université de Strasbourg, France