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Effect of age, haemosporidian infection and body condition on pair composition and reproductive success in Great Tits *Parus major*

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Several factors shape lifetime reproductive success, including genetic background, body condition, environmental conditions and ecological interactions such as parasitism. Adults often show higher reproductive success than their young conspecifics, especially in long-lived bird species, and this may be explained by the cumulative effects of an increase in reproductive experience and the selection of high-quality individuals from one year to another. To test whether this pattern also exists in short-lived bird species, we used 13 years of monitoring data from two Great Tit *Parus major* populations. The effects of male and female age on several reproductive parameters were analysed in 419 pairs of Great Tits, whilst accounting for body condition and infection by haemosporidian parasites. Reproductive success was mainly affected by the age-class of males. Pairs containing a sub-adult male fledged one-third fewer chicks than pairs containing an adult male. The difference was not caused by variation in male fertility but could have been caused by better parental care provided by adult birds. In addition to lower reproductive success, first-year males also had reduced access to mating compared to adult males, suggesting an avoidance of sub-adult males by females. Nestling body condition was positively correlated with parental body condition, and the body condition of male and female members of breeding pairs was positively correlated. Finally, the number of fledged chicks was mainly affected by the infection status of males. This results temper our previously published results showing an effect of infection on Great Tit reproduction regardless of their sex. In this previous study, and as in most cases, the status of the partner was not taken into account and we show here that this is essential because it can lead to a biased interpretation of the results.

Keys words: life history traits, sexual selection, *Plasmodium*, *Haemoproteus*, *Leucocytozoon*

Many processes affect the annual reproductive success of birds. Individuals in a population are exposed to the same biotic and abiotic factors, such as weather conditions, food abundance, predation risk or local density. These factors directly affect reproductive success (Skinner *et al.* 1998, Scheuerlein *et al.* 2001, Nielsen & Møller 2006), but their effects depend heavily on the intrinsic quality of male and female parents (Erikstad *et al.* 1997, Sanz *et al.* 2000, Magallanes *et al.* 2018).

Parents with better body condition will be more likely to successfully defend their territory, protect their brood against predators and find resources than parents with a lower body condition (Chastel *et al.* 1995, Dearborn 2001, Milenkaya *et al.* 2015). Ecto- and blood parasite infection may in turn negatively impact parental body condition (Marzal *et al.* 2008, Sánchez *et al.* 2018 but see Tripet & Richner 1997). It may also affect parental behaviour (Christe *et al.* 1996a, Mukhin *et al.* 2016), leading to a lower feeding rate (Knowles *et al.* 2010, Wegmann *et al.* 2015, but see Christe *et al.* 1996b) and ultimately lower reproductive success (Marzal *et al.* 2008, Asghar *et al.* 2015). Although most studies have used correlative data, several experimental studies have confirmed these findings (Merino *et al.* 2000, Christe *et al.* 2001). For example, a medication experiment showed that Blue Tit *Cyanistes caeruleus* infection by haemosporidian parasites reduce parental working capacity to feed nestlings (Merino *et al.* 2000).

The age of both parents may also affect their capacity to deal with fluctuating environmental constraints. Indeed, iteroparous species can increase their breeding experience (Gochfeld & Burger 1984, Pärt 2001) and ultimately enhance their reproductive performance (Forslund & Pärt 1995). For instance, experienced birds may be better at synchronizing their breeding schedules with food availability than first-time breeders (Low *et al.* 2007, Goutte *et al.* 2010, Zhang *et al.* 2015, Harris *et al.* 2016). The quality of a reproductive territory also influences reproduction (Matechou *et al.* 2015) and, in some species, older birds have been shown to occupy higher quality territories (Hill 1988, Hiebert *et al.* 1989, Pärt 2001, Smith & Moore 2005). Higher reproductive success in older birds may also be explained by the fact that surviving to an advanced age is evidence of high intrinsic quality. For instance, adult birds could demonstrate their superior quality simply by showing that they have survived at least one year longer than sub-adult individuals (Kokko & Lindstrom 1996, Brooks & Kemp 2001, but see Beck & Promislow 2007). The age-related increase in breeding performance should be influenced by both the cumulative effects of an increase in skill and by the selection of high-quality birds from one year to another (Forslund & Pärt 1995, Kokko & Lindstrom 1996). However, the effect of age can eventually be negative, especially for fertility (Holmes *et al.* 2003, Møller *et al.* 2009), for example as a result of reduced sperm and egg quality (Richard *et al.* 2005, Beamonte-Barrientos *et al.* 2010).

The Great Tit *Parus major* is a biological model species that has been intensively studied since the mid-20th century (Kluyver 1957, Perrins 1970, Bosse *et al.* 2017, Pigeault *et al.* 2018). Tit breeding is strongly influenced by weather conditions (Van Noordwijk *et al.* 1995) and food availability (Martin 1987, Van Noordwijk *et al.* 1995), but also by the intrinsic quality of males and females (McGregor *et al.* 1981, Sanz *et al.* 2000, Cauchard *et al.* 2017). For instance, a relationship was observed between parental body condition and reproductive success, mainly driven by the quality of the environment: in a poor environment, lower parental body condition is associated with a lower quality of food brought to the chicks (Riddington & Gosler 1995). In some Great Tit populations, one-year-old females and females aged five and over lay later than other females (Dhondt 1989). Laying date also affects reproductive success, with a reduction of clutch size as the breeding season progresses (Perrins 1970, Perrins & McCleery 1985, Wawrzyniak *et al.* 2016). Finally, different studies have shown negative (Allander & Bennett 1995, Ots & Hõrak 1996), positive (Oppliger *et al.* 1997, Pigeault *et al.* 2018) or no association (Ots & Hõrak 1996) between haemosporidian infection and Great Tit reproductive success, depending on the population studied (Ots & Hõrak 1996) and the parasite genus involved in infection (Pigeault *et al.* 2018).

These previous studies of the impact of age, haemosporidian infection and body condition on reproductive success usually did not consider the status of the partner (but see Perrins & McCleery 1985, Riddington & Gosler 1995). Yet age, reproductive status or body condition of both breeding partners may affect overall reproductive success. For example, female Great Tits and Black-legged Kittiwakes *Rissa tridactyla* breeding for the first time but paired with an older male begin to lay their eggs earlier than first time breeder females paired with sub-adult males (Coulson 1966, Harvey *et al.* 1979). In addition, and according to the compensation hypothesis (Gowaty 2008), individuals constrained by ecological factors or social status to reproduce with a second-choice partner may compensate or attempt to compensate for offspring viability deficits through enhanced investment in parental care (Bluhm & Gowaty 2004, Gowaty 2008, but see Haaland *et al.* 2017).

In a previous study (Pigeault *et al.* 2018), we used a long-term data set to test the effect of infection and co-infection on individual reproductive traits of Great Tit. Here we use a subsample of the same long-term data set (with one additional year of data) to focus on the effect of the pair composition according to age, body condition or haemosporidian infection on reproductive parameters. Contrary to our previous study (Pigeault *et al.* 2018), the replication unit is here the breeding pair rather than the individual parents taken separately. This subsample is thus made of pairs for which complete information (*i.e.* age-class, body condition and infection status) on both parents is available. Our chosen measures of reproductive success were laying date (as this is known to influence Great Tit reproductive success (e.g., Perrins 1970, Perrins & McCleery 1985), clutch size, the number of hatched chicks and brood size when chicks were 14-days old, the latter reflecting parental ability to feed their chicks at the point of peak food demand (Gibb 1950), and the number of fledged chicks. At 14-days, we also measured chick body mass and tarsus length in order to derive a measure of nestling body condition.

Based on previous studies of Great Tit pairs, assortative mating by age, body condition or infection status is not predicted to occur (Perrins & McCleery 1985, Isaksson *et al.* 2006, Bischoff *et al.* 2009). Laying date, however, should vary with female age, body condition and infection status (Perrins & McCleery 1985, Dhondt 1989). Sub-adult females, females with poorer body condition and females infected by haemosporidian parasite should lay later than adult and uninfected birds or individuals in better body condition. An effect of age could also be observed (Harvey *et al.* 1979). We predict a lower reproductive success in pairs composed of two sub-adult birds than in pairs with at least one adult parent (Riddington & Gosler 1995). However, we hypothesize a more pronounced effect of male than female age on reproductive success (Perrins & McCleery 1985) because experienced males are predicted to be better at managing the energy expenditure associated with territory defence and attraction of females. Finally, we previously showed that co-infection of a parent by haemosporidian parasites reduced Great Tit survival rate but increased their current reproductive success (Pigeault *et al.* 2018). In this study, we used the infection status of both parents to investigate their combined effects on breeding parameters and reproductive success.

METHODS

Study area and host species

A total of 238 nest-boxes, designed to allow temporary extraction of the nest and contents, were installed in two study sites in the canton of Vaud in western Switzerland: (1) Dorigny, a 17.6 ha forest patch on the campus of the University of Lausanne (46°31’25.607”N 6°34’40.714”E, alt: 380 m) and (2) Monod, a 1180 ha rural forest (46°34’19.953”N 6°23’59.204”E, alt: 660 m). Each year, all nest-boxes were cleaned before the breeding season by removing nesting material. Nest-boxes were regularly inspected from mid-March to June for thirteen consecutive breeding seasons (2005-2017). Laying date, clutch size, number of hatched chicks, number of chicks alive 14 days post-hatching and number of fledged chicks were recorded. To correct for between-year differences in average breeding time, laying date was standardized by using the first year of the study as reference (2005, April 1st = day 1). The differences in laying date between 2005 and each subsequent year (2006-2017) were subtracted from the actual laying date of that particular year (see Allander & Bennett 1995). Because the infestation of nests by ectoparasites as hen fleas *Ceratophyllus gallinae* is associated with a reduction in fledging success (Richner *et al.* 1993), all nests were heat-treated in a microwave oven two days after hatching for two minutes to kill all ectoparasites, in order to avoid this source of variance in reproductive success. During this procedure, we kept the chicks altogether in a bird bag and kept them warm close to our body. We placed the chicks back in their nest when the latter had cooled (usually after 2-3 minutes).

When nestlings were fourteen days old, adult Great Tits were caught in their nest-boxes by using door traps mounted inside the nest-boxes. To avoid nest desertion, we spent at most 90 minutes per nest-box trying to catch both parents; if after that time one of the parents was not caught, we tried again the following day for another 45 minutes before giving up. Fourteen-day-old chicks and their parents were marked with an individually numbered aluminium ring (Swiss Ornithological Institute) and weighed with an electronic balance (0.1 g). The tarsus was measured using digital calipers (0.01 mm). The scaled mass index, which allows for allometry by including a scaling component, was used as a metric of body condition (Peig & Green 2009). It was computed as $W_{\mathrm{ind}} * (\frac{T_{\mathrm{mean}}}{T_{\mathrm{ind}}})^{m}$, where W_{ind} is the individual's body weight, T_{mean} the population's mean tarsus length, T_{ind} the individual's tarsus length and m the slope of the regression between the logarithms of body weights and tarsus lengths in the population (see Peig & Green 2009). The sex and age of both parents were determined using plumage characteristics (Svensson 1984) or ringing records when available. Because an exact age could not be assigned for a large proportion of parents (414 of 848 birds), individuals were allocated as either sub-adults (first-year) or adults (second-year or older). To investigate avian haemosporidian infection in parents, 30-50 µl of their blood was sampled by brachial venipuncture and collected in lithium-heparin lined Microvettes. Blood samples were stored at -20°C in SET buffer until molecular analysis.

Molecular diagnosis of haemosporidian infections

Plasmodium, *Haemoproteus* and *Leucocytozoon* were detected from blood samples using molecular methods. Briefly, DNA extraction from blood was achieved using the DNeasy tissue extraction kit (QIAGEN) according to the manufacturer's protocol. A nested PCR, from the original protocol of Hellgren *et al.* (2004), was performed on all DNA samples. Nested

PCR products were sequenced in both directions as in van Rooyen *et al.* (2013). The sequences were assembled and edited and were then identified by performing a local BLAST search with the MalAvi database (Bensch *et al.* 2009). The birds were assigned to one of the following groups: uninfected, infected with a single genus (*Plasmodium*, *Haemoproteus* or *Leucocytozoon*) of parasite, or co-infected by two parasite genera (*Plasmodium* and *Leucocytozoon* or *Haemoproteus* and *Leucocytozoon*). Double peaks observed on DNA chromatograms were considered to be indicators of mixed infections. Because *Plasmodium* and *Haemoproteus* gene fragments (Cytb) were amplified with the same primer pair, we were not able to differentiate co-infections by *Plasmodium* and *Haemoproteus* lineages (*i.e.* co-infection by two genera) from mixed infections by *Plasmodium* or *Haemoproteus* lineages (*i.e.* infection by a single genus). For this reason, we excluded *Plasmodium*/*Haemoproteus* mixed/co-infections from the analyses (26 of 848 birds).

Statistical analyses

All statistical analyses were carried out using R statistical software (v. 3.3.1; R Core Team, 2014). We used a subset of the data set used by Pigeault *et al.* (2018) for which all information on both the male and the female within a pair were available (the number of pairs per year and per population is given in the **Table S1**). Although the study populations were monitored over the whole study period (2005-2017), two experiments were performed during this time; one in 2006 and 2007 (Christe *et al*. 2012) and the second in 2011 (see Delhaye *et al*. 2016). Only control pairs or pairs not included in these experiments were used in the dataset for these three specific years (Table S1).

As preliminary analyses, we tested whether there was assortative mating in Great tits according to their age-class (sub-adult and adult), haemosporidian infection and body condition. For the categorical variables age-class and haemosporidian infection, the proportion of each age-class pair combination was compared using chi-square tests. Similar analyses were performed to compare the proportion of the infection status of male and female involved in the pairs. The effect of body condition, a continuous variable, was investigated using a mixed modelling procedure with the female body condition as a response variable and male body condition and age-class as fixed factors. Year of capture and individual (ring number) were used as random factors to account for temporal and spatial pseudo-replication.

Laying date and reproductive parameters (clutch size, brood size at hatching, number and body condition of 14-days old chicks and number of chicks fledged) were analysed using mixed modelling procedures with a normal error structure (*lme* procedure, Pinheiro *et al.* 2018). Population (Dorigny, Monod), laying date (when it was not a response variable), male and female body condition, infection status and age-class were fitted as fixed factors. The interactions between male and female age class, male and female body condition, and laying date and population were also fitted in the models. Given the large number of possible combinations of male and female infection status and the large disparity in the frequency of these different combinations, it was not possible to add the interaction between infection status of both parents in the models. Year of capture and individual (ring number) were fitted as random factors to account for temporal and spatial pseudo-replication. Nest-box identity was also used as random factor in order to control for nest-box location. Nest location may impact the reproductive success and the health status (*e.g.* susceptibility to infections, food availability) of wild birds (Li & Martin 1991, Riddington & Gosler 1995, Wood *et al.* 2007).

In order to test the independence of predictor variables included in each model, pairwise correlations were tested. In all cases, the absolute value of correlation coefficients was lower than 0.1, suggesting that there were no co-linearity problems (Dormann *et al.* 2013). Maximal models were simplified by sequentially eliminating non-significant interactions and terms to establish a minimal adequate model (model containing only the significant terms, Crawley 2012). The significance of the explanatory variables was established using a likelihood ratio test (LRT, Bolker 2008) and using $P = 0.05$ as a cut-off. The significant chi-square given in the text was for the minimal models, whereas non-significant values correspond to those obtained before the deletion of the interaction or the deletion of the explanatory variable from the model. When an explanatory variable had a significant effect on a response variable and this explanatory variable had more than two levels (e.g., infection status) a posteriori contrasts were carried out by aggregating factor levels and by testing the goodness of fit of the simplified model (with the aggregating factor levels) using LRT. Maximal models are showed in Table S2 and the Minimal models in Table 1.

RESULTS

Pair composition according to age, infection status and body condition

We collected data on 419 pairs: 275 from Dorigny and 144 from Monod. The composition of pairs by age-class and infection status was similar between the two populations (age-classes: $\chi^2_1 = 0.570$, $P = 0.643$; infection status: $\chi^2_1 = 0.948$, $P = 0.414$). Approximately half of the pairs were made up of two adults (49%), but sub-adult females were more often associated with adult males (25%) than with sub-adult males (17%, $\chi^2_1 = 12.375$, $P = 0.0004$), and only 9% of adult females were paired with sub-adult males (Figure 1). More sub-adult females reproduced than sub-adult males (42% of sub-adult females and 26% of sub-adult males). Haemosporidian single- and co-infection prevalence was very high in both populations (Dorigny: single-infection = 31%, co-infection = 65%, Monod: single-infection = 28%, co-infection = 70%). In almost half of cases (48%), pairs contained two co-infected parents (Figure S1); 35% of pairs contained one single- and one co-infected parent; and in 11% of the cases, both parents were single-infected. An uninfected parent was involved in 4% of pairs, with only two pairs composed of two uninfected birds. The proportion of each infection status did not differ between males and females ($\chi^2_{20} = 24$, $P = 0.2424$). Assortative mating according to body condition was observed; male and female body condition within pairs was positively correlated ($\chi^2_1 = 24.949$, $P < 0.0001$, minimal model named model 1 in Table 1, Figure S2), independently of male age-class ($\chi^2_1 = 0.001$, $P = 0.927$, Table S2).

Association of parental age, infection and body condition with laying date

The laying date of pairs was significantly correlated with female body condition (model 2: $\chi^2_1 = 5.104$ $P = 0.024$, Table 1). Females with the highest body condition laid eggs earlier than females with the lowest body condition (Figure S3 A). Laying date was not associated with male body condition (Figure S3 B), infection status or age-classes of either sex (model 2, Table S2).

Association of parental age, infection and body condition with clutch and brood size

Both clutch size and brood size at hatching correlated inversely with laying date (Figure S4) and differed between populations (clutch size: model 3: laying date $\chi^2_1 = 44.624$ $P < 0.0001$, population $\chi^2_1 = 52.100$ $P < 0.0001$, Brood size at hatching: model 4: laying date $\chi^2_1 = 53.778$

P < 0.0001, population χ^2_1 = 41.515 *P* < 0.0001, Table 1). Pairs from Monod laid more eggs and had more hatched chicks than pairs from Dorigny (mean ± s.e., clutch size: Monod = 9.05 ± 0.14, Dorigny = 7.90 ± 0.10, number of hatched chicks: Monod = 8.11 ± 0.15, Dorigny = 6.89 ± 0.12). Body condition, infection status and age-classes of both parents were not associated with clutch size and brood size at hatching (model 3 & 4, Table S2).

Association of parental age, infection and body condition with 14-day-old chick number and quality

The number of 14-day-old chicks varied inversely with laying date in Dorigny (Figure S5 A) but not in Monod (Figure S5 B, model 5: χ^2_1 = 12.849 p = 0.0003, Table 1). Sub-adult males had fewer 14-day-old chicks than adult males (mean ± se: sub-adult = 6.02 ± 0.25, adult = 6.80 ± 0.14, model 5: χ^2_1 = 10.338 *P* = 0.001, Table 1, Figure 2 A). Male infection status was also associated with the number of 14-day-old chicks (model 5: χ^2_1 = 14.517 *P* = 0.013, Table 1). Infected and co-infected males had a higher number of 14-day-old chicks than uninfected birds (Figure S6). No effect of the female age-class, infection status or body condition of either parent was observed (model 5, Table 2, Figure 2 B).

The average body condition of 14-day-old chicks within a brood was positively correlated to the body conditions of both females and males (model 6: χ^2_1 = 4.42 *P* = 0.035, χ^2_1 = 6.258 *P* = 0.012, respectively, Figure 3). No effect of the other studied parameters was observed (Table S2).

Association of parental age, infection and body condition with reproductive success

The number of fledged chicks varied inversely with laying date in Dorigny (Figure S7 A), but not in Monod (model 7: χ^2_1 = 7.863 *P* = 0.005, **Table 1**, Figure S7 B). In addition, the number of fledged chicks was higher in Monod (5.86 ± 0.26) than in Dorigny (4.69 ± 0.19). Pairs containing an adult male fledged more chicks than pairs containing a sub-adult male (3.75 ± 0.31, 5.59 ± 0.17, respectively, model 7: $\chi^2_1 = 33.412$ $P < 0.0001$, Table 1, Figure 2 C). Male infection status was also associated with the number of fledged chicks (model 7: $\chi^2_1 = 12.970$ $P = 0.025$, Table 1). Males co-infected by *Haemoproteus* and *Leucocytozoon* had a higher number of fledged chicks than males infected by either or both of *Plasmodium* and *Leucocytozoon* (Figure 4). The number of fledged chicks was not related to the female age class or infection status (model 7: $\chi^2_1 = 0.002$ $P = 0.964$, $\chi^2_1 = 7.422$ $P = 0.191$, respectively, Table S2, Figure 2 D).

DISCUSSION

In this study, we collected reproductive data on 419 pairs of Great Tits to evaluate the effects of pair composition and the relative importance of maternal and paternal status (age, body condition and haemosporidian infection) as correlates of laying date and reproductive parameters. The infection status of males was positively associated with the reproductive success of pairs, irrespective of the infection status of the female. In addition, while female age-class was not associated with any reproductive parameters, sub-adult and adult males differed in their access to reproduction and in their ability to fledge chicks. First-year males had lower reproductive success than older males.

Association of male age with pair composition and reproductive success

In the two studied populations, there were fewer sub-adult breeding males than females. Indeed, both adult and sub-adult females were predominantly paired with adult males. A long-term study carried out on the breeding of the Oxfordshire (UK) Great Tit population also showed a lower proportion of sub-adult males than sub-adult females, but contrary to our study, no evidence for assortative pair formation was observed (Perrins & McCleery 1985). Great Tit populations typically have a balanced sex ratio at fledging (Lessells *et al.* 1996, Kabasakal & Albayrak 2012), which suggests that sub-adult males have either (i) a lower survival rate than sub-adult females or (ii) reduced access to reproduction when compared to older males, as shown in other bird species (Loffredo & Borgia 1986, Enstrom 1993, Lozano *et al.* 1996, Freeman-Gallant & Taff 2017). No sex-biased survival rate was observed in the two Great Tit populations used in this study (Pigeault *et al.* 2018), which suggests that the latter explanation is more likely. Limited access to reproduction for young males might be caused by a female preference for adult males. Sexual selection in Great Tits is based on plumage and song characteristics (Richner 2016), and these traits vary with age (Evans *et al.* 2010, Rivera-Gutierrez *et al.* 2010 but see Isaksson *et al.* 2008). Females may prefer traits values that are found in adult males. Indeed, an important result of our study is that pairs including a sub-adult male fledged one-third fewer chicks than pairs with an adult male. This difference was already apparent with the number of 14-day-old nestlings. However, this was not due to a variation in clutch or brood size between pairs composed by adults or sub-adults males. The higher survival rate observed in chicks reared by adult males might be explained by better direct benefits, such as better territories (Krebs 1971, Pärt 2001, but see Low *et al.* 2007), but also by better parental care (Shealer & Burger 1995, Limmer & Becker 2009) provided by adult males due to their experience. The cumulative effects of an increase in experience (Krebs 1971, Shealer & Burger 1995, Pärt 2001, Limmer & Becker 2009) and the selection of high-quality males from one year to another (Kokko & Lindstrom 1996) may explain the increase in reproductive success between sub-adult and adult males.

Only the age-class of males was associated with the reproductive success of a pair. This might be due to the allocation of resources in mate attraction and territory defence (Ydenberg & Krebs 1987, Thomas 2002) that can improve with age (Forslund & Pärt 1995). Breeding experience could exacerbate the differences between sub-adult and adult males with adult males better at dealing with these energy expenditures than sub-adult males.

Effect of parental body condition and differences between study populations

Another important result of our study was the positive relationship observed between the body condition of the two parents. Males with better body condition, independently of their age-class, tend to have a better access to the higher quality females. This assortative mating may have strong impact on the reproductive success of pairs. Indeed, we observed a positive correlation between parental and nestling body condition at day 14 post-hatching. Previous studies of Great Tits have shown that fledglings with higher body condition have a higher survival rate (Norris 1990, Naef-Daenzer *et al.* 2001). The body condition of females was also associated with laying date. Better-condition females laid eggs earlier. This relationship has a significant effect on the reproductive success of pairs since we have shown that, as in many bird species, earlier laying dates result in higher reproductive success (Desrochers & Magrath 1993, Oppliger *et al.* 1994, Christians *et al.* 2001, Harriman *et al.* 2017). This result may be explained by a better overlap between chick energy requirements and food availability early in the season (Van Noordwijk *et al.* 1995). However, this relationship was observed only in the Dorigny population. The main difference between our two populations is habitat: Dorigny is a peri-urban forest patch on the campus of the University of Lausanne while Monod is a large forest massif. Environmental quality has a significant effect on bird reproduction (Riddington & Gosler 1995) and, as expected, we observed a higher overall reproductive success in the rural population. This difference may be partly explained by both the quality and the quantity of resources present at each site. Abundance and quality of food change drastically according to environment. For instance, urban environment is usually associated with a lack of protein-rich diet (Schoech & Bowman 2003, Seress *et al.* 2018), carotenoids (Giraudeau *et al.* 2015) and calcium (Heiss *et al.* 2009), which may impact chick nutrition (Riddington & Gosler 1995, Toledo *et al.* 2016) and ultimately negatively affect their development (Toledo *et al.* 2016, Biard *et al.* 2017). Such differences may explain the different relationships between laying date and reproductive success observed in our rural (Monod) and urban (Dorigny) populations. However, only population replicates would confirm this result.

Parental haemosporidian infection and pair reproductive success

Haemosporidian infection may have a negative (Merino *et al.* 2000, Knowles *et al.* 2010, Asghar *et al.* 2015), positive (Richner *et al.* 1995, Oppliger *et al.* 1997, Norte *et al.* 2009, Christe *et al.* 2012, Pigeault *et al.* 2018) or no effect (Bensch *et al.* 2007, Asghar *et al.* 2011, de Jong *et al.* 2014) on reproductive success, depending on host and parasite genotypes. In the two populations of Great Tits used in this study, the prevalence of haemosporidian infection and co-infection was very high and similar among sexes and age-classes (Pigeault *et al.* 2018). It is therefore difficult to estimate the advantage of being uninfected in terms of reproductive success without an experimental approach. Nevertheless, in a recent study with a sample size of approximately 1200 Great Tits, we found that birds co-infected had a higher reproductive success than single-infected individuals (Pigeault *et al.* 2018). In this previous study, no interaction between sex and infection was observed, suggesting a similar effect of haemosporidian infection on the reproductive success of birds of both sexes. However, the results obtained here temper our previously published results. Using the pairs and not the individuals as a replication unit in the models leads to more contrasting observations. Only the infection status of males impacted significantly the number of chicks alive 14 days after hatching and ultimately the number of chicks fledged. The effect of the infection on Great tit reproduction therefore seems to be mainly driven by male parameters. In our populations, most infected females were paired with an infected male. Therefore, analysing the effect of a parent's status without considering the effect of its partner can lead to biased interpretation of the results.

In addition, experimental tests are needed to assess the causal links between the modelled variables and their interactions. As we observed a higher reproductive success in older birds, and considering the fact that the probability of being infected increases with age (in our populations, the annual probability for an uninfected bird to become infected or for a single-infected bird to become co-infected is 37.9% +- 17.8 and 52.9% +- 5.6 respectively, Pigeault *et al.* 2018), the roles of each parameter and the directionality of their effect are unclear. While the positive association between infection and reproductive success might reflect an increased investment in reproduction of birds whose survival prospects are challenged by infection (Agnew *et al*. 2000), it might also be the result of the immunological cost of a higher allocation of resources into current reproduction (for a review see Knowles *et al*. 2009). This result could also be a derived consequence of a differential mortality in infected and uninfected birds related to their quality (Sánchez *et al.* 2018, Jiménez-Peñuela *et al.* 2019). Low-quality individuals would experience a high mortality rate caused by infection while the high-quality individuals are able to both achieve high reproductive success and tolerate infection (Sánchez *et al.* 2018).

Other factors potentially influencing pair reproductive success

Other factors may influence the reproductive success of Great Tits in our study populations. For instance, we eliminated the ectoparasites a few days after hatching in order to reduce the variance caused by their effect on reproductive success, as demonstrated by Richner *et al.* (1993). Indeed, these authors experimentally showed that hen flea infestation caused an increase in nestling mortality and a reduction of fledging number and success. Although we did not directly observe fleas, lice or blowflies in the nests at the time of fledging, infestation may occur after the cleaning of the nests and we cannot exclude that some ectoparasites still affected reproductive success. In addition, we observed some ticks around the eyes of adult birds, which were not quantified but could also affect reproductive success. Another limitation of this study is that we did not consider a potential second brood (Smith *et al.* 1987). Even if only a very small proportion of pairs initiated a second brood in our study populations, this may influence our results. Finally, to reach a better understanding of the effect of haemosporidian infection on reproductive success, haemosporidian infection intensity using a qPCR protocol should be performed to investigate the potential association between infection intensity and reproductive success.

Conclusion

In conclusion, we show that the reproductive success of Great Tit pairs was mainly associated with the age-class of males; the number of fledged chicks increased markedly between pairs containing a sub-adult male and pairs with an adult male. We highlighted that this difference was not caused by differences in male fertility. A cross-fostering experiment could help to disentangle the relative influence of genetic quality (adult males having proven their quality by surviving) and experience (adult males having learnt how to take care of a brood) on pair reproductive success. The reproductive success of pairs was also higher when the males were co-infected by *Leucocytozoon* and *Haemoproteus*. In our Great Tit populations, these two parasite genera were composed of several parasite lineages (*Leucocytozoon* = 24 lineages, *Haemoproteus* = 7 lineages) and future work should focus on the effect of each haemosporidian lineage but also on the effect of the most prevalent combinations on reproductive success.

References

Allander, K. & Bennett, G.F. 1995. Retardation of breeding onset in great tits (*Parus major*) by blood parasites. *Funct. Ecol.* **9**: 677–682.

Asghar, M., Hasselquist, D. & Bensch, S. 2011. Are chronic avian haemosporidian infections costly in wild birds? *J. Avian Biol.* **42**: 530–537.

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.

Beamonte-Barrientos, R., Velando, A., Drummond, H. & Torres, R. 2010. Senescence of maternal effects: Aging influences egg quality and rearing capacities of a long-lived bird. *Am. Nat.* **175**: 469–480.

Beck, C.W. & Promislow, D.E.L. 2007. Evolution of female preference for younger males. *PLoS One* **2**: e939.

Bensch, S., Hellgren, O. & Pérez-Tris, J. 2009. MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. *Mol. Ecol. Resour.* **9**: 1353–1358.

Bensch, S., Waldenström, J., Jonzén, N., Westerdahl, H., Hansson, B., Sejberg, D. & Hasselquist, D. 2007. Temporal dynamics and diversity of avian malaria parasites in a single host species. *J. Anim. Ecol.* **76**: 112–122.

Biard, C., Brischoux, F., Meillère, A., Michaud, B., Nivière, M., Ruault, S., Vaugoyeau, M. & Angelier, F. 2017. Growing in cities: An urban penalty for wild Birds? A study of phenotypic differences between urban and rural great tit chicks (*Parus major*). *Front. Ecol. Evol.* **5**.

Bischoff, L.L., Tschirren, B. & Richner, H. 2009. Long-term effects of early parasite exposure on song duration and singing strategy in great tits. *Behav. Ecol.* **20**: 265–270.

Bluhm, C.K. & Gowaty, P.A. 2004. Reproductive compensation for offspring viability deficits by female mallards, *Anas platyrhynchos*. *Anim. Behav.* **68**: 985–992.

Bolker, B.M. 2008. *Ecological Models and Data in R*. Princeton University Press.

Bosse, M., Spurgin, L.G., Laine, V.N., Cole, E.F., Firth, J.A., Gienapp, P., Gosler, A.G., McMahon, K., Poissant, J., Verhagen, I., Groenen, M.A.M., Oers, K. van, Sheldon, B.C., Visser, M.E. & Slate, J. 2017. Recent natural selection causes adaptive evolution of an avian polygenic trait. *Science* **358**: 365–368.

Bouwhuis, S., Choquet, R., Sheldon, B.C. & Verhulst, S. 2012. The forms and fitness cost of senescence: age-specific recapture, survival, reproduction, and reproductive value in a wild bird population. *Am. Nat.* **179**: E15-27.

Bouwhuis, S., Sheldon, B.C., Verhulst, S. & Charmantier, A. 2009. Great tits growing old: selective disappearance and the partitioning of senescence to stages within the breeding cycle. *Proc. R. Soc. Lond. B Biol. Sci.* **276**: 2769–2777.

Brooks, R. & Kemp, D.J. 2001. Can older males deliver the good genes? *Trends Ecol. Evol.* **16**: 308–313.

Cauchard, L., Angers, B., Boogert, N.J., Lenarth, M., Bize, P. & Doligez, B. 2017. An experimental test of a causal link between problem-solving performance and reproductive success in wild great tits. *Front. Ecol. Evol.* **5**.

Chastel, O., Weimerskirch, H. & Jouventin, P. 1995. Body condition and seabird reproductive performance: A study of three petrel species. *Ecology* **76**: 2240–2246.

Christe, P., Richner, H. & Oppliger, A. 1996b**.** Of great tits and fleas: sleep baby sleep *Anim. Behav.*, **52**: 1087-1092.

Christe, P., de Lope, F., Gonzalez, G., Saino, N., Møller, A. P. 2001**.** The influence of environmental conditions on immune responses, morphology and recapture probability of nestling house martins (*Delichon urbica*). *Oecologia* **126:** 333-338.

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. *Proc R Soc B* **279**: 1142–1149.

Christe, P., Richner, H. & Oppliger, A. 1996b. Begging, food provisioning, and nestling competition in great tit broods infested with ectoparasites. *Behav. Ecol.* **7**: 127–131.

Christians, J.K., Evanson, M. & Aiken, J.J. 2001. Seasonal decline in clutch size in European starlings: A novel randomization test to distinguish between the timing and quality hypotheses. *J. Anim. Ecol.* **70**: 1080–1087.

Coulson, J.C. 1966. The influence of the pair-bond and age on the breeding biology of the kittiwake gull *Rissa tridactyla*. *J. Anim. Ecol.* **35**: 269–279.

Crawley, M.J. 2012. *The R Book*. John Wiley & Sons.

de Jong, M.E., Fokkema, R.W., Ubels, R., van der Velde, M. & Tinbergen, J.M. 2014. No evidence for long-term effects of reproductive effort on parasite prevalence in great tits *Parus major*. *J. Avian Biol.* **45**: 179–186.

Daunt, F., Wanless, S., Harris, M.P., Money, L. & Monaghan, P. 2007. Older and wiser: improvements in breeding success are linked to better foraging performance in European shags. *Funct. Ecol.* **21**: 561–567.

Dearborn, D.C. 2001. Body condition and retaliation in the parental effort decisions of incubating great frigatebirds (*Fregata minor*). *Behav. Ecol.* **12**: 200–206.

Delhaye, J., Jenkins, T. & Christe, P. 2016. *Plasmodium* infection and oxidative status in breeding great tits, *Parus major*. *Malar. J.* **15**: 531.

Desrochers, A. & Magrath, R.D. 1993. Age-specific fecundity in european blackbirds (*Turdus merula*): Individual and population trends. *The Auk* **110**: 255–263.

Dhondt, A.A. 1989. The effect of old age on the reproduction of great tits *Parus major* and blue tits *P. caeruleus*. *Ibis* **131**: 268–280.

Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J.R.G., Gruber, B., Lafourcade, B., Leitão, P.J., Münkemüller, T., McClean, C., Osborne, P.E., Reineking, B., Schröder, B., Skidmore, A.K., Zurell, D. & Lautenbach, S. 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography* **36**: 27–46.

Enstrom, D.A. 1993. Female choice for age-specific plumage in the orchard oriole: implications for delayed plumage maturation. *Anim. Behav.* **45**: 435–442.

Erikstad, K.E., Asheim, M., Fauchald, P., Dahlhaug, L., Tveraa, T. & Dahlhaug, P. 1997. Adjustment of parental effort in the puffin; the roles of adult body condition and chick size. *Behav. Ecol. Sociobiol.* **40**: 95–100.

Evans, S.R., Hinks, A.E., Wilkin, T.A. & Sheldon, B.C. 2010. Age, sex and beauty: methodological dependence of age- and sex-dichromatism in the great tit *Parus major. Biol. J. Linn. Soc.* **101**: 777–796.

Forslund, P. & Pärt, T. 1995. Age and reproduction in birds — hypotheses and tests. *Trends Ecol. Evol.* **10**: 374–378.

Freeman-Gallant, C.R. & Taff, C.C. 2017. Age-specific patterns of infection with haemosporidians and trypanosomes in a warbler: implications for sexual selection. *Oecologia* **184**: 813–823.

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. *Behav. Ecol. Sociobiol.* **69**: 957–970.

Gochfeld, M. & Burger, J. 1984. Age differences in foraging behavior of the american robin (*Turdus migratorius*). *Behaviour* **88**: 227–239.

Goutte, A., Antoine, É., Weimerskirch, H. & Chastel, O. 2010. Age and the timing of breeding in a long-lived bird: A role for stress hormones? *Funct. Ecol.* **24**: 1007–1016.

Gowaty, P.A. 2008. Reproductive compensation. *J. Evol. Biol.* **21**: 1189–1200.

Haaland, T.R., Wright, J., Kuijper, B. & Ratikainen, I.I. 2017. Differential allocation revisited: when should mate quality affect parental investment? *Am. Nat.* **190**: 534–546.

Harriman, V.B., Dawson, R.D., Bortolotti, L.E. & Clark, R.G. 2017. Seasonal patterns in reproductive success of temperate-breeding birds: Experimental tests of the date and quality hypotheses. *Ecol. Evol.* **7**: 2122–2132.

Harris, M.P., Albon, S.D. & Wanless, S. 2016. Age-related effects on breeding phenology and success of Common Guillemots *Uria aalge* at a north sea colony. *Bird Study* **63**: 311–318.

Harvey, P.H., Greenwood, P.J., Perrins, C.M. & Martin, A.R. 1979. Breeding success of great tits *Parus major* in relation to age of male and female Parent. *Ibis* **121**: 216–219.

Heiss, R.S., Clark, A.B. & McGowan, K.J. 2009. Growth and nutritional state of american crow nestlings vary between urban and rural habitats. *Ecol. Appl.* **19**: 829–839.

Hellgren, O., Waldenström, J. & Bensch, S. 2004. A new pcr assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. *J. Parasitol.* **90**: 797–802.

Hiebert, S.M., Stoddard, P.K. & Arcese, P. 1989. Repertoire size, territory acquisition and reproductive success in the song sparrow. *Anim. Behav.* **37**: 266–273.

Hill, G.E. 1988. Age, plumage brightness, territory quality, and reproductive success in the black-headed grosbeak. *The Condor* **90**: 379–388.

Holmes, D.J., Thomson, S.L., Wu, J. & Ottinger, M.A. 2003. Reproductive aging in female birds. *Exp. Gerontol.* **38**: 751–756.

Isaksson, C., Ornborg, J., Prager, M. & Andersson, S. 2008. Sex and age differences in reflectance and biochemistry of carotenoid-based colour variation in the great tit *Parus major*. *Biol. J. Linn. Soc.* **95**: 758–765.

Isaksson, C., Uller, T. & Andersson, S. 2006. Parental effects on carotenoid-based plumage coloration in nestling great tits, Parus major. *Behav. Ecol. Sociobiol.* **60**: 556–562.

Jiménez-Peñuela, J., Ferraguti, M., Martínez-de la Puente, J., Soriguer, R., Figuerola, J. 2019. Urbanization and blood parasite infections affect the body condition of wild birds. *Sci Total Environ*. **15**:3015-22.

Kabasakal, B. & Albayrak, T. 2012. Offspring sex ratios and breeding success of a population of the great tit, *Parus major*. *Zool. Middle East* **57**: 27–34.

Kluyver, H.N. 1957. Roosting habits, sexual dominance and survival in the great tit. *Cold Spring Harb. Symp. Quant. Biol.* **22**: 281–285.

Knowles, S.C.L., Nakagawa, S. & Sheldon, B.C. 2009. Elevated reproductive effort increases blood parasitaemia and decreases immune function in birds: a meta-regression approach. *Funct. Ecol.* **23**: 405-415.

Knowles, S.C.L., Palinauskas, V. & Sheldon, B.C. 2010. Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *J. Evol. Biol.* **23**: 557–569.

Kokko, H. & Lindstrom, J. 1996. Evolution of female preference for old mates. *Proc. Biol. Sci.* **263**: 1533–1538.

Krebs, J.R. 1971. Territory and breeding density in the great tit, *Parus Major. Ecology* **52**: 3–22.

Lessells, C.M., Mateman, A.C. & Visser, J. 1996. Great tit hatchling sex ratios. *J. Avian Biol.* **27**: 135–142.

Li, P. & Martin, T.E. 1991. Nest-site selection and nesting success of cavity-nesting birds in high elevation forest drainages. *The Auk* **108**: 405–418.

Limmer, B. & Becker, P.H. 2009. Improvement in chick provisioning with parental experience in a seabird. *Anim. Behav.* **77**: 1095–1101.

Loffredo, C.A. & Borgia, G. 1986. Male courtship vocalizations as cues for mate choice in the satin bowerbird (*Ptilonorhynchus violaceus*). *The Auk* **103**: 189–195.

Low, M., Pärt, T. & Forslund, P. 2007. Age-specific variation in reproduction is largely explained by the timing of territory establishment in the New Zealand stitchbird *Notiomystis cincta*. *J. Anim. Ecol.* **76**: 459–470.

Lozano, G.A., Perreault, S. & Lemon, R.E. 1996. Age, arrival date and reproductive success of male american redstarts setophaga ruticilla. *J. Avian Biol.* **27**: 164–170.

Magallanes, S., López-Calderón, C., Balbontín, J., Møller, A.P., de Lope, F. & Marzal, A. 2018. Reproductive success related to uropygial gland volume varies with abundance of conspecifics in barn swallows *Hirundo rustica*. Behav. Ecol. Sociobiol. **72**: 183.

Martin, T.E. 1987. Food as a limit on breeding birds: A life-history perspective. *Annu. Rev. Ecol. Syst.* **18**: 453–487.

Marzal, A., Bensch, S., Reviriego, M., Balbontin, J. & De Lope, F. 2008. Effects of malaria double infection in birds: one plus one is not two. *J. Evol. Biol.* **21**: 979–987.

Matechou, E., Cheng, S.C., Kidd, L.R. & Garroway, C.J. 2015. Reproductive consequences of the timing of seasonal movements in a nonmigratory wild bird population. *Ecology* **96**: 1641–1649.

McGregor, P.K., Krebs, J.R. & Perrins, C.M. 1981. Song repertoires and lifetime reproductive success in the great tit (*Parus major*). *Am. Nat.* **118**: 149–159.

Merino, S., Moreno, J., Sanz, J.J. & Arriero, E. 2000. Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). *Proc. R. Soc. Lond. B Biol. Sci.* **267**: 2507–2510.

Milenkaya, O., Catlin, D.H., Legge, S. & Walters, J.R. 2015. Body condition indices predict reproductive success but not survival in a sedentary, tropical bird. *PLOS ONE* **10**: e0136582.

Møller, A.P., Mousseau, T.A., Rudolfsen, G., Balbontin, J., Marzal, A., Hermosell, I. & de Lope, F.D. 2009. Senescent sperm performance in old male birds. *J. Evol. Biol.* **22**: 334–344.

Mukhin, A., Palinauskas, V., Platonova, E., Kobylkov, D., Vakoliuk, I. & Valkiūnas, G. 2016. The strategy to survive primary malaria infection: An experimental study on behavioural changes in parasitized birds. *PLoS ONE* **11**.

Naef-Daenzer, B., Widmer, F. & Nuber, M. 2001. Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. *J. Anim. Ecol.* **70**: 730–738.

Nielsen, J.T. & Møller, A.P. 2006. Effects of food abundance, density and climate change on reproduction in the sparrowhawk *Accipiter nisus*. *Oecologia* **149**: 505.

Norris, K.J. 1990. Female choice and the quality of parental care in the great tit *Parus major*. *Behav. Ecol. Sociobiol.* **27**: 275–281.

Norte, A.C., Araújo, P.M., Sampaio, H.L., Sousa, J.P. & Ramos, J.A. 2009. Haematozoa infections in a great tit *Parus major* population in central Portugal: relationships with breeding effort and health. *Ibis* **151**: 677–688.

Oppliger, A., Christe, P. & Richner, H. 1996. Clutch size and malaria resistance. *Nature* **381**: 565.

Oppliger, A., Christe, P. & Richner, H. 1997. Clutch size and malarial parasites in female great tits. *Behav. Ecol.* **8**: 148–152.

Oppliger, A., Richner, H. & Christe, P. 1994. Effect of an ectoparasite on lay date, nest-site choice, desertion, and hatching success in the great tit (*Parus major*). *Behav. Ecol.* **5**: 130–134.

Ots, I. & Hõrak, P. 1996. Great tits *Parus major* trade health for reproduction. *Proc R Soc Lond B* **263**: 1443–1447.

Pärt, T. 2001. The effects of territory quality on age-dependent reproductive performance in the northern wheatear, *Oenanthe oenanthe*. *Anim. Behav.* **62**: 379–388.

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.

Perrins, C.M. 1970. The timing of birds‘ breeding seasons. *Ibis* **112**: 242–255.

Perrins, C.M. & McCleery, R.H. 1985. The effect of age and pair bond on the breeding success of Great Tits *Parus major*. *Ibis* **127**: 306–315.

Pigeault, R., Cozzarolo, C.-S., Choquet, R., Strehler, M., Jenkins, T., Delhaye, J., Bovet, L., Wassef, J., Glaizot, O. & Christe, P. 2018. Haemosporidian infection and co-infection affect host survival and reproduction in wild populations of great tits. *Int. J. Parasitol.* **48**: 1079–1087.

Pinheiro, J., to 2007), D.B. (up, to 2002), S.D. (up, to 2005), D.S. (up, authors (src/rs.f), E., sigma), S.H. (Author fixed, sigma), B.V.W. (Programmer fixed & R-core. 2018. *nlme: Linear and Nonlinear Mixed Effects Models*.

Richard, M., Lecomte, J., Fraipont, M.D. & Clobert, J. 2005. Age-specific mating strategies and reproductive senescence. *Mol. Ecol.* **14**: 3147–3155.

Richner, H. 2016. Interval singing links to phenotypic quality in a songbird. *Proc. Natl. Acad. Sci.* **113**: 12763–12767.

Richner, H., Christe, P. & Oppliger, A. 1995. Paternal investment affects prevalence of malaria. *Proc. Natl. Acad. Sci.* **92**: 1192–1194.

Richner, H., Oppliger, A. & Christe, P. 1993. Effect of an ectoparasite on reproduction in great Tits. *J. Anim. Ecol.* **62**: 703–710.

Riddington, R. & Gosler, A.G. 1995. Differences in reproductive success and parental qualities between habitats in the Great Tit *Parus major*. *Ibis* **137**: 371–378.

Rivera-Gutierrez, H.F., Pinxten, R. & Eens, M. 2010. Multiple signals for multiple messages: great tit, *Parus major*, song signals age and survival. *Anim. Behav.* **80**: 451–459.

Sánchez, C.A., Becker, D.J., Teitelbaum, C.S., Barriga, P., Brown, L.M., Majewska, A.A., Hall, R.J. & Altizer, S. 2018. On the relationship between body condition and parasite infection in wildlife: a review and meta-analysis. *Ecol. Lett.* **21**: 1869–1884.

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*). *J. Anim. Ecol.* **69**: 74–84.

Scheuerlein, A., Hof, T.V. & Gwinner, E. 2001. Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). *Proc. R. Soc. Lond. B Biol. Sci.* **268**: 1575–1582.

Schoech, S.J. & Bowman, R. 2003. Does differnetial acces to protein influence difrences in timing of breeding of florida scrub-jays (*Aphelocoma coerulescens*) in suburban and wildland habitats? *The Auk* **120**: 1114–1128.

Seress, G., Hammer, T., Bókony, V., Vincze, E., Preiszner, B., Pipoly, I., Sinkovics, C., Evans, K.L. & Liker, A. 2018. Impact of urbanization on abundance and phenology of caterpillars and consequences for breeding in an insectivorous bird. *Ecol. Appl.* **28**: 1143–1156.

Shealer, D.A. & Burger, J. 1995. Comparative foraging success between adult and one-year-old roseate and sandwich terns. *Colon. Waterbirds* **18**: 93–99.

Skinner, W.R., Jefferies, R.L., Carleton, T.J. & Abraham, R.F.R.K.F. 1998. Prediction of reproductive success and failure in lesser snow geese based on early season climatic variables. *Glob. Change Biol.* **4**: 3–16.

Smith, H.G., Källander, H. & Nilsson, J.-Å. 1987. Effect of experimentally altered brood size on frequency and timing of second clutches in the great tit. *The Auk* **104**: 700–706.

Smith, R.J. & Moore, F.R. 2005. Arrival timing and seasonal reproductive performance in a long-distance migratory landbird. *Behav. Ecol. Sociobiol.* **57**: 231–239.

Svensson, L. 1984. Identification guide to European Passerines. (3 ed.). Stockholm.

Thomas, R.J. 2002. The costs of singing in nightingales. *Anim. Behav.* **63**: 959–966.

Toledo, A., Andersson, M.N., Wang, H.-L., Salmón, P., Watson, H., Burdge, G.C. & Isaksson, C. 2016. Fatty acid profiles of great tit (*Parus major*) eggs differ between urban and rural habitats, but not between coniferous and deciduous forests. *Sci. Nat.* **103**: 55.

Tripet, F. & Richner, H. 1997. Host responses to ectoparasites: Food compensation by parent blue tits. *Oikos* **78**: 557–561.

van Noordwijk, A.J., McCleery, R.H. & Perrins, C.M. 1995. Selection for the timing of great tit breeding in relation to caterpillar growth and temperature. *J. Anim. Ecol.* **64**: 451–458.

van Rooyen, J., Lalubin, F., Glaizot, O. & Christe, P. 2013. Avian haemosporidian persistence and co-infection in great tits at the individual level. *Malar. J.* **12**: 40.

Wawrzyniak, J., Kaliński, A., Glądalski, M., Bańbura, M., Markowski, M., Skwarska, J., ZielińSki, P., Cyżewska, I. & Bańbura, J. 2016. Long-term variation in laying date and clutch size of the great tit *Parus major* in central Poland: A comparison between urban parkland and deciduous forest.

Wegmann, M., Voegeli, B. & Richner, H. 2015. Physiological responses to increased brood size and ectoparasite infestation: Adult great tits favour self-maintenance. *Physiol. Behav.* **141**: 127–134.

Wood, M.J., Cosgrove, C.L., Wilkin, T.A., Knowles, S.C.L., Day, K.P. & Sheldon, B.C. 2007. Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. *Mol. Ecol.* **16**: 3263–3273.

Ydenberg, R.C. & Krebs, J.R. 1987. The tradeoff between territorial defense and foraging in the great tit (*Parus major*). ***Am. Zool.* 27: 337–346.**

Zhang, H., Rebke, M., Becker, P.H. & Bouwhuis, S. 2015. Fitness prospects: effects of age, sex and recruitment age on reproductive value in a long-lived seabird. *J. Anim. Ecol.* **84**: 199–207.

Table 1: Summary table of minimal mixed effect models

Response variable	**Explanatory variables**	**χ^2_1**	**Pr(χ^2_1)**		**Estimate**	**s.e**
model 1: Female body condition (N = 419)	Intercept				12.94	0.86
	Body_conditon_Male	24.949	5.885E-07	***	0.26	0.05
model 2: Laying date (N = 364)	Intercept				51.99	7.02
	Body_conditon_Female	5.104	0.024	*	-0.96	0.4
model 3: Clutch size (N = 364)	Intercept				10.9	0.43
	Laying_date	44.624	2.39E-11	***	-0.08	0.01
	Population	52.1	5.28E-13	***	Monods = 1.14	Monods = 0.16
model 4: Number of hatched chicks (N = 350)	Intercept				10.48	0.5
	Laying_date	53.778	2.25E-13	***	-0.1	0.01
	Population	41.515	1.17E-10	***	Monod = 1.27	Monod = 0.19
model 5: Number of 14-day old chicks alive (Box-Cox transformation, λ = 1.33) (N = 271)	Intercept				25.33	3.31
	Infection_Status_Male	14.517	0.013	*	Ni = -10.9, Lcz = -7.99, Plsm = -8.88 Lcz/Hm = -7.88, Lcz/Plsm = -8.24	Ni = 3.27, Lcz =3.13, Plsm = 3.12 Lcz/Hm = 3.15, Lcz/Plsm = 3.08
	AgeClass_Male	10.338	0.001	**	Sub adult = -1.39	0.43
	Laying_date : Population	12.849	0.0003	***	Monod = 0.22407	Monod = 0.06201
model 6: 14-day-old chick body condition (N = 309)	Intercept				8.15	1.79
	Bodyconditon_Male	6.258	0.012	*	0.21	0.08
	Bodyconditon_Female	4.42	0.035	*	0.17	0.08
model 7: Number of fledged chicks (N = 341)	Intercept				11.42	2.64
	Infection_Status_Male	12.790	0.025	*	Ni = -1.51, Lcz = -1.49, Plsm = -2.72 Lcz/Hm = -0.81, Lcz/Plsm = -1.86	Ni = 2.60, Lcz =2.53, Plsm = 2.50 Lcz/Hm = 2.53, Lcz/Plsm = 2.49
	AgeClass_Male	33.412	7.455e-09	***	Sub adult = -1.68	0.3
	Laying_date : Population	7.863	0.005	**	Monod = 0.12	0.04

Notes. In each minimal mixed effect models, year of capture, individual (ring number) and nest-box identity were used as random factors. The response variable was not transformed unless otherwise stated (superscript indicates λ in Box-Cox transformation). Minimal models are given with intercept as well as estimates, standard errors (s.e.), Chi-square and *P* values for each specific term. N gives the number of birds included in each analysis. *P* value < 0,1, **P* value < 0.05, ***P* value < 0.001, ****P* value < 0.0001. Abbreviation: Single infection = Plsm: *Plasmodium sp.*, Lcz : *Leucocytozoon sp.*, Hm : *Haemoproteus sp.* Co-infection = Lcz/Plsm: *Leucocytozoon/ Plasmodium*, Lcz/Hm: *Leucocytozoon/Haemoproteus sp.*

Figures

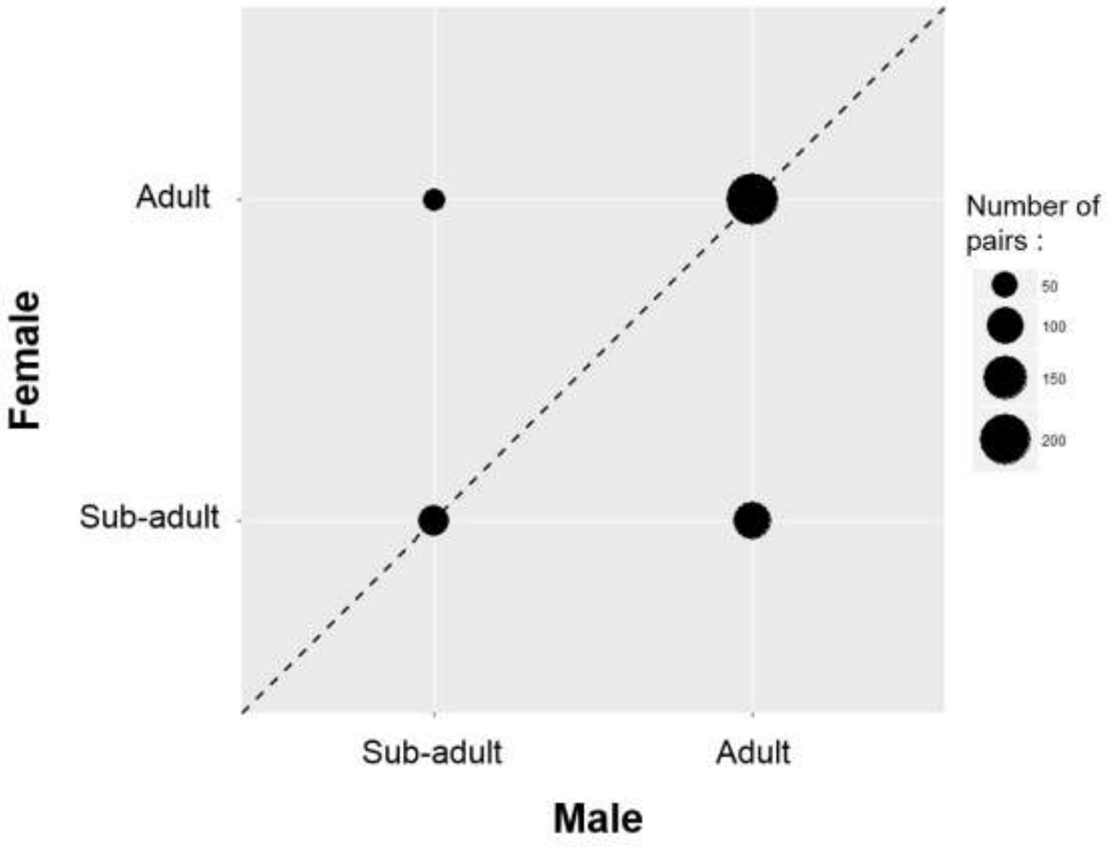

Figure 1: Female age-class plotted as a function of male age-class within breeding pairs. The diameter of the symbol is proportional to the sample size.

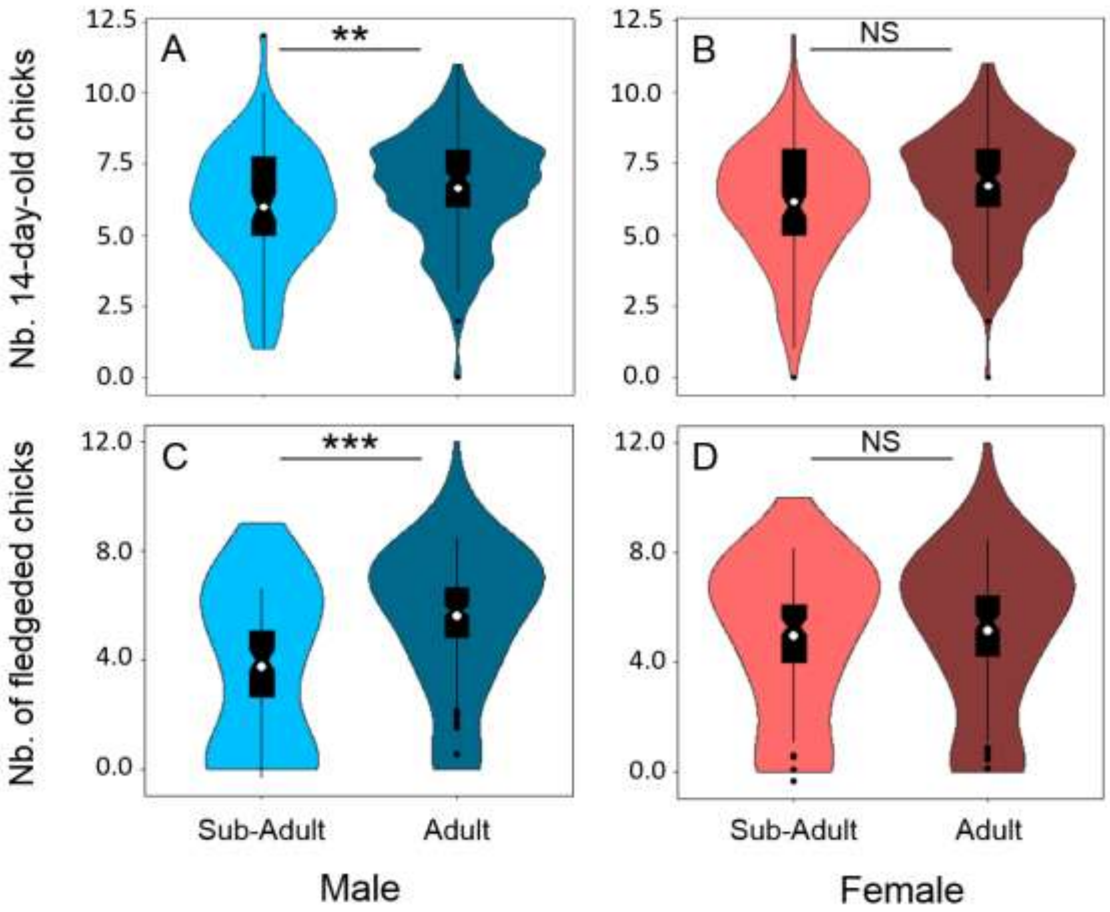

Figure 2: Effect of male and female age-classes on annual reproduction. **A, B** Effect of parent age-classes on the number of 14-day-old chicks. **C, D** Effect of parent age-classes on the number of fledged chicks. Violin plots were constructed to show the spread and density of the raw data. Boxplots were constructed to show the predicted value from the minimal models. Boxes above and below the medians (horizontal lines) show the first and third quartiles, respectively. White points represent the means. Light blue: sub-adult male, dark blue: adult male, light red: sub-adult female, and dark red: adult female. NS: non-significant, **: $P < 0.01$, ***: $P < 0.001$.

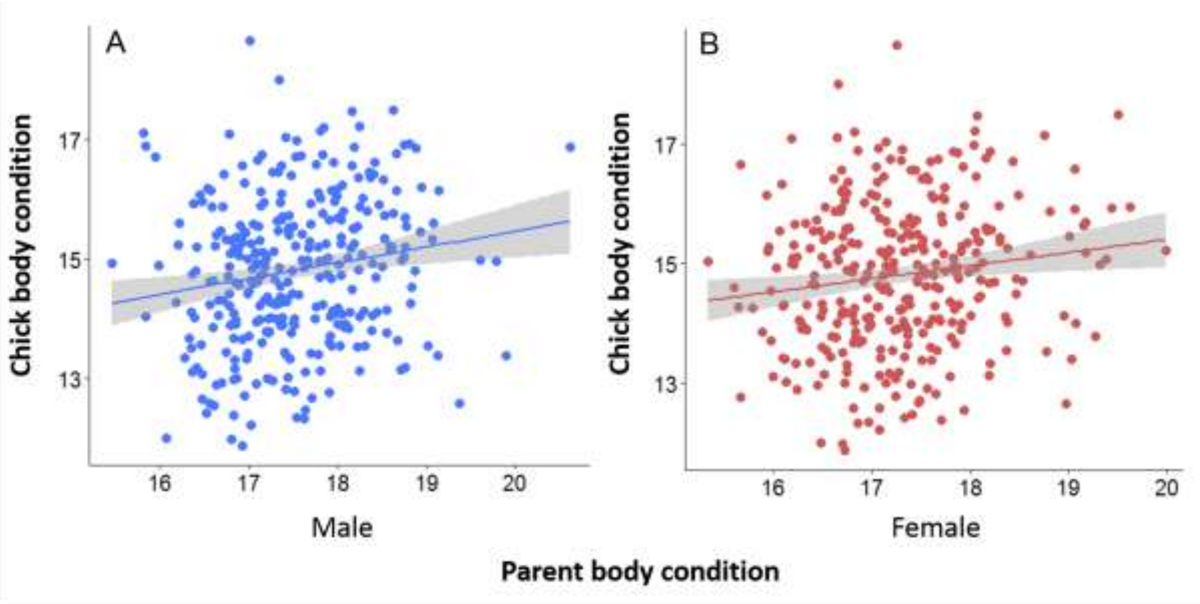

Figure 3: Chick body condition plotted as a function of (A) male and (B) female body condition.

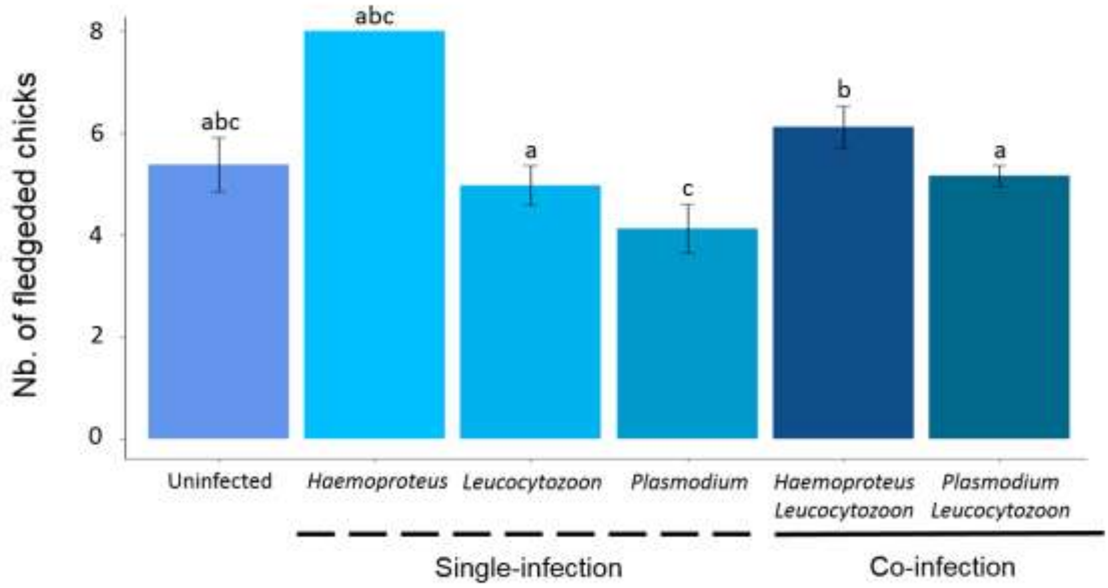

Figure 4: Effect of male great tit haemosporidian infection on the number of fledged chicks. Levels not connected by the same letter are significantly different. Error bars represent ± s.e.

Supporting information

Additional supporting information may be found online in the supporting Information section at the end of the article

Table S1. Number of pairs used per great tit population and per year.

Table S2. Summary table of maximal mixed effect models

Figure S1: Female haemosporidian infection status plotted as a function of male haemosporidian infection status within breeding pairs. Acronyms: Hm: *Haemoproteus*, Lctz: *Leucocytozoon*, and Plsm: *Plasmodium.* The diameter of the symbol is proportional to the sample size.

Figure S2: Female body condition plotted as a function of male body condition within breeding pairs.

Figure S3: Relationship between (A) female and (B) male body condition and laying date.

Figure S4: (A) Relationship between the clutch size of pairs and their laying date. (B) Relationship between the number of hatched chicks and the laying date.

Figure S5: Relationship between the number of 14-day-old chicks in the nest and the laying date in (A) Dorigny and (B) Monod population.

Figure S6: Effect of male great tit haemosporidian infection on the number of 14-day-old chicks.

Figure S7: Relationship between the number of fledged chicks and the laying date in (A) Dorigny and (B) Monod population.