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Sibling rivalry and vocal negotiation in the barn owl (*Tyto alba*)

Charlène-Aurore Ruppli

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SIBLING RIVALRY AND VOCAL NEGOTIATION IN THE BARN OWL TYTO ALBA

Thèse de doctorat ès sciences de la vie (PhD)

présentée à la

Faculté de biologie et de médecine
de l'Université de Lausanne

par

Charlène-Aurore RUPPLI

Master de l'Université des Sciences et Techniques de Montpellier II

Jury

Prof. Mehdi Tafti, Président
Prof. Alexandre Roulin, Directeur de thèse
Dr. Amélie N. Dreiss, Co-directrice
Dr. Michel Chapuisat, expert
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SIBLING RIVALRY AND VOCAL NEGOTIATION IN THE BARN OWL *TYLO ALBA*

Lausanne, le 8 février 2013

M. Tafti

pour Le Doyen
de la Faculté de Biologie et de Médecine

Prof. Mehdi Tafti

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Chez les animaux, les jeunes dépendant des parents durant leur développement sont en compétition pour obtenir la nourriture, qu'ils quémandent par des cris et postures ostentatoires et se disputent physiquement. Les frères et sœurs n'ont pas la même compétitivité, en particulier s'ils diffèrent en âge, et leur niveau de faim fluctue dans le temps. Comme dans tout type de compétition, chacun doit ajuster son investissement aux rivaux, c'est à dire aux besoins et comportements de ses frères et sœurs. Dans le contexte de la famille, selon la théorie de sélection de parentèle, les jeunes bénéficient de leur survie mutuelle et donc de la propagation de la part de gènes qu'ils ont en commun. L'hypothèse de la « négociation frères-sœurs » prédit que, sous certaines conditions, les jeunes négocient entre eux la nourriture, ce qui réduit les coûts de compétition et permet de favoriser les frères et sœurs les plus affamés. La littérature actuelle se focalise sur les signaux de quémande entre enfants et parents et les interactions compétitives frères-sœurs sont étudiées principalement au sein de paires, alors que les nichées ou portées en comprennent souvent de nombreux. Cette thèse vise à mieux comprendre comment et jusqu'à quel point plusieurs jeunes ajustent mutuellement leurs signaux de besoin. C'est une question importante, étant donné que cela influence la répartition de nourriture entre eux, donc la résolution du conflit qui les oppose et à terme leur valeur évolutive. Le modèle d'étude est la chouette effraie (*Tyto alba*), chez laquelle jusqu'à neufs poussins émettent des milliers de cris chacun par nuit. Ils négocieraient entre eux la prochaine proie indivisible rapportée au nid avant que les parents ne reviennent : un poussin affamé crie plus qu'un autre moins affamé, ce qui dissuade ce dernier de crier en retour et par la suite de quémander la nourriture aux parents. L'investissement optimal correspondrait donc à écarter son frère en permanence vu que l'arrivée des parents est imprévisible, mais à moindre coût.

Dans un premier axe, nous avons exploré au sein de dyades les mécanismes acoustiques permettant aux poussins de doser leur effort vocal durant les heures de compétition où ils sont laissés seuls au nid. Nous avons trouvé que les poussins évitent de crier simultanément, ce qui optimiserait la discrimination du nombre et de la durée de leurs cris, lesquels reflètent de façon honnête leur niveau de faim et donc leur motivation. L'alternance des cris paraît particulièrement adaptée au fait que les poussins se fient à des variations temporelles subtiles dans le rythme et la durée de leurs vocalisations pour prendre la parole. En particulier, allonger ses cris tout en criant moins dissuade efficacement le rival de répondre, ce qui permet de monopoliser la parole dans de longs « monologues ». Ces règles seraient universelles puisqu'elles ne dépendent pas de la séniorité, de la faim, ni de la parenté et les poussins répondent à un playback de façon similaire à un vrai frère. Tous ces résultats apportent la première preuve expérimentale que les juvéniles communiquent de façon honnête sur leurs besoins, ajustent activement le rythme de leurs cris et utilisent des composantes multiples de leurs vocalisations d'une façon qui réduit le coût de la compétition. De plus, il s'agit de la première démonstration que des règles de conversation régissent de longs échanges vocaux chez les animaux de façon comparable aux règles basiques observées chez l'Homme.

Dans un second axe, nous avons exploré les stratégies comportementales que les poussins adoptent pour rivaliser avec plusieurs frères et sœurs, par le biais d'expériences de playback. Nous avons trouvé que les poussins mémorisent des asymétries de compétitivité entre deux individus qui dialoguent et répondent plus agressivement au moins compétitif une fois qu'ils sont confrontés à chacun isolément. Dans la même ligne, quand ils entendent un nombre variable d'individus criant à un taux variable, les poussins investissent le plus contre des rivaux moins nombreux et moins motivés. En accord avec les prédictions des modèles théoriques, les poussins de chouette effraie escaladent donc les conflits pour lesquels leur chance de gagner contrebalance le plus l'énergie dépensée. Nous révélons ainsi que 1) les jeunes frères et sœurs 'espionnent' les interactions de leurs rivaux pour évaluer leur compétitivité relative, ce qui est sans doute moins coûteux qu'une confrontation directe avec chacun, et 2) dosent leur investissement vocal en fonction du nombre de rivaux actuellement en compétition et de leur motivation de façon concomitante. Ces résultats montrent que les interactions entre frères et sœurs au nid reposent sur des mécanismes similaires à ceux observés, mais encore de façon anecdotique, chez les adultes non apparentés qui se disputent les territoires et partenaires sexuels.

Cette thèse souligne donc combien il est crucial de considérer dorénavant la famille comme un réseau de communication à part entière pour mieux comprendre comment les jeunes résolvent les conflits autour du partage des ressources parentales. Plus généralement, elle révèle l'importance de la dynamique temporelle des vocalisations dans les conflits et la communication des animaux. A la lumière de nos résultats, la chouette effraie apparaît comme un modèle clé pour de futures recherches sur la résolution des conflits et la communication acoustique.

In species with parental care, offspring contest priority access to food by begging through conspicuous postures and vocalisations and by physically jockeying. Siblings differ in their competitiveness, especially in the case of age and size hierarchies, and their hunger level fluctuates in time. As in competition in general, each individual should adjust its investment to opponents that is to say to its siblings' needs and behaviours. In the particular context of family, according to kin selection theory, siblings derive extra fitness benefits from their mutual survival and hence the spreading of the genes they share. The "sibling negotiation" predicts that, under certain conditions, young would negotiate among them priority access to food, which reduces competition costs and enables promoting the most hungry siblings. To date, the literature focuses on signals of need between parents and offspring and competitive interactions (in particular among siblings) are mostly studied within pairwise interactions, yet they commonly involve more numerous rivals. This PhD aims at better understanding how and the extent to which several young siblings compete through signalling. This is important since this influences how food is allocated among them, thus the outcome of sibling rivalry and ultimately their fitness. I use the barn owl (*Tyto alba*) as a model, in which the one to nine nestlings emit a simple noisy call thousands of times per night. Thereby, they would negotiate among them priority access to the indivisible food next delivered prior to parents' feeding visits. A hungry nestling emits more calls than a less hungry sibling, which deters it to call in return and ultimately beg food at parents. The optimal investment thus corresponds to constantly deterring the rival to compete, given that parents' arrival is unpredictable, but at the lowest costs.

In the first axis of my thesis, we explored within dyads the acoustic mechanisms by which owlets dose vocal effort when competing during the hours they are left alone. We found that owlets avoid overlapping each other's calls. This would enhance the discrimination of both call number and duration, which honestly reflect individuals' hunger level and hence motivation to compete. Such antiphony seems best adapted to the fact that siblings actually use subtle temporal variations in the rhythm and duration of their calls to take or give their turn. Owlets alternate monologs, in which lengthening calls efficiently deters the rival to respond while reducing call number. Such rules depend neither on seniority, hunger level nor kinship since nestlings responded similarly to a live sibling and an unrelated playback individual. Taken together, these findings provide the first experimental proof that dependent young honestly communicate about their need, actively adjust the timing of their calls and use multicomponent signals in a way that reduces vocal costs. Moreover, this is the first demonstration of conversational rules underlying animal long-lasting vocal exchanges comparable to the basic turn-taking signals observed in humans.

In the second axis, we focused on the behavioural strategies owlets adopt to compete with more than one sibling, using playback experiments. We found that singleton bystanders memorised competitive asymmetries between two playback individuals dialoguing and responded more aggressively to the submissive one once they later faced each of both alone. Moreover, when hearing a varying number of nestlings calling at varying rates, owlets vocally invested the most towards fewer and less motivated rivals. In line with predictions from models on conflict settlement, barn owls thus escalate contests in which their chance of winning best counterbalances the energy spent. These results reveal that young socially eavesdrop on their siblings' interactions to assess their relative competitiveness at likely lower costs than direct confrontation, and dose vocal effort relative to both their number and motivation. This shows that young siblings' interactions imply mechanisms similar to those observed, yet still anecdotally, in unrelated adults that contest mates and territories.

This PhD therefore highlights how crucial it is to further consider family as a communication network to better understand how siblings resolve conflicts over the share of parental resources. More generally, it provides important insights into the role of the temporal dynamics of signalling during animal contests and communication. In the light of our findings, the barn owl emerges as a key model for future research on conflict resolution and acoustic communication in animals.

GENERAL INTRODUCTION

COMMUNICATION TO RESOLVE CONFLICTS

In nature, because space, either as territory or mating sites, mates and food are limited, conspecifics are often in conflict over the share of resources. Evolutionary theory predicts that to maximise their own fitness, i.e. survival and reproduction, individuals should compete so as to derive the greatest benefits at the lowest costs (Maynard Smith 1976). While competing is easier for the strongest rivals, resources are more valuable for the most needy individuals. Whether opponents engage in or retreat from a contest will then depend on their relative ‘resource holding potential’ (i.e. fighting ability) and motivation (Parker 1974). Direct confrontation, despite representing a straightforward means of assessing a rival’s strength and motivation to compete, may lead to serious or lethal injuries. Animals thus commonly challenge each other over priority access to mates and territories using signals such as vocalisations (Schwartz and Freeberg 2008; Todt and Naguib 2000), scent-marking (Peters and Mech 1975; Rich and Hurst 1998), body coloration (Dijkstra et al. 2005; Senar 2006) and behavioural displays (Hofmann and Schildberger 2001; Mattiangeli et al. 1999; Mercier and Dejean 1996). Provided that communication is costly to prevent cheating, signals reliably reflect competitors’ dominance and need (Grafen 1990), allowing opponents to optimally adjust competing effort to each other. Hence, examining how animals modulate signals to one another is central to understanding their evolutionary function and the resolution of conflicts over resources.

To date, the role of communication in agonistic social interactions has been described in depth in the context of sexual conflicts and contests for territories. In a wide range of species, males and females use various signals that reflect their dominance and physiological state to gain access to and defend mates and territories (Maynard-Smith and Harper 2004). Despite an extensive theoretical and empirical framework on conflicts among adults, knowledge about how communication regulates competition at younger ages is more elusive, even though competition might be intense for space and food among juveniles (Mock and Parker 1997) and early survival is crucial to later reproduce. Particularly in species with parental care, siblings, who are no more than half related, are assumed to be in conflict over the amount of care and food provisioning they receive. Each individual is predicted to demand a larger than equal portion of parental resources (Godfray 1995a,b). Yet, because relatives share some genes, kin selection theory predicts that they may derive indirect fitness benefits from each other's survival and reproduction (Hamilton 1964). Given that communication enables avoiding lethal injuries, it should play an important role in sibling competition over parental resources, specifically in altricial species where offspring are reared in close proximity.

SIGNALLING IN THE CONTEXT OF SIBLING RIVALRY

Young animals physically compete to the point of siblicide (Drummond 2006; Mock and Parker 1997), but most commonly solicit food from parents through ostentatious begging postures and other signals such as loud vocalisations in birds and mammals (Hudson and Trillmich 2008; Kilner and Johnstone 1997) or chemicals in insects (Mas and Kolliker 2008). Because parents face a trade-off between self-maintenance and investment in reproduction (Stearns 1992), begging has historically been considered a reliable signal of need that parents use to optimally allocate food among their offspring (Harper 1986; Kilner and Johnstone 1997). Indeed, empirical works are consistent with the hypothesis that offspring modulate begging intensity in relation to their own hunger level and condition (Kitaysky et al. 2001; Manser et al. 2008; Sacchi et al. 2002; Smiseth and Moore 2007; Villasenor and Drummond 2007). However, they further reveal that young modulate the intensity of signals of need also to their siblings' (Marques et al. 2011), as well as their hunger level (Price et al. 1996; Roulin et al. 2001; Smith and Montgomerie 1991), condition (appendice 1, Vallarino et al. 2006) size

(Price et al. 1996; Roulin 2004a), number (Kacelnik et al. 1995; Price 1996) and physical behaviours (Dreiss et a. 2010b) of their siblings. This indicates that offspring not only use vocal, postural and physical displays to communicate their need to parents, but also as competitive signals to siblings.

Broodmates intrinsically differ in need and condition, which fluctuate across feedings, and in competitiveness. This last difference is even accrued in the case staggered birth establishes age and size hierarchies. Due to such asymmetries, offspring may parasitize parental decisions in how food is distributed (Parker et al. 2002). Extensive fieldwork in birds showed that stronger and / or older young may be better able to monopolise food than their smaller and younger siblings, notably because they physically outcompete them to attract parental attention (e.g. Smith et al. 2005). Lower competitive individuals may then compensate for their submissiveness by begging at higher levels, which proves useful depending on whether parents rely on brood/litter or individual signalling level to distribute food (Bonisoli-Alquati et al. 2011; Cotton et al. 1999; Smiseth and Amundsen 2002). Because this so-called ‘scramble competition’ determines signalling strategies and interferes with parental feeding decisions over the outcome of conflict, i.e. within brood or litter food distribution, it is crucial to examine how siblings mutually modulate signalling level to compete for food. Though a vast body of theoretical and empirical works has tackled this issue, authors usually consider begging signals to be uniquely directed at parents, hence disregarding a potential communication among offspring. In consequence, the extent to which and how offspring adjust signalling levels to each other is poorly understood.

Typically, empiricists measure individuals’ behaviours in live siblings, which does not allow disentangling their mutual influence of one on another (but see Marques et al. 2011). In more, authors consider young behaviours mostly during begging sessions, which they treat as one-off encounters although these are interdependent over time. While game theory predicts signalling contests to be iterative (McNamara et al. 1999), little remains known about how siblings assess their relative competitiveness and need from signals and cues, and about how their physical and vocal behaviours are interconnected across, between and during feeding sessions. Notwithstanding, the 'sibling negotiation hypothesis' predicts that siblings might actively induce each other to withdraw from begging food from parents. By communicating their need to each other, young would reduce competition costs while guaranteeing food to the most needy and allowing the least hungry to save energy to be reallocated later (Roulin 2002a). This sib-sib communication is predicted to evolve when the outcome of competition is predictable, i.e. when food is indivisible and asymmetries well pronounced among siblings (Johnstone and Roulin 2003). To date, it has been shown to occur prior to parents' arrival between feeding visits in the barn owl (*Tyto alba;* Roulin et al. 2000; Roulin 2002a) and in the spotless starling (*Sturnus unicolor*; Bulmer et al. 2008). To properly understand how and to which extent altricial young interact with parents' provisioning decisions and resolve conflicts over the sharing of resources, it is crucial to further consider the family as a whole communication network (Horn and Leonard 2005).

THE CASE STUDY OF BARN OWLS' SIB-SIB NEGOTIATION

The barn owl is an ideal model to study signalling interactions among altricial young because owlets vocally communicate among themselves to negotiate feeding prior to parents' arrival (in fact, this species prompted the 'sibling negotiation' hypothesis). Extra-pair copulations being rare (Roulin et al. 2004), the two to nine owlets are most often full siblings and have common inclusive fitness interests in avoiding costly competition and promoting hungry siblings (see also appendix 2). Furthermore, given that siblings hatch asynchronously and strongly differ in age and size, and that parents deliver indivisible prey items (micro-mammals), the outcome of competition is predictable. Between the sporadic parental feeding visits at night, nestlings repeat a single noisy call up to thousands of times each (fig. 2) to challenge each other for priority access to the indivisible prey item next delivered.

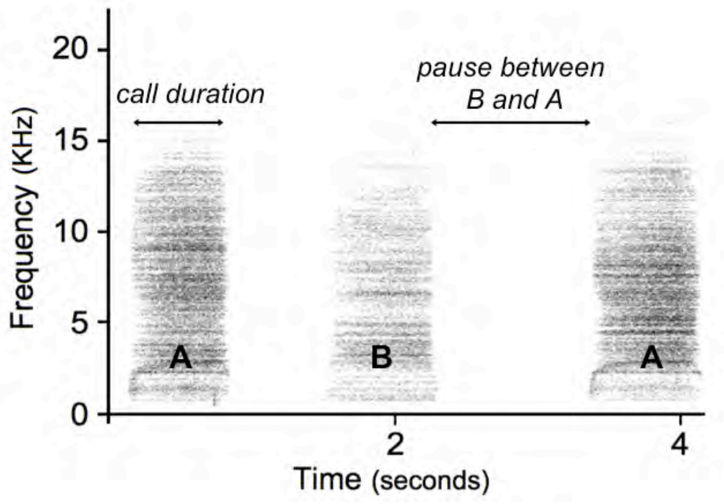

Figure 2. Sonograms of negotiation calls from two barn owl nestlings (A and B).

Published correlative results indeed give evidence that:

1) Siblings communicate with one another, not with their parents. The latter generally forage too far from their nest to hear owlets (pers. obs.) and do not adjust provisioning rate to the number of calls owlets produce (Roulin et al. 2000).

2) An owlet modulates its vocal output not only relative to its own needs but also in function of the needs and vocal output of a sibling, which ultimately determines which obtains food. When food deprived, nestlings emit on average more and longer calls than when sated, indicating that both the rate and duration of calls reflect their motivation to compete (Roulin et al. 2000a; Roulin 2001; Roulin et al. 2009). Within a pair of siblings, the owlet that emits the most and longest calls ultimately begs more intensely to the parents and receives the prey item (Dreiss et a. 2010b; Roulin 2001; Roulin et al. 2000). However, owlets do not call desperately above the level of a sibling but dose investment in competition proportionally to their own chance of succeeding. When facing a hungry sibling, that is to say highly motivated to compete, a hungry owlet will call less than when the sibling is sated, that is to say when its

own chance of compensating the costs incurred by signalling by food obtaining is greater (Roulin et al. 2000). Older owlets (‘seniors’), which are better able to physically impose themselves, are also less sensitive to their younger siblings’ (‘juniors’) negotiation behaviour than the reverse and produce fewer and shorter calls (Roulin 2004a; Roulin et al. 2009). Interestingly, recent correlative results suggest that owlets would not, or not only, escalate signalling above the level of a sibling, but also - or rather - induce it to withdraw from negotiating and ultimately from begging (Dreiss et a. 2010b; fig. 3).

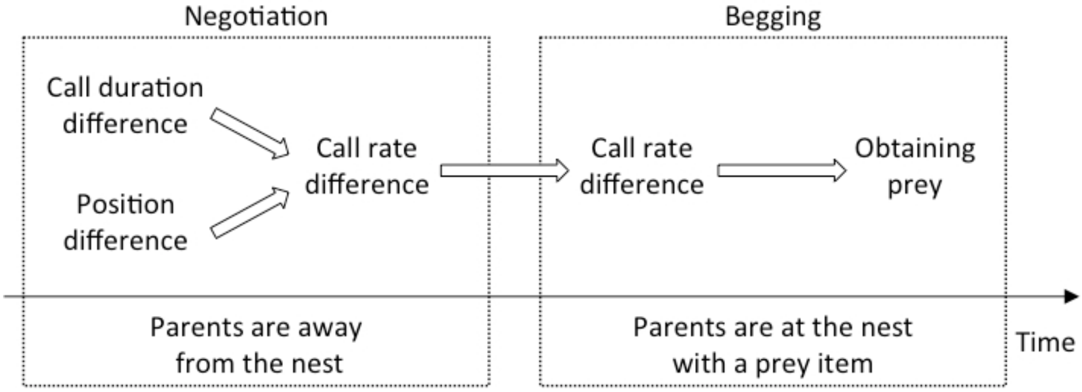

Figure 3. **Synthesis of the hypothetical role of the different behaviours leading to a nestling obtaining prey** (taken from Dreiss et a. 2010b). In the absence of parents the individual that produces longer negotiation calls (i.e. ‘Call duration difference’ during the negotiation phase) and is positioned closer to the nest boy entrance (i.e. ‘Position difference’ during the negotiation phase) induces its sibling to negotiate at a lower rate (i.e. ‘Call rate difference’ during the negotiation phase). The individual that negotiates more than its sibling (i.e. ‘Call rate difference’ during the negotiation phase) induces its sibling to beg at a lower rate (i.e. ‘Call rate difference’ during the begging phase), which positively influences the likelihood of obtaining a prey item from its parents (i.e. ‘Obtaining prey’).

GOAL & OUTLINE OF MY THESIS

The focus of my PhD is to examine how and to which extent several barn owl young siblings resolve conflicts over parental resources through signalling with each other. To this aim, I develop my thesis along two main axes. Because results have been correlative until now, I first experimentally explore, using correlative and playback procedures, the acoustic rules governing sib-sib vocal exchanges within dyads. I examine how individual bystanders modulate the timing of their calls (Chapter I) and average call rate and duration (Chapter II) to those of a nestmate. Then, I investigate how owlets continuously adjust these acoustic parameters during their naturally long lasting vocal exchanges (Chapter III). Second, based on the findings within chapters I through III, I test some of the behavioural tactics by which owlets compete with more numerous nestmates (Chapters IV & V).

Part 1 - Acoustic rules governing dyadic vocal exchanges

1) Global adjustment of multiple acoustic components

In altricial young begging comprises different components, such as postures and calls in birds and mammals. Across and within species, different behaviours and different call features (i.e. frequencies, duration) encode for various aspects of individual need (Duckworth et al. 2009; Gladbach et al. 2009; Leonard and Horn 2006). Siblings might then differentially compete over these multi- or uni-sensory components (Glassey and Forbes 2002; Jacob et al. 2011). Some studies suggest that the acoustic structure and timing of begging calls might have a great influence on the within-brood food allocation. For instance, field works in black-headed gulls (*Larus ridibundus*) reveal that during development, chicks progressively synchronise their calls, which cooperatively enhances brood signal and parents' provisioning rate (Blanc et al. 2010). In the meerkat (*Suricata suricatta*), adult feeders best discriminate and provision pups that call alternately compared to synchronously (Madden et al. 2009). To my knowledge, however, a single playback study has experimentally tested how siblings adjust their vocalisations to each other (Marques et al. 2011). The authors found that tree swallow chicks (*Tachycineta bicolor*) would deter siblings to beg by increasing call intensity, yet they did not examine for other acoustic components such as call number, duration, rhythm or frequencies.

In the barn owl, both the number and duration of calls are positively correlated with nestlings' level of hunger and relate to their resource holding potential, with juniors producing more and longer calls than seniors (Roulin 2004a, Roulin et al. 2009). To impose oneself, one could expect owlets to interrupt its siblings by overlapping their calls, a signal linked to aggressiveness in some songbirds (Mennill and Ratcliffe 2004; Naguib and Todt 1997). To optimally behave, however, each hungry individual should display proportionally to its siblings' motivation and resource holding potential, and hence discriminate the number and duration of their calls. Barn owl siblings thus should rather optimise vocal effort by avoiding interferences that could jam the information encoded in each other's vocalisations. If owlets vocalise altogether, they could shift individual acoustic features similarly to chorusing males of some anuran species (Schwartz and Freeberg 2008). If siblings call alternately, this should promote individual recognition while discriminating both call rate and duration. This so-called 'antiphony' is common in competitive or cooperative interactions, especially at long-range distances or in dark environments where visual signals are barely available, such as in songbirds' contests or duets (Brumm 2006; Luther 2008) or the exchange of contact calls in mammals (Carter et al. 2008; Versace et al. 2008; Yosida et al. 2007). In **Chapter I**, I thus explore owlets' propensity to avoid overlapping each other's vocalisations, using both playbacks and food-deprivation experiments.

Now, if owlets mutually adjust the timing of their negotiation calls to distinguish call number and duration, what are the respective functions of these two acoustic components in sib-sib vocal competition? Multiple-component signalling across or within sensory modalities is frequent in animal courtship and territorial displays (Bro-Jørgensen 2010; Partan and Marler 2005). Because evolving several signals may seem *a priori* wasteful if one type efficiently repels rivals and predators or attract mates, hypotheses have been proposed to explain the maintenance of multiple signalling. Broadly, cheaper ‘back-up’ or ‘redundant’ signals may serve to enhance message transmission in environments with visual or acoustic interferences (Johnstone 1996; Partan and Marler 1999) or to facilitate learning in receivers (‘receiver psychology', Rowe 1999). Alternatively, distinct signal components may convey ‘multiple messages’. They could reflect different aspects of 1) the signaller’s quality, which reinforces signal costs and reliability to rivals and mates about its resource holding potential or ‘good genes’, or 2) the message content, if the full meaning relies on the combination of several signal types (so-called ‘emergent signal’; Rowe 1999, Bro-Jørgensen 2010).

Several empirical papers give support to these various hypotheses among taxa, but most concentrate on multimodal signals, i.e. from different sensory channels (e.g. Partan et al. 2009; Smith and Evans 2008). Knowledge on the concomitant function of multiple acoustic components in vocal contests remains more elusive (but see Miller and Hauser 2004; Richardson and Lengagne 2010; Rivera-Gutierrez et al. 2010). In **Chapter II**, I investigate the mutual roles of call number and duration in barn owls’ sib-sib vocal competition. I examine how live siblings respectively modulate these acoustic components according to their motivation to compete (i.e. hunger level) and the response of bystander individuals to playback calls of varying durations broadcast at different rates.

2) Temporal dynamics of long-lasting dyadic vocal exchanges

During barn owls’ sib-sib negotiation, nestlings may be left alone several hours in a row during which parents can come back with food at any time. Hence, each hungry individual should constantly call more than its less hungry siblings so as to induce them to refrain from calling back and ultimately from begging, but at the lowest costs (energy, potential predation). In other words, a motivated nestling should emit enough calls that last long enough to efficiently induce siblings to withdraw from responding, but not above the level required to do so. In more, it should lower its vocal effort once siblings stop calling, unless what it would waste energy. Notwithstanding, its hungry siblings might profit from such a decrease in effort to monopolise again the acoustic space. The focus of **Chapter III** is then to unravel how barn owl siblings manage to optimally impose themselves during several hours of negotiation. To this aim, I examine the acoustic cues on which owlets rely to preferentially give or take their turn during these competitive long lasting vocal exchanges.

Game-theory models predict that in repeated interactions using one signal type, such as owlets' negotiation calls, the way opponents modulate signalling intensity over time depends on how receivers assess it. If the 'rule of assessment' of signallers' resource holding potential depends on average signal intensity, signallers should display at fixed levels with the fewest repetitions possible (e.g. 'sequential assessment game'; Enquist and Leimar 1983). This is unlikely in barn owls because young modulate call rate and duration with hunger level, which fluctuates both among individuals and across feeding sessions. Another possibility is that owlets assess each other's motivation to compete from the calls of highest intensity / duration to adjust the next one (so-called 'best-so-far' rule; Payne and Pagel 1997). In such situation, owlets are predicted to escalate call rate and duration only if lower values are inefficient in deterring siblings from competing. Hungry siblings should thus continuously escalate the contest until parents come back to the nest with a prey item. Alternatively, if owlets assess each other's motivational states from the cumulative information of their call number and call duration, maintaining many long calls over time could reflect vocal endurance, as suggested in red deers (*Cervus elaphus*, Clutton-Brock and Albon 1979).

According to the course of the interaction, owlets might then either mutually escalate or de-escalate in one or both parameters over time (Payne 1998).

The growing body of empirical literature testing predictions from these three ‘assessment rules’ in agonistic iterative contests mostly focus on physical interactions (e.g. Briffa and Elwood 2009; Domhnall et al. 2005; Morell et al. 2005). In this case, the fitness costs of competing strategies are indeed straightforward. Although many authors report the role of the number, rhythm and acoustic features of vocalisations in deterring opponents to vocally compete across species (see reviews in Arark 1983; Todt and Naguib 2000), they usually examine bystanders’ responses to live or playback rivals over short periods of several minutes, and more scarcely over long lasting exchanges (e.g. Foote et al. 2008). In all cases authors measure average level in bystanders’ response to signal types. To my knowledge, no study has addressed the question of how individuals continuously assess and adjust concomitant variations in different acoustic call features over long periods of time, yet courtship or territorial contests can last long, during which rivals’ motivation to compete and condition fluctuate.

In the barn owl, several nestlings vocalise altogether in parents’ absence. At first glance, nestlings’ vocal patterns do not present any obvious temporal organisation of rhythm, of call duration and alternation of turns. As revealed by the extensive study of human conversations, temporal modulations in the pitch, intonation, frequency and timing of vocal output are crucial to allow the smooth course of vocal interactions over time. In humans, both parties, with coincident interests or not, usually alternatively take and lead the floor using such fluctuations in their speech, which is referred to as basic ‘turn-taking rules’ (Duncan 1972). In **Chapter III**, I examine if owlets have evolved turn-taking rules, that is to say whether and how pairs of siblings continuously assess and mutually adjust their call rate (rhythm) and call duration during long-lasting vocal interactions. I use both food-deprivation and several playback experiments to test whether potential turn-taking rules vary relative to hunger level, seniority, familiarity and relatedness.

Part 2 – Vocal competition within a network of several nestmates

If the current framework of evolutionary game theoretical models predicts animals' stable signalling strategies in detail, they mostly remain based, for practical reasons, on pairwise interactions, including models on sibling rivalry. Paradoxically, altricial offspring, as chorusing anurans or insects, birds living in flocks and mammals, fish and invertebrates living in aggregates or social groups compete with several conspecifics. In what concerns begging young, authors have examined so far how bird chicks modulate signalling level toward several siblings with respect to the signalling level of the whole brood (e.g. Smith and Montgomerie 1991), including barn owls (Roulin 2002a). Young, however, could modulate their competitive effort as a function of their several siblings' individual effort and / or of what occurs during their siblings' interactions.

Although this issue was formally raised several decades ago about agonistic encounters in general, only recently have researchers addressed the question of how animals compete within 'communication networks' (McGregor 2005). In particular, some studies reveal that competitors would not only use 'interceptive eavesdropping' to adjust competitive effort to a rival's - whereby they assess their relative resource holding potential from the latter's traits or behaviours - but also 'social eavesdropping' (Amy and Leboucher 2007; Peake et al. 2005; Valone 2007). Social eavesdropping corresponds to a situation when an individual spies on rivals' interactions to assess their relative strength and motivation without engaging in immediate competition. It thereby offers a relatively cost-free means of gathering useful information on potential opponents to ultimately dose competitive effort without paying the cost of direct confrontation (Peake and McGregor 2004). To date, social eavesdropping has been empirically tested and shown to occur in contests such as the long or middle-range agonistic interactions of songbird and teleost fish males (Peake 2005). Situations where individuals compete with one another in close proximity as in altricial young could have facilitated the evolution of social eavesdropping even more. Therefore, in **Chapter IV**, I examine whether barn owl nestlings acoustically eavesdrop on siblings' vocal interactions to adjust their negotiation effort. Using a playback procedure, I test whether bystander individuals assess the relative resource holding potential (seniority and vocal dominance) of two nestmates from their vocal exchange and accordingly modulate vocal investment in subsequent interactions.

Surprisingly, despite their growing interest in examining agonistic interactions within social networks, both theoreticians and empiricists neglect whether animals are able to count the number of current competitors, intruders or mates. Notwithstanding this shortcoming, ability to assess fluctuations in the number of rivals or mates regarding their motivation or quality is crucial to optimally allocating competitive effort. A large body of laboratory experiments, usually based on pre-training procedures, supports that animal species have evolved numerical abilities well beyond humans, including not only highly cognitive species such as apes (Boysen and Hallberg 2000), rats (Davis and Hiestand 1992), dogs (West and Young 2002), horses (Uller and Lewis 2009), cetaceans (Kilian et al. 2003), but also birds (Pepperberg 2006; Rugani et al. 2007), amphibians (Uller et al. 2003), fish (Agrillo et al. 2009) and insects (Carazo et al. 2009; Dacke and Srinivasan 2008). Evidence for numerical assessment of competitors in the field is still restricted to anecdotal, but thereby interesting, papers on adult lions (McComb et al. 1994), birds (Seddon and Tobias 2003) and frogs (Reichert 2011), which are shown to gauge the number of territory intruders from vocalisations or visual cues. In altricial species, young may be able to recognise the vocal signature of parents (Jacot et al. 2010). In crowded and dark nests or litters, offspring might also rely on siblings’ calls to discriminate the number of which that are currently competing and optimally adjust signalling level towards parents and / or siblings. Accordingly, in **Chapter V**, using a playback procedure, I test whether barn owl nestlings modulate negotiation effort not only to the total call rate of a brood, but also to fluctuations in both the number of call rates and motivation of nestmates.

CHAPTER I

CHAPTER I

Barn owls do not interrupt their siblings

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ABSTRACT

Animals communicate with conspecifics to resolve conflicts over how resources are shared. Since signals reflect individuals' resource holding potential and motivation to compete, it is crucial that opponents efficiently transmit and receive information to optimally adjust competing effort to each other Acoustic communication is particularly flexible as it can be modulated according to background and social feedback. Diverse mechanisms have evolved to minimise acoustic signal interference, one being the avoidance of signal overlap by adjusting the timing of call production to alternate each others' calls. Though avoidance of interference would be particularly important to resolve competition among relatives, its occurrence has been barely studied. Here, we investigated overlap avoidance in barn owl young siblings (*Tyto alba*), who vocally negotiate with each other the share of food provided by parents. Using both correlative and playback approaches, we found that owlets overlapped their live siblings' and broadcasted calls at least five times less often than expected at random. We conclude that behaviour to reduce signal interference has evolved to optimise communication among kin.

Key-words: animal communication, overlap, sibling negotiation, signal interference

INTRODUCTION

Animals are often in conflict over limited resources. In order to reduce the cost of physical competition, animals communicate among each other their motivation and their competitive ability to contest resources (Maynard Smith 1982; Parker 1974). Provided that communication entails costs, and hence reliably reflects signallers' motivation to compete, the individuals that invest more effort in signalling have priority access to resources. Individuals facing competitors that display a high motivation are more likely to give up the contest for which the outcome is predictable (Parker 1974). This phenomenon is reinforced when competitors are kin (Hamilton 1964), since a less motivated individual derives benefits by giving up a contest not only because it avoids competing for an unlikely outcome, but also because the contested resources are consumed by a genetically related individual. During a contest, to advertise their motivation to compete, body condition or social status, conspecifics need to efficiently emit signals, but also to perceive the signals of opponents. The avoidance of signal interference is thus important component of animal communication.

Animals can communicate using different channels. Acoustic communication is particularly interesting because animals can modulate vocal signalling rapidly in relation to environmental and social cues (e.g. Remage-Healey and Bass 2006), and they have the possibility to adopt a large range of signalling strategies (e.g. Todt and Naguib 2000). A very important aspect of acoustic communication is that it can be blurred by background noise and conspecific interference. In various animal species, mechanisms have evolved to ensure that signals of different individuals can be discriminated by conspecifics. For instance, the human auditory system has the ability to discriminate between different speakers in a crowd even when the sounds are produced simultaneously, the so-called cocktail party problem (Bee and Micheyl 2008). Among species of insects, frogs and birds that vocalise in large groups or in noisy environment, individuals shift their call features to avoid overlapping in frequency, so that their vocalisations differentiate from others and are thereby distinguishable (Narins and Zelick 1988; Römer and Bailey 1998; Slabbekoorn and Peet 2003). Emission of acoustic signals in groups can also be set by temporal organisation rules (Ficken et al. 1974; Gerhardt 1994) and antiphonal calling has been documented groups of bats (Carter et al. 2008) and in numerous mated birds (Stokes and Williams 1968). Some primates can even detect and wait for silent windows to vocalise (Versace et al. 2008). This temporal organisation leads to an alternation of vocal signals reducing the risk of individuals calling simultaneously.

A particular situation occurs when individuals communicating between each other belong to the same family. Although a previous study suggested that siblings would avoid overlapping their vocalisations when parents are away (Chaiken 1990), no experimental test has been performed. In some altricial species, siblings vocalise in the absence of parents to communicate their motivation to compete among each other (Bulmer et al. 2008; Johnstone and Roulin 2003; Roulin et al. 2000). This so-called sibling negotiation can be considered as a form of cooperation between related individuals, since its primary function is to reduce the level of sibling competition. Accordingly, a game-theoretical model showed that sibling negotiation is more likely to evolve in species in which nestmates are full- rather than half-siblings and when the cost of sibling competition increases (Johnstone & Roulin 2003). Such vocal exchanges are usually not heard by parents and therefore, cannot be interpreted as a form of begging behaviour that evolved to convey honest information to parents (Roulin et al. 2000). In the barn owl (*Tyto alba*), the single food item brought by a parent is indivisible and only one offspring is fed per parental feeding visit. Therefore, each nestling should invest more effort in negotiation when its chance of outcompeting its siblings increases (Johnstone and Roulin 2003). Hence, it is essential that each nestling assesses the level of competitiveness and motivation of all surrounding siblings, to optimally invest effort into sibling competition. Hungrier individuals signal their higher motivation to compete for the next impending food item to nestmates by vocalising at a higher rate with long calls. This vocal behaviour induces siblings to reduce their vocalisation and momentarily withdraw from the contest over the impending food item (Dreiss et al. 2010b; Roulin 2002a). Thus, in the absence of parents, nestlings need to hear and be heard, to optimally adjust investment in sibling competition once parents arrive with food. During a single night nestlings can produce thousands of calls, implying that this communication system may be costly in terms of energy and time invested in calling (Roulin 2002a). Thus, to maximise transmission of vocal signals between siblings, we propose that nestlings avoid calling simultaneously otherwise they may have to call even more often to transfer the same amount of information.

Here, we report an experimental test of this hypothesis of overlap avoidance in nestling barn owls, by studying free vocal interactions between pairs of nestlings (i.e. dyads). Siblings differ in age due to a pronounced hatching asynchrony, which results in asymmetries in the competitiveness of nestmates and different vocal behaviours. Given their stronger competitive abilities, seniors are usually less prompt to invest in vocalisations and are less sensitive to the vocal behaviour of their junior siblings (Roulin 2004a). We thus recorded naturally occurring vocal interactions between pairs of siblings that were either both food-deprived or both food-satiated, each pair comprising one senior and one junior owlet. We tested whether the degree to which an individual avoids calling simultaneously as its siblings varies with motivation, i.e. their level of hunger, and with competitiveness, i.e. between juniors and seniors, and with development stage (i.e. absolute age).

In such a design, each owlet produces calls at variable rhythm and hence the observation of siblings not calling simultaneously may simply result from the fact that siblings have different time-dependent activity patterns. Hence, to further tackle the hypothesis that barn owl siblings actively avoid calling simultaneously, we performed additional playback experiments with variable call rate. In natural vocal interactions, owlets produce calls at variable rhythm, from loose clusters of rapid calls to a relatively regular rhythm (see for instance Roulin et al. 2009). We thus analysed the vocal response of singleton nestlings to two different playback experiments in which we either broadcasted negotiation calls at random (unpredictable) time points, and at a constant (predictable) rhythm, within the natural ranges. If competing barn owl nestlings minimise acoustic signal interference, we expect that individuals call simultaneously as their sibling or the playback less often than expected at random, regardless of the rhythm at which calls are emitted.

METHODS

General procedure

The study was performed in western Switzerland (46°4'N, 6°5'E) on a population of wild barn owls breeding in nest-boxes located in barns. When nestlings were 18- to 45-days old (mean ± s.e.: 34.5 ± 0.3), we brought them to the university where they vocally behave as in nature (Roulin et al. 2009) and were not physiologically stressed (Dreiss et al. 2010a). We always left one or several nestlings in the natural nest to make sure parents did not abandon their brood. At that age, owlets are thermo-independent and able to eat prey items without maternal help. For these reasons, the mother is not sleeping with her offspring during the daylight hours and comes back only at night to deliver food items. Thus, we carried out our laboratory experiments on offspring that were used to the mother's absence during the day.

Recording setup

Dyadic vocal interactions between pairs of siblings

In 2008, we recorded 78 pairs of siblings (21-45 days old, 83 males, 71 females, 2 individuals of unknown sex) issuing from 41 different broods, implying that more than one pair of siblings was sometimes used per nest. Siblings were hosted during three nights in an experimental nest-box similar to the one where they were reared in natural conditions (62 x 56 x 37 cm), except that the box was divided into two areas by a thin wooden wall pierced with five holes at the top (fig. 1A). We placed one nestling on each side of the wall, randomly chosen, so that siblings could hear each other without visually or physically interacting. Each pair of nestlings comprised a senior individual and a junior sibling, which was on average 5 days younger (range: 1-15 days). After a first night of acclimation, each pair of siblings was recorded over two nights in a row, from the beginning of the night (19h30) until 23h30. One night we food-deprived the nestling pair (no food given during the preceding 28 hours) and the other night we food-satiated them (from 00h00 to 16h00 on the recording day we offered 130 g of laboratory mice, i.e. 3-4 mice, which exceeds their daily food requirement of about 67 g; Durant and Handrich 1998), with the order of the two treatments being randomly assigned in time across pairs. Individuals that were starved on the first night were randomly chosen, since their mean body mass at the start of the experiment was similar to the mean body mass of individuals receiving the *ad libitum* treatment the first night (Student's t-test: $t = 0.63$, d.f. = 202, $P = 0.53$).

As in natural conditions (pers. obs.), it happened that one of the two individuals did not vocalise during the 4.5 hours-recording period. Among the 78 pairs of siblings this occurred in 10 pairs (12.8%) when food-deprived and 20 pairs (25.6%) when food-satiated. Since our goal was to study vocal interactions between two individuals, we excluded these pairs of siblings to perform statistical analyses (table 1).

Table 1. Summary of the laboratory experiments used to assess the occurrence of call overlap in nestling barn owls. For each experiment, we analysed the recordings for which both siblings of a pair called, or the singleton nestlings responding to a playback vocalised.

Experiment	**Year**	**Total number of nestlings**	**Number of recorded nestlings**	**Number of broods**	**Hours of recording**	**Number of calls recorded per nestling** (mean ± s.e.)
Natural vocal interaction between food-deprived siblings	2008	156 (78 pairs)	136 (68 pairs)	41	19h30-23h30	1136 ± 70
Natural vocal interaction between food-satiated siblings	2008	156 (78 pairs)	116 (58 pairs)	41	19h30-23h30	895 ± 88
Unpredictable playback	2009	54	48	16	21h00-00h45	59 ± 3
Predictable playback	2010	96	65	26	21h00-01h30	62 ± 3

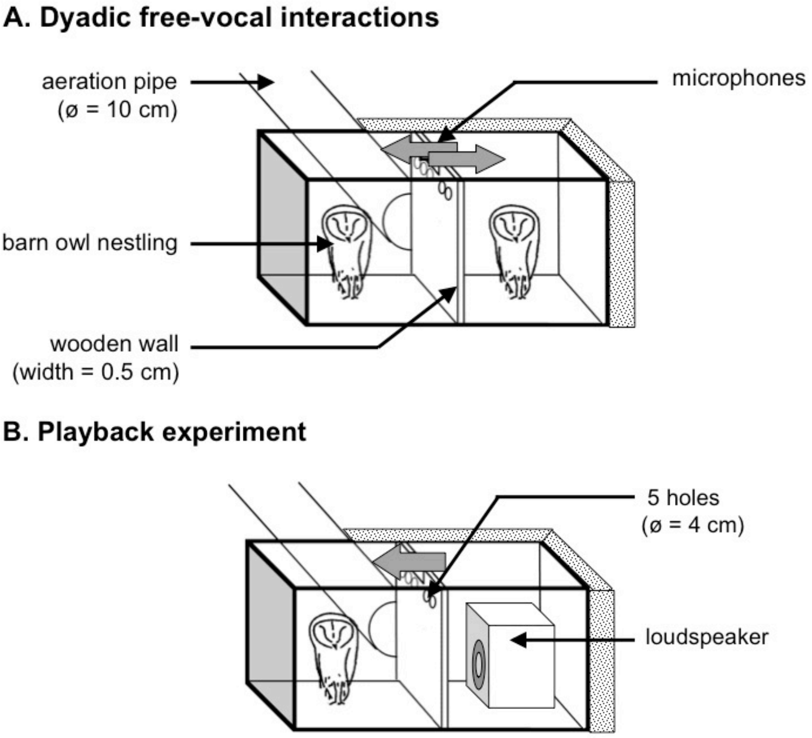

Figure 1. **Experimental nest boxes in which barn owl nestlings were recorded (A) in pairs of siblings and (B) individually while responding to playback soundtracks.**

Playback experiment on singleton nestlings

In 2009 and 2010, we brought free-living nestlings to the university for the playback experiments, in the same rooms as the ones where we recorded dyadic vocal interactions (fig. 1B). Nestlings were placed alone in one side of the same experimental nest-boxes, with a loudspeaker (near05 experience, ESI Audiotechnik GmbH, Leonberg, Germany) in the other side to broadcast a pre-recorded playback sequence. In 2009, we broadcasted pre-recorded calls at unpredictable time points (hereafter denoted “unpredictable playback”) to 54 nestlings (27 to 44 day-old, 20 males, 33 females, 1 individual of unknown sex) from 16 different broods. Six of the 54 nestlings (11.1%) did not call during the recording session taking place between 21h00 and 00h45. In 2010, we broadcasted calls at a constant rhythm (hereafter denoted “predictable playback”) to 96 nestlings (18 to 41 days old, 47 males, 48 females, 1 individual of unknown sex) from 26 broods. 31 of them (32.3%) did not vocalise during the recording session taking place from 21h00 to 01h30. In both the unpredictable and predictable playback experiments, nestlings were not fed between the preceding morning 08h00 until the start of the experiment at 21h00 as it usually happens in natural conditions. The experiments occurred on the third night nestlings were hosted at the laboratory for the unpredictable playback (2009) and on the second night for the predictable playback (2010). These differences are unlikely to create a difference between years in nestlings’ stress level, as it does not differ from baseline level from the second night (Dreiss et al. 2010a).

Playback soundtracks

In 2009, we broadcasted these calls to 54 single nestlings at three different rates (2, 6 and 10 calls per minute) and three different durations (0.6, 0.8 and 1 sec). These values of call duration and call rate correspond to the mean values and to the lowest and highest 10% of the distributions observed in the two-chick broods. These two acoustic variables are the most important parameters used in sibling negotiation (Dreiss et al. 2010b; Roulin et al. 2009). We built nine playback sequences lasting 15 minutes each, which were separated from one another by 10 min silence. These sequences correspond to the combination of the three different call rates, with the three different call durations. We inserted calls randomly and thus unpredictably in the soundtracks, except that two successive calls were separated by at least 1 sec interval. A pause of one sec or less between two successive calls produced by the same individual was observed in only 0.03% of the cases (mean ± SD = 15.3 ± 106.4 s, range = 0.7 – 7259.2 s).

In 2010, we broadcasted sequences of 20 min each to 96 singleton nestlings at the same three different rates (2, 6 and 10 calls per minute), each sequence containing the calls of 1, 2 or 5 different playback individuals, with the calls of different individuals being allocated randomly in the playback sequence (in each sequence all playback individuals produced the same number of calls). By broadcasting the calls from one to five playback individuals, we tried to mimic the several siblings naturally present in the nest (up to 9; Dreiss and Roulin 2010). This experiment, thus consisted of nine sequences of 20 minutes each, separated by 10 min silence, corresponding to the combination of three different call rates by three different numbers of playback individuals. Calls were inserted in a random order, but at regular time intervals; every 30, 20 or 6 sec to obtain playback sequences that differ in call rate. Detail methods for the construction of playback are presented in Supplementary material.

Statistical analyses

We considered that nestlings overlapped a call of their counterpart (their sibling or the playback) when they initiated their calls before the call of their counterpart ended. Negotiation calls are simple noisy sounds with unique temporal characteristics (see fig.10 in Bühler and Epple 1980). Means are provided ± standard error.

Propensity of overlapping siblings' calls

We used a randomization procedure to investigate whether nestlings overlap each other differently than if they call at random time points. For each vocal exchange between pairs of siblings recorded in 2008, we randomised the calls produced by both siblings with respect to time of call onset. Duration of the vocal exchange as well as the number and duration of the calls were thus kept unchanged. Since barn owl nestlings can naturally produce two successive calls with barely any pause between them (we recorded five pauses of 0 to 0.1 sec among 10,000 recorded pauses), we did not constrain this randomisation with respect to pause duration between two calls of the same individual. The randomisation of the calls of each sibling of a pair was repeated 1,000 times to generate a null distribution of call overlaps for each pair of siblings. This null distribution was then compared with the observed call overlap in the pair of siblings to calculate a *P*-value. Global probability of overlap across all pairs of siblings was determined with a Fisher combined probability test. We analysed junior and senior separately in order to have the seniority effect and because they are not independent. We corrected for multiple testing with Bonferroni (see Results). We also performed distinct analyses for pairs of food-deprived and food-satiated siblings, as each nestling was recorded in both states.

Propensity of overlapping broadcasted calls

We applied a similar randomization procedure to assess whether nestlings avoid overlapping the broadcasted calls. For each playback sequence, we randomised the calls produced by the focal nestling with respect to time of onset 1000 times and we compared the obtained null distribution of call overlap with the playback calls to the observed call overlap in the experiment. To analyse independent data in Fisher combined probability tests, so that each nestling appears only once per test, we performed separated analyses for each nine combination of playback treatment (call rate and call duration for unpredictable playback; call rate and number of individuals for predictable playback).

Influence of age and seniority on the propensity of vocally overlapping others

We investigated the effect of age on the proportion of nestling calls that overlapped the calls produced by a sibling during vocal exchanges between pairs of siblings. For this aim, we ran a generalised mixed model with a binary error distribution and a logit link, with as dependent term the number of calls produced by the focal nestling that overlapped a call of its counterpart, divided by the total number of calls produced by the focal nestling, using SAS v.9.1 (SAS Institute Inc., Cary, NC, USA). We included the independent term age and the cofactors food treatment and seniority, i.e. whether the nestling was the junior (younger) or the senior (older) of the pair. To control for the repeated measurements per broods, pairs of siblings and nestlings, we included nestling identity nested in the identity of sibling pair and in the broods from which individuals were issued as random factors.

Ethical note

Removing several nestlings from a nest during two or three nights never induced parents to abandon their brood. We always left one or two nestlings in the natural nest and we had already observed that parents do not adjust feeding rate to short-term variations in food need (Roulin et al. 2000). Nestlings were transported in opaque aerated plastic boxes, with foam floor. In the laboratory, nestlings were not physiologically stressed, as shown by the absence of a rise in baseline corticosterone level compared to the situation prevailing under natural, undisturbed conditions (Dreiss et al. 2010a) and we did not observe behavioural signs of stress. Blood samples (around 20 µL) for corticosterone assay were taken by puncturing the brachial vein and collecting the blood with heparinised capillary tubes (see methdos and results details in Dreiss et al. 2010a). We feed nestlings with laboratory mice *Mus musculus* euthanized by CO_2, bought frozen from an animal house (Reptiles Farm, Servion, Switzerland). Barn owls can naturally fasted for one or two nights when the weather is bad (personal observations). Keeping owlets at the university did not negatively affect their body condition since mean body mass at fledgling and survival up to fledgling did not significantly differ between nestlings brought to the university and nestlings left in their nest (Wilcoxon test on body mass at fledgling stage of recorded and non-recorded siblings: $Z = 1.6$, $P = 0.11$ [recorded: 358 ± 2 g; non-recorded: 353 ± 3 g] and on mortality: $Z = 1.6$, $P = 0.10$ [recorded: 10 % of mortality; non-recorded: 16 %]). The experiments were approved by the veterinary services of Canton de Vaud (Form No 2109.1).

RESULTS

Siblings avoid vocally overlapping each others' calls

Food-deprived and food-satiated nestlings overlapped the calls of their siblings (who were in a similar food state) in only 1.04 ± 0.13 % and 0.81 ± 0.04 % of the cases, respectively, which is 7.3 and 4.6 times less often than expected at random (fig. 2; Fisher combined probability tests: $\chi^2 = 908$ and 897 for junior and senior food-deprived siblings respectively; $\chi^2 = 553$ and 594 for junior and senior food-satiated siblings; all $P < 0.0001$). Food treatment, absolute age and seniority (junior or senior) did not have any significant effect on the proportion of observed overlapped calls (generalised mixed model, food treatment: $F_{1,95} = 2.12$, $P = 0.15$; absolute age: $F_{1,95} = 0.75$, $P = 0.39$; seniority: $F_{1,95} = 2.89$, $P = 0.09$). If we removed the term "absolute age" from the model, the variable "seniority" remained not significant ($P = 0.08$).

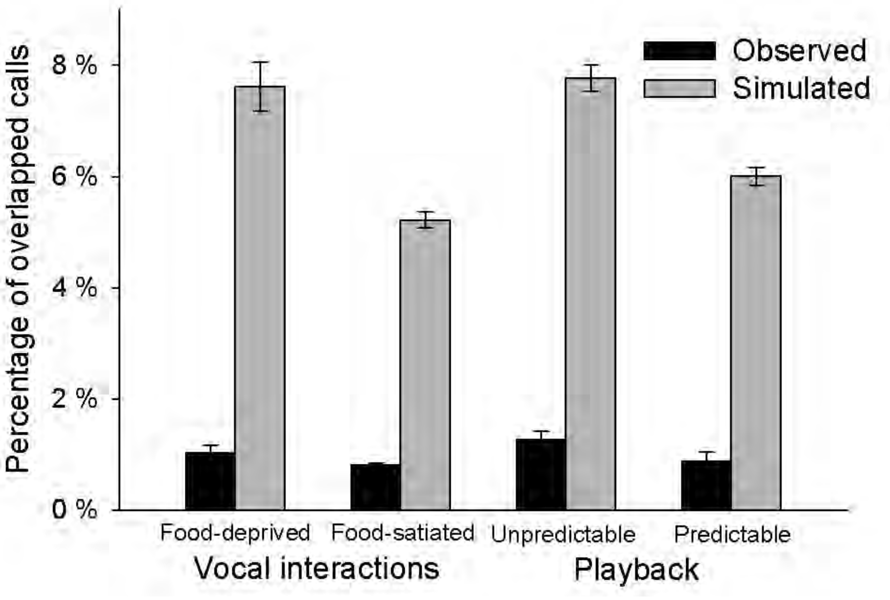

Figure 2. Observed (black) and expected (grey) percentage of overlapped calls (± s.e.) of barn owl nestlings. By definition, a nestling produced "overlapped calls" when it starts the emission of a call before a call of its sibling or before a broadcasted call has ended. Nestlings either exchanged vocalisations with a sibling during free vocal dyadic interactions, in a food-deprived or food-satiated state, or responded to pre-recorded playback sequences containing calls inserted at an unpredictable random timing or, at a predictable fixed timing. In all situations, observed call overlap was significantly lower than expected at random.

Nestlings avoid vocally overlapping playback calls

The proportion of calls that overlapped playback calls was 1.27 ± 0.15 % and 0.88 ± 0.16 % for unpredictable and predictable playbacks respectively, which is 4.7 and 8.8 times less often than expected at random (fig. 2; Fisher combined probability tests for the 9 sequences of the unpredictable playback: all $\chi^2 > 142$; all $P < 0.005$ [*P*-value threshold for 9 tests according to Bonferroni], except for the sequence that comprised 2 calls/min and calls of 0.6 sec: $\chi^2 > 104$; $P = 0.061$; for the 9 sequences of the predictable playback: all $\chi^2 > 199$; all $P < 0.005$, except for the 3 sequences that comprised 2 calls/min: $\chi^2 > 111$; $0.005 < P < 0.04$). At random, the proportion of calls that overlapped the playback during the sequences containing 2 calls/min was on average 2.4 ± 0.3 %. This very low proportion explains why the difference between observed overlap and random overlap was not significant.

DISCUSSION

Here we show that nestling barn owls, which are known to vocally negotiate among each other for food resources delivered by parents (Roulin 2002a; Roulin et al. 2000), have developed a mechanism to avoid overlap of their vocal signals. Using both correlative and playback approaches, we found that nestlings preferentially alternate their calls in time rather than overlapping them. This phenomenon of overlap avoidance suggests that accuracy of signal transmission is essential in this sibling communication system.

Our results show that nestlings do not vary in the extent of overlap avoidance according to their age, which could reflect dominance status, or hunger level, which reflects motivation to compete for food. This absence of variation across individuals and food states suggests that overlap is not used as an aggressive signal, to deter competitors from calling, contrary to some observations in songbirds (Todt and Naguib 2000). Alternating acoustic signals, referred to as antiphonal patterns, enables interacting individuals to avoid signal interference and thus the jamming of information contained in signals. In some communication system, the end of calls is more variable and thus carries more information than the beginning (Todt and Naguib 2000). An individual that overlaps a counterpart vocal signal, may benefit from masking its competitor signal, without losing much of its own signal information (Hultsch and Todt 1982). This is not the case in the barn owl where calls are simple noisy sounds, thus the same information is conveyed at the beginning as at the end of calls (Bühler and Epple 1980). Overlappers that start calling before their counterpart has terminated its call would thus suffer the same signal masking than their overlapped counterpart, and thus hinder similar information loss. Call duration is an important features of communication between sibling barn owls (Dreiss et al. 2010b; Roulin et al. 2009), and overlap would thus blur this signal.

In sibling negotiation process, although individuals are in conflicts for the same resource, two elements promote the evolution of overlap avoidance: the importance of signal exchange for food sharing and the relatedness between competitors (Johnstone and Roulin 2003). Genetically related sibling should assess each other motivation to obtain the next delivered prey item, thus resolve peacefully the conflict over food sharing. Vocal overlap would blur the signal and the negotiation system would be less efficient. Furthermore, the avoidance of overlap should be particularly developed in vocal exchanges between kin, because kin selection would promote the evolution of altruistic behaviour. Sibling negotiation can be considered as a form of altruism between related individuals, since its primary function is to reduce the level of sibling competition. In the barn owl where offspring mainly compete with full-sibling (Roulin et al. 2004). A game-theoretical model showed that sibling negotiation is more likely to evolve in species in which nestmates are full- rather than half-siblings and when the cost of sibling competition increases (Johnstone & Roulin 2003). By avoiding signal overlap, individuals let their needy relatives the opportunity to transmit their signals.

Sibling competition is particularly important in offspring that still depend on their parents for food resources (Wright and Leonard 2002) and they often share the same acoustic space to signal their motivation to compete. Offspring have to efficiently signal their need towards their parents, which allocate food among their progeny based on these signals (Godfray 1995b). Social interactions between siblings play an important role on how each individual communicates with family members. Accordingly, several studies have shown that individuals not only adjust their level of signalling to their own needs, but also to the signalling level of their siblings (Blanc et al. 2010; Smith and Montgomerie 1991). These adjustments can be interpreted as means to increase individual signal efficiency. For instance in meerkats (*Suricata suricatta*) pups beg one after the other to avoid interference with littermates (Madden et al. 2009) and this increases adult provisioning. Alternatively, change of signalling behaviour when facing a nestmate can be a form of altruistic behaviour. For instance nestling barn swallows *Hirundo rustica* moderate their begging when facing related rather than unrelated nestmates, suggesting that kin selection modulates sibling competition (Boncoraglio et al. 2009). In the barn owl where offspring mainly compete with full-siblings (Roulin et al. 2004), nestlings may refrain from calling to let their sibling the opportunity to call and/or favour their own signal efficiency.

The present study highlights an interesting characteristic of the communication system of young birds still dependent on their parents. Nestlings favour the accuracy of acoustic communication over loud vocal demonstration. Systems in which related individuals exchange signals that participate in enhancing fitness would thus favour the development of mechanisms to avoid call overlap.

Acknowledgements

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Supplementary material

Acoustic recordings

We simultaneously recorded vocal interactions of pairs of siblings using two microphones (MC930, Beyerdynamic GmbH & Co KG, Heilbronn, Germany) oriented in opposite directions, each facing one bird and connected to a multichannel recording system managed by Cubase v.5.2 software (Steinberg Media Technologies GmbH, Hamburg, Germany). The two recording soundtracks were analysed with Matlab v.7.7 (MathWorks, Natick, MA, USA.) to assign calls to each individual of a pair and to measure precise timing of call onset and call duration (see Matlab script below).

We recorded the vocal response of singleton nestlings responding to playback using a single microphone placed in its direction. We again used Matlab v.7.7 to assign calls to the singleton nestling and the playback and to measure the timing of call onset as well as the duration of each call (see script below).

Construction of playback experiments

We extracted the calls used to build playback soundtracks from the dialogs of siblings pairs recorded in 2008. Calls were selected for their duration and not modified, except for magnitude, which was standardized using Audacity v.1.3 Beta freeware (http://audacity.sourceforge.net). This manipulation did not affect other acoustic parameters. Measuring variation of amplitude necessitate positioning the individual at a constant distant from the microphone, which is biologically difficult. Hence, the recorded calls differ in amplitude especially because individual were not at the same distance from microphone, as they freely move in the box. We have thus corrected for this difference in amplitude.

We built playback sequences in both years using an automatic program in Matlab v.7.7 (MathWorks, Natick, MA, USA.) that inserted the calls at random in the experiment performed in 2009 (unpredictable playback) or at constant intervals in the experiment performed in 2010 (predictable playback).

Experiment session 2 – Unpredictable playback experiment

We selected calls from 16 nestlings, with 30 calls each (6 males, 10 females; aged 28 to 45 days), corresponding to 10 calls of three different categories of call duration. Across the 9 sequences played-back to each singleton nestling, we used the calls from two different individuals out of the 16 possible ones, which we chose randomly, with one sequence of 15 minutes always containing the calls of one single playback individual. To avoid pseudo-replication (Kroodsma et al. 2001), The playback soundtracks broadcasted were unique, as call order and timing and the order of the nine sequences were randomised for each singleton nestling.

Experiment session 2 – Predictable playback experiment

We selected 10 calls of 0.6 sec from 16 nestlings (6 males, 10 females; aged 29-45 days). We built a unique playback soundtrack for each nestling by randomising the order of the nine sequences and the order of the calls of playback individuals in each sequence.

CHAPTER II

CHAPTER II

Social adjustment of multiple vocal signals in nestling barn owls (*Tyto alba*)

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This chapter is being prepared for publication.

Experimental design: personal contribution
Data collection personal contribution, Amélie N. Dreiss
Acoustic analysis: personal contribution, Arnaud Da Silva
Statistical analysis: personal contribution
Writing of the article: personal contribution, with Alexandre Roulin

ABSTRACT

Young animals compete for parental resources by displaying various acoustic signals that may differentially affect the outcome of sibling competition. We propose the hypothesis that young should primarily compete using the vocal component that is most closely associated with hunger level. We tested this hypothesis in the barn owl (*Tyto alba*) in which nestlings vocally compete by producing more calls of longer duration than siblings to negotiate priority access to the indivisible prey item their parents will deliver next. Because food-deprived nestlings increase call rate proportionally more than call duration compared to when sated, an individual should invest more effort in call rate than call duration in order to influence sibling behaviour, and therefore call rate should be more sensitive to variations in the intensity of sibling competition. To singleton nestlings, we broadcasted calls of varying durations at different rates. When hearing calls at higher frequency or of longer duration, bystanders reduced vocalisation rate to a larger extent than call duration. Variation in the rate at which we broadcasted calls also influenced bystanders' responses to a greater extent than variation in the duration of calls. Young animals thus actively and differentially use multiple signalling components to compete with their siblings over parental resources.

Key-words: begging, call rate, call duration, multiple signalling, negotiation**,** competition

INTRODUCTION

Offspring typically request more resources than parents are willing to provide (Trivers 1974). This parent-offspring conflict is thought to have promoted the evolution of honest signals of need by offspring allowing parents to optimally adjust provisioning rate and allocate food among them (e.g. Teather 1992; Glassey and Forbes 2002; Whittingham et al. 2003; Rosivall et. al 2005; Tanner et al. 2008). Social interactions also play a prominent role in how young animals adjust solicitation level, since the parental decision to feed a particular young depends on the behaviour displayed by the other offspring. In the case where parents feed several offspring per visit, scramble competition triggers siblings to escalate begging behaviour relative to one another to obtain a larger than equal share of the parental resources (Godfray 1995). For instance, bird nestlings typically increase begging level when their nestmates become hungrier (Smith and Montgomerie 1991, Price and Ydenberg 1995, Leonard and Horn 1998). When parents feed only one offspring per visit, the individual that is the most motivated to compete will deter its siblings from competing since the outcome of the competition is predictable (Johnstone and Roulin 2003).

Multiple components of begging behaviour may have evolved to convey detailed information about fine-tuned variation in competitive interactions. For instance, call rate, duration, amplitude and frequency can indicate identity (Saino et al. 2003; Yasukawa et al. 2008; Quillfeldt et al. 2010; Reer and Jacot 2011), body condition (e.g. Gladbach et al. 2009), hunger level (Roulin et al. 2000; Marques et al. 2009; Reer and Jacot 2011), size (e.g. Sacchi et al. 2002; Roulin et al. 2009) and health (Saino et al. 2001). Although multiple begging components can be correlated to each other (e.g. Leonard et al. 2003), they differentially influence how food is shared among the progeny (Royle et al. 2002; Tanner et al. 2008). Because each single acoustic feature may not be similarly associated with an individual’s need and resource holding potential, young may differentially adjust acoustic features in relation to their siblings' behaviour. For this reason, animal offspring should differentially adjust the various components of begging signals to one another. In systems where the primary function of solicitation behaviour is to signal need to parents or siblings, we predict that the begging component most closely associated with the need for food is more sensitive to the prevailing social environment and also impacts sibling behaviour to a larger extent.

The barn owl (*Tyto alba*) is suitable to investigate the relative role played by different acoustic features in sibling competition. In this species, young not only beg from their parents but also vocally communicate among each other in the prolonged absence of parents to resolve the contest over access to the next indivisible food item that parents will deliver. Each nestling vocally informs its siblings about the willingness to compete over the impending indivisible food item. The hungriest individual is highly vocal, which deters siblings from begging conspicuously at the arrival of their parents. Owlets are said to vocally negotiate to reduce the level of competition taking place once parents have returned with food (Roulin 2002a). Published correlative results suggest that both the rate and duration of vocalisations play a major role in this sib-sib communication system. By producing longer negotiation calls a hungry individual would induce siblings to refrain from vocally negotiating and by emitting more negotiation calls it would induce siblings to refrain from begging once parents return with food (Dreiss et al. 2010b).

In the present study, we propose the hypothesis that to be successful in sibling negotiation nestlings have to invest more effort in the vocal component that is more finely modulated in relation to variation in hunger level. Because this component best signals hunger level and hence motivation to outcompete siblings, it should have a stronger influence on how sibling contests are resolved. In order to test this, we performed two experiments. First, we recorded vocal interactions at night in pairs of live siblings in which we manipulated hunger level by alternatively food-depriving them and offering them food *ad libitum*. This enabled us to investigate whether nestlings naturally increase call number to a larger extent than call duration (or the opposite) when in greater need. Secondly, we experimentally tested two predictions of the hypothesis that nestlings primarily compete by using the acoustic component that is more sensitive to their need. To do so, we recorded the rate and duration of vocalisations of singleton nestlings responding to pre-recorded calls of varying durations broadcasted at various rates. (1) We expected that bystander nestlings would primarily modulate the vocal component that most strongly reflects hunger level in relation to variation in the rate and duration of the broadcasted calls. Thus, when listening to more calls and calls of longer duration singleton nestlings should reduce the vocal component that is more closely associated with hunger level to a larger degree than other vocal components. (2) Variation in this playback component, rather than variation in the other component, should have a greater influence on vocal behaviour of bystander nestlings. For instance, if call rate is more sensitive to hunger level than call duration we would expect that when we broadcasted calls at a greater frequency, bystander nestlings should decrease the rate at which they call and the duration of their calls to a larger extent than when we broadcasted longer calls.

METHODS

Study site and animals

The study was performed in western Switzerland (46°49’N/06°56’E) in a population of wild barn owls breeding in nest-boxes. Parents hunt small mammals at night to feed their one to nine offspring. Once offspring are thermo-independent at two to three weeks of age, the mother begins to hunt in order to provision for the brood. We carried out the experiments after this age, when parents were naturally sleeping outside their nest-box in another barn, and before nestlings take their first flight at around 55 days. We estimated nestling age shortly after hatching by measuring the length of the left flattened wing from the bird's wrist to the tip of the longest primary (Roulin 2004b).

Experiment 1: differential effect of hunger on call rate and call duration

To investigate whether call rate or call duration is more sensitive to variation in hunger level, we manipulated food supply in 98 nestlings, 51 males, 45 females and two individuals of unknown sex, issued from 35 broods in 2008 (c.f. Chap. III). When aged 25 to 45 days (mean ± SD: 35 ± 5 days) we brought them back to the laboratory in the afternoon to be kept in a similar wooden nest-box (100 x 60 x 50 cm) as the one where they were reared in natural conditions (fig.1A in Chap.I). Nestlings were not physiologically stressed, as shown by the absence of a rise in baseline corticosterone levels compared to the situation prevailing under natural, undisturbed conditions (Dreiss et al. 2010a). Nest-boxes were divided in two parts with a thin wooden wall pierced with five holes at the top so that two siblings, placed in each part of the box, could vocally communicate without interacting physically. We kept nestlings in these boxes for two days and three nights before taking them back to their original nest in the field. After a first night of acclimation, we analysed the vocal exchange of each pair of siblings from 21:00 until 23:40 on the second and third nights. On one of the two nights, chosen randomly, we food-deprived the two individuals (no food given during the preceding 28 hours) or food-satiated them (from midnight to 16:00 on the recording day we offered 130 g of laboratory mice, which exceeds their daily food requirement of about 67 g). Over 24 hours food-deprived individuals lost on average 42 ± 1 grams and when fed *ad libitum* they gained 16 ± 2 grams. To avoid superfluous disturbance, we manipulated nestlings only once per day at 16:00 and opened nest-boxes again at midnight to add food.

Experiment 2: relative role of call rate and call duration on sibling negotiation

In 2009, we brought 19 male and 35 female nestlings issued from 15 broods to the laboratory at 16:00. They were 35 ± 4 days of age (range: 25-44). We hosted them in a similar wooden nest-box as in 2008, except that an individual was placed in one side of the box, while we put a loudspeaker (near05experience, ESI Audiotechnik GmbH, Leonberg, Germany) in the other side behind the wooden separation (fig.1B in Chap.I). At 9:00 on the first morning following their arrival, we provided food *ad libitum* and the second morning at 09:00 we removed the remaining mice, so that nestlings were food-deprived until 21:00 when we started the playback experiment.

We broadcasted nine playback sequences in a row, each sequence lasting 15 minutes, with periods of 10 minutes of silence between two sequences, as described in fig. 1. The nine sequences corresponded to the nine combinations of calls of three different durations (0.6, 0.8 and 1.0 second) broadcasted at three different rates (2, 6 and 10 calls/min). These values correspond to the mean values and to the lowest and highest 10% of the distributions observed in the two-chick broods recorded in 2008 (fig. 2). To avoid pseudo-replication, we built a unique soundtrack of 9 sequences for each bystander individual. We allocated the nine combinations in a random order, except that we limited the possibility that the bystander individual heard the same call rate and call duration in two consecutive sequences. We built the nine sequences using natural calls from two randomly chosen starved nestlings (donors I1 and I2 in fig. 1) recorded in 2008, out of 16 possible ones (6 males and 10 females; aged 28-45 days). We built the first three and last three sequences with the calls of donor I1, and the fourth, fifth and sixth sequences with the calls of donor I2. We used 10 possible distinct calls of a given duration (0.6, 0.8 or 1.0 sec) of a donor to build each sequence. Each of the nine sequences was built with 10 unique calls. We randomly inserted the calls within each sequence, with the constraint that two successive calls were separated by an interval of at least one-second. In the two-chick broods recorded in 2008, a pause of one second or less between two successive calls produced by the same individual was observed in only 0.03% of the cases (mean ± SD = 15.3 ± 106.4 s, range = 0.7 – 7259.2 s). We standardised intensity of playback calls using free Audacity software v.1.3 Beta (http://audacity.sourceforge.net), a procedure that does not affect other acoustic parameters.

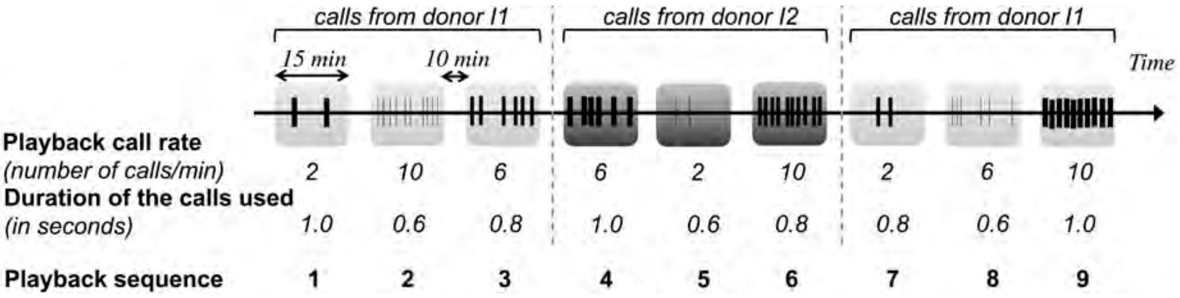

Figure 1. Design of the playback experiment. A unique combination of nine playback sequences lasting 15 minutes each and separated by 10 minutes of silence was broadcasted to each bystander nestling. These sequences corresponded to the nine combinations of calls of three different durations (0.6, 0.8 and 1.0 sec) broadcasted at three different rates (2, 6 and 10 calls/min). We used the calls of two pre-recorded individuals: donors I1 and I2, with 10 possible distinct calls from each donor in each of the three durations. We used the calls of I1 to build the three first and three last sequences, and of I2 to build the sequences 4, 5, and 6. For a given duration, the 10 calls of I1 used in the first three sequences were different from the 10 calls of the same duration used in the last three sequences. In this example, the 10 calls lasting 1.0 sec in the first sequence were different from the 10 calls of 1.0 sec used to build the ninth sequence.

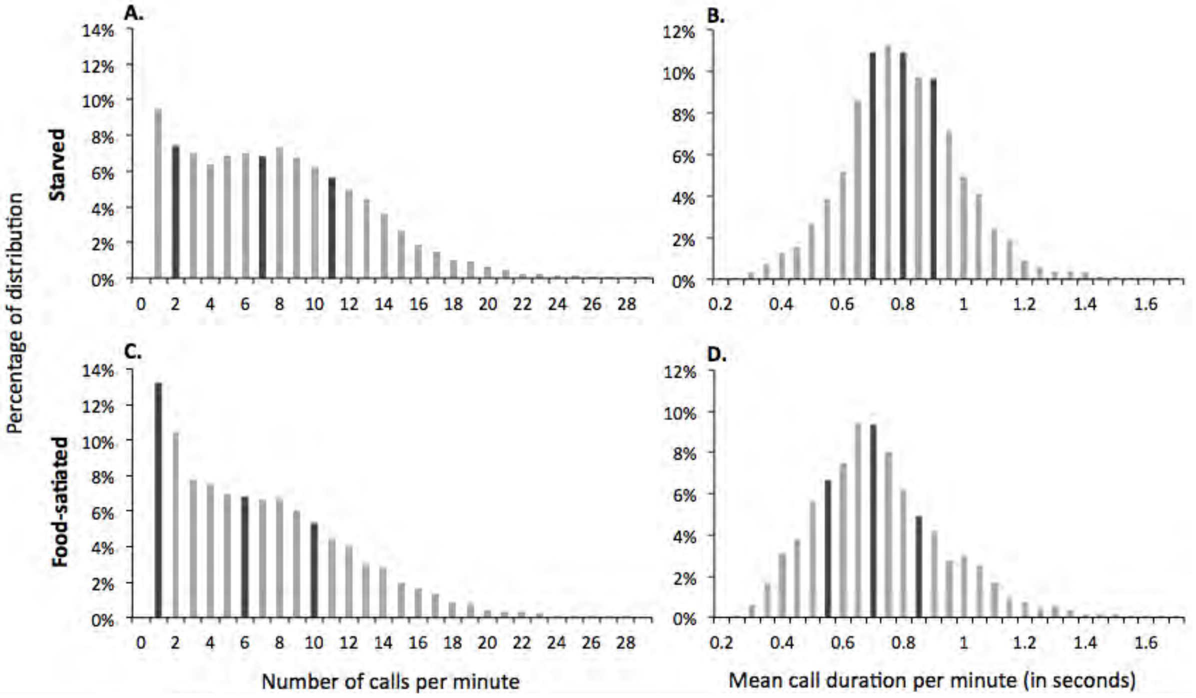

Figure 2. Distributions of call rate (A and C) and mean call duration per minute (B and D) in barn owl siblings interacting in pairs, which were alternatively starved (A and B) and satiated (C and D). Recordings were made between 21h00 and 23h40 and the 160-minute long soundtrack was divided in one-minute intervals. We then considered only those minutes during which nestlings produced at least one call. Sample size is 98 individuals from 49 pairs of siblings in each food treatment. In each diagram darker bars correspond, from left to right, to quartiles, i.e. values corresponding to 25, 50 and 75 % of the total distributions.

Acoustic analyses

In 2008, we recorded calls using two microphones (MC930, Beyerdynamic GmbH & Co KG, Heilbronn, Germany) oriented in opposite directions, each facing one nestling, and in 2009, we used a similar microphone oriented towards singleton nestlings. We could thus easily assign calls to each individual, or to the singleton nestling and the playback, based on intensity differences between paired soundtracks. We used Cubase® software versions 4 and 5.1 (Steinberg Media Technologies GmbH, Hamburg, Germany), to simultaneously record the two siblings placed in the same box in 2008 and to simultaneously broadcast the playback and record the vocal response of bystander nestlings in 2009. We measured call duration using a semi-automatic program in Matlab v.7.7 (MathWorks, Natick, MA, USA).

Statistical analyses

Experiment 1: differential effect of hunger on call rate and call duration

We analysed the relative effect of food supply on call rate and call duration in 49 pairs of nestlings (n = 98 individuals). For each individual and food treatment we calculated the number of calls and the mean call duration (in seconds). Because long periods during which an individual does not call will strongly reduce mean call rate but not affect mean call duration, considering these periods will blur the results. We thus divided the soundtracks recorded between 21h00 and 23h40 in one-minute intervals, and considered only those minutes during which nestlings produced at least one call to compute an overall mean call rate and mean call duration to be compared. We ran a generalised linear mixed model with Poisson error distribution to analyse the effect of food treatment on call rate and a linear mixed model to analyse its effect on mean call duration, using the GLIMMIX and MIXED procedure in SAS V9.2 (SAS Institute Inc., Cary, NC, USA), respectively. Both models included nestling identity nested in the experimental nest-box and in the brood where they were raised in the field as random intercept to control for pseudo-replication. As independent factors, we included food treatment (starved *vs.* satiated), the order of the treatment across the two nights of experimentation as well as their interaction.

To investigate whether nestlings differentially increase the rate and duration of their calls with hunger level, we computed the percentage of increase in call rate and in mean call duration per minute per individual between a starved and satiated state (i.e. difference in call rate between the two food states divided by call rate measured when food-satiated; similar procedure for call duration). We performed a within-individual pairwise comparison using a Student’s *t*-test.

Experiment 2: relative role of call rate and call duration on sibling negotiation

We first investigated the mutual effects of the rate and duration of broadcasted calls on the vocal response of bystander nestlings. To do so, for each of the 54 bystander individuals, we computed their call rate for each of the nine 15-minutes long playback soundtracks, i.e. the number of calls divided by 15 minutes, and the mean call duration (in seconds). Because in 90 recorded sequences nestlings did not call, we analysed more call rates (n = 486 sequences) than mean call durations (n = 396 sequences). To investigate the carry-over effect of each playback sequence, we also measured nestling vocal behaviour during the 10-minutes long periods of silence separating playback sequences. For each bystander nestling and for each of these 10-minutes long periods, we computed the call rate, i.e. the number of calls divided by 10 minutes of silence, and mean call duration.

We ran four separate linear mixed models for the vocal response (call rate and mean call duration) measured during the 15-minutes long playbacks and during the following 10-minutes long period of silence, using the MIXED procedure in SAS V9.2 (SAS Institute Inc., Cary, NC, USA), to quantify the immediate and carry-over effect of the playback treatments. In each model, we fitted call rate or mean call duration of bystander nestlings as dependent variable and included nestling identity nested in brood where they were reared in the field as random intercept. We also fitted the identity of the playback individuals used to generate sequences as an extra random variable. As independent variables, we included two factors (i.e. 3 levels of playback call rate and 3 levels of playback call duration) plus their interaction, and three covariates, namely the order at which each of the nine playback sequences were broadcasted, and bystander nestling sex and age. We initially included as covariates the age and sex of the playback individuals, but since these two covariates proved not significant, we removed them from the saturated models for the sake of clarity. To investigate the carry-over effect of the playback treatments on bystander call rate during the following silence, we also included as covariate the call rate of this bystander during the playback. Similarly, we included bystander mean call duration during the playback as covariate in the model of the bystander's mean call duration during the following silence. For all models, we performed backward model selection; final models only contained significant effects ($P < 0.05$), and main effects involved in significant interactions. Residuals were systematically checked for normality.

Linear mixed models enabled us to investigate the effect of variations in playback call rate and in playback call duration on bystander vocal response. In a second step, we compared the magnitude to which nestlings modulated call rate (and call duration) in response to variations in playback call rate and call duration. Finally, we only considered the extreme playback values, i.e. when we broadcasted 2 and 10 calls/min and when we broadcasted calls lasting 0.6 and 1.0, leaving out the playbacks of 6 calls/min and 0.8 sec. We adopted this procedure because the change in bystander vocal response was linear across the three-levels for both call rate and mean call duration. We thus computed the percentage of change in bystander response, i.e. call rate or mean call duration, as the average call rate (or average call duration) for the playback at 2 calls/min minus the average call rate (or average call duration) for the playback at 10 calls/min divided by the average call rate (or average call duration) for the playback 10 calls/min. In each case, nestling average call rate was computed over all the three possible playback call durations, since interaction between both the playback call rate and the playback call duration proved non significant in linear mixed models. A similar procedure was applied for playback call durations of 0.6 and 1.0 sec. Then, across all individuals we compared the within-individual percentage of change in average call rate and in average call duration according to varying playback call rates and according to varying playback call durations using a Wilcoxon Signed Rank test. Similarly, we compared the percentages of change in bystander average call rate and in its average call duration according to playback call rates and then according to playback call durations.

RESULTS

Experiment 1: differential effect of hunger on call rate and call duration

Food-deprived owlets produced more calls than food-satiated ones (mean ± SE = 8.30 ± 0.04 *vs.* 7.54 ± 0.05 calls per minute; linear mixed models, food treatment: $F_{1,24651} = 711.5$, $P < 0.0001$; order of food treatment: $F_{1,24651} = 132.8$, $P < 0.0001$; interaction: $F_{1,24651} = 0.2$, $P = 0.63$; fig. 2). Owlets also produced longer calls when food-deprived than food-satiated (0.818 ± 0 0.002 sec *vs.* 0.750 ± 0.002 sec; food treatment: $F_{1,24651} = 2787.2$, $P < 0.0001$; order of food treatment: $F_{1,24651} = 505.8$, $P < 0.0001$, interaction: $F_{1,24651} = 0.03$, $P = 0.83$; fig. 2). The within-individual percentage of increase in call rate from a food-satiated to a food-deprived state was proportionally greater than the percentage of increase in call duration (34 ± 6% *vs.* 14 ± 2%; Student's *t*-test, $t_{97} = 3.8$, $P = 0.0003$, $n = 98$ nestlings).

Experiment 2: relative role of call rate and call duration on sibling negotiation

1. Immediate vocal adjustment by bystanders during the 15-minutes long playbacks

During the playback both the duration of the broadcasted calls and the rate at which they were broadcasted exerted a significant effect on the bystander's call rate and on its call duration (table 1). When we broadcasted calls at a higher rate and for a longer duration, we observed a linear reduction in the rate at which nestlings vocalised and in the duration of their calls (table 1; fig. 3). Owlets produced 0.7 ± 0.2 (estimate ± SE*;* $P = 0.002$) and 1.5 ± 0.2 ($P < 0.0001$) fewer calls per minute when hearing 10 compared to six and two calls per minute respectively (table 1, effect of 'PB call rate', fig. 3A). Similarly, when hearing calls of 1.0 second, nestlings emitted 0.3 ± 0.2 (*P* = 0.09) and 0.9 ± 0.2 (*P* < 0.0001) fewer calls per minute than when playback calls lasted 0.8 and 0.6 seconds (table 1, effect of ’PB call duration’, fig. 3C). Owlets also produced calls that were shorter by 0.04 ± 0.01 second (*P* = 0.0004) when hearing 10 compared to 2 calls/min (table 1, ‘effect of PB call rate’, 10 *vs*. 2 calls/min: *P* = .23; fig. 3B), and of 0.02 ± 0.01 (*P* = 0.07) and 0.03 ± 0.01 second (*P* = 0.01) when the broadcasted calls lasted 1.0 compared to 0.8 and 0.6 second respectively (table 1, ‘effect of PB call duration’; fig. 3D). The effects of the rate at which calls were broadcasted and of the duration of playback calls on the nestlings’ response were independent from each other, as shown by the absence of significant interaction between these two factors (table 1, both *P* > 0.09). The sequence order covariate indicates that with time bystanders increased both the rate at which they called and the duration of their vocalisations (table 1, both *P* ≤ 0.007, estimate ± SE = 0.1 ± 0.03 calls/min, 0.01 ± 0.02 second).

To deter a sibling to negotiate an individual could therefore produce many long calls. However, a high call rate appears to be more efficient than long calls as suggested by the following two arguments. First, variation in the rate at which calls were broadcasted exerted a stronger effect on nestling vocal behaviour than variation in the duration of broadcasted calls. Accordingly, owlets were more dissuaded to vocalise when hearing higher call rates than longer calls (within-individual pairwise comparison between the percentage of decrease in nestling mean call rate while hearing calls broadcasted at 2 and 10 calls/min and the decrease while hearing broadcasted calls of 0.6 and 1.0 sec, Wilcoxon Signed Rank test, V = 800, *P* = 0.03, *n* = 48 nestlings). Similarly, they shortened their calls much more when hearing calls broadcasted at a higher rate than longer calls (similar comparison of the percentage of decrease in nestling mean call duration: V = 786, *P* = 0.04). Second, call rate of singleton bystanders was more sensitive to variations in our playbacks than was their call duration. Owlets reduced the rate of their vocalisations to a higher magnitude than their duration in response to an increase in both playback call rate and call duration (within-individual pairwise comparison between the percentage of decrease in bystander mean call rate and mean call duration, while hearing calls broadcasted at 2 and 10 calls/min, V = 987, $P < 0.0001$; while hearing calls of 0.6 and 1.0 sec: V = 932, $P = 0.0004$).

Table 1. Linear mixed models on call rate and mean call duration of bystander barn owl nestlings during the 15-minute long playbacks and the 10-minute long period of silence following the playback. Models are based on a total of 486 observations for call rate, 396 and 385 for mean call duration during playback and silence, respectively. The 54 nestlings were issued from 15 nests. Nestling identity nested in brood where they were raised in the field was fitted as a random intercept, as well as the identity of the playback individual from which we used the calls. Model selection was based on a stepwise elimination of non-significant effects, beginning with interactions. Estimates are indicated for significant playback effects ($P < 0.05$).

	nestling call rate (calls / min)		nestling call duration (sec)	
Fixed effects	F_{df}	*P*-value	F_{df}	*P*-value
	DURING PLAYBACK			
Nestling sex	$F_{1,412} = 4.2$	0.04	$F_{1,325} = 0.4$	0.55
Nestling age	$F_{1\ 412} = 9.1$	0.003	$F_{1,325} = 1.9$	0.17
Sequence order	$F_{1\ 412} = 11.6$	0.0007	$F_{1,325} = 43.5$	<0.0001
Playback (PB) call rate	$F_{2,412} = 28.5$	<0.0001	$F_{2,325} = 6.7$	0.001
PB call duration	$F_{2,412} = 9.3$	0.0001	$F_{2,325} = 3.4$	0.04
PB call rate x PB call duration	$F_{4,408} = 0.9$	0.44	$F_{4,321} = 2.0$	0.09
	DURING SILENCE			
Call rate / duration during playback	$F_{1,414} = 156.8$	<0.0001	$F_{1,304} = 56.7$	<0.0001
Nestling sex	$F_{1,411} = 0.0$	0.98	$F_{1,300} = 0.0$	0.92
Nestling age	$F_{1,414} = 6.3$	0.01	$F1_{,302} = 1.8$	0.18
Sequence order	$F_{1,413} = 1.2$	0.27	$F_{1,304} = 12.0$	0.0006
PB call rate	$F_{2,414} = 21.6$	<0.0001	$F_{2,302} = 1.4$	0.26
PB call duration	$F_{2,411} = 0.2$	0.79	$F_{2,300} = 0.1$	0.89
PB call rate x PB call duration	$F_{4,407} = 0.4$	0.84	$F_{4,296} = 0.8$	0.56

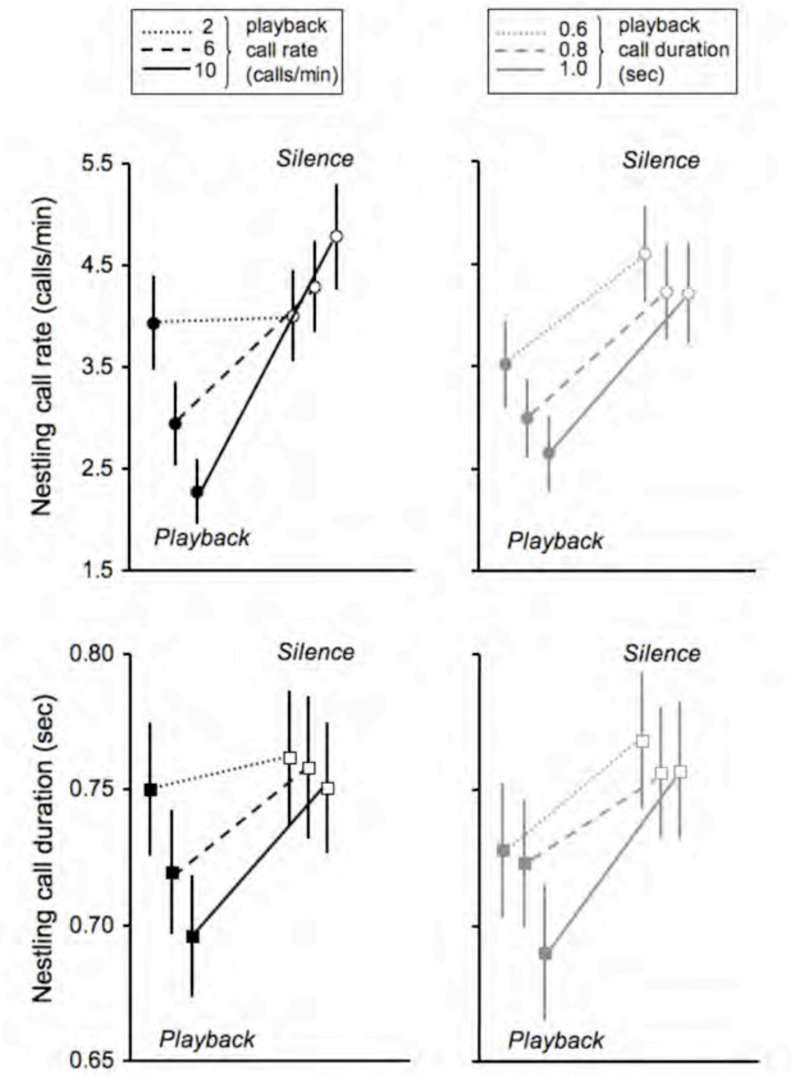

Figure 3. Vocal response of singleton barn owl nestlings exposed to playbacks of pre-recorded nestlings. Mean call rate during the 15-minute long playback (filled circles) and the 10-minute long period of silence just after the playback was stopped (open circles) and mean call duration (filled and open squares), according to the three broadcasted call rates (black, A and C) and call durations (grey, B and D). Interactions in final linear mixed models between playback call rates and durations being non significant (table 1), the average for each broadcasted call rate was computed over the corresponding three call durations for each individual pooled together. A similar procedure was applied for call duration over the corresponding three call rates. Means are given ± SE and were computed over 54 nestlings issued from 15 nests.

2. Carry-over effect of the playback on bystander vocal behaviour during the 10-minutes long periods of silence

As can be seen in fig. 3, for all but one playback sequence, nestlings increased the rate at which they called as well as the mean duration of their calls after the playback ended (Wilcoxon Signed Rank tests for within-individual pairwise comparison of nestling’s call rate and mean duration during and after a playback sequence: $P \leq 0.002$). When hearing 2 calls/min bystander nestlings maintained a similar call rate and duration (both $P > 0.14$). Nestlings that produced many calls of longer duration during playback also emitted longer calls at higher frequency during the silence that followed (table 1; effect of nestling call rate during playback on the call rate during silence: +0.56 ± 0.04 call/min; effect of call duration during playback on the call duration during silence: +0.34 ± 0.04 sec, both $P < 0.0001$). Independently, nestlings still significantly modulated their call rate during the silence following the playback in function of the rate at which we had broadcasted calls (table 1, effect of 'PB call rate'). Interestingly, the effect of variation in playback call rates on nestling call rate during the silence was the reverse compared to when calls were being broadcasted (fig. 3). On average, after having heard 10 calls/min, bystanders emitted 0.8 ± 0.2 ($P = 0.0002$) and 1.5 ± 0.2 ($P < 0.0001$) more calls per minute than after having heard 6 and 2 calls/min. The increase in call rate between the period when bystanders were listening to the playback and the period just after the playback ended was thus proportional to the rate at which we broadcasted the pre-recorded calls (linear mixed model with increase in mean call rate during each of the nine sequences as dependent variable, i.e. nestling call rate during silence minus call rate during playback: nestling identity nested in brood of origin and identity of the playback individual as random intercepts; effect of playback call rate: $F_{2,414} = 93.4$, $P < 0.0001$; effect of nestling call rate during playback as covariate: $F_{1,414} = 22.1$, $P < 0.0001$; the playback call duration, the order at which each playback sequence was broadcasted, nestling sex and age were not significant: all $P > 0.10$). This was the only carry-over effect of the playback, since after we stopped broadcasting calls nestlings did not adjust the duration of their vocalisations to the rate and duration of the playback calls previously heard (table 1; fig. 3B, C and D).

DISCUSSION

We investigated the role of multiple components in vocal signalling, i.e. number and duration of calls, in the resolution of sibling competition over the share of parental food resource. In the barn owl, nestlings vocally compete with each other in the absence of parents over the next indivisible food item to be delivered. Previously published correlative data revealed that by producing more calls of longer duration hungrier owlets deter their less needy siblings from begging food from parents (Roulin et al. 2001; Roulin et al. 2009; Dreiss et al. 2010b). Accordingly, pairs of siblings increased both the rate and duration of their vocalisations when they were food-deprived compared to when they were food-satiated, showing that both components reflect nestling need and motivation to compete over food resources. Furthermore, bystander individuals reduced both the rate and duration of their calls when responding to playbacks of longer calls broadcasted at higher rates. To our knowledge, only the playback study by Marques and colleagues (2011) in tree swallow chicks (*Tachycineta bicolor*) experimentally demonstrated that young birds actively use the acoustic features of their siblings' begging calls to adjust their own signalling level, yet the authors investigated a single vocal component, i.e. call intensity. Here, our findings clearly show that young may actively use multiple acoustic components to compete with each other over the sharing of parental resources. Our results also indicate that they adjust distinct components in relation to the competitive situation, but the component that best reflects variations in the current need being more finely adjusted than the other vocal component.

Experimentally starved, barn owl nestlings increased call rate to a larger extent than call duration compared to when sated. This indicates that the number of calls is a more reliable signal of food requirements than the duration of calls and thus indicates a greater motivation to outcompete siblings. As a consequence, we predicted that call rate should play a more important role in the resolution of sibling competition than call duration. Accordingly, our playback experiment revealed that bystander nestlings modulated the number of their vocalisations in function of the rate and the duration of broadcasted calls to a larger degree than the duration of their vocalisations. Complementarily, the rate at which we broadcasted pre-recorded calls exerted a stronger influence on how bystanders vocalised than variation in the duration of the broadcasted calls. Our study therefore suggests that in the barn owl, the most important vocal component to outcompete siblings is call rate followed by call duration. This conclusion is consistent with a previously published correlative study showing that producing longer calls deters siblings from vocally negotiating in the absence of parents. In contrast, producing more negotiation calls more directly affects the outcome of sibling rivalry, since it induces siblings to refrain from begging food from parents, which directly influences which offspring is fed first (Dreiss et al. 2010b). Studies performed in other species have shown that young nestlings adjust multiple begging components in relation to hunger level, but the exact function of each single component is usually not entirely clear (e.g. Iacovides and Evans 1998; Villasenor and Drummond 2007; Roulin et al. 2009). Our experimental study thus adds new information in this context. Although several components of begging behaviour may redundantly signal food needs honestly, they may not necessarily be used interchangeably given that they differentially affect the outcome of sibling competition. This is likely the case in most animals, since offspring concurrently adjust vocal and physical behaviours, such as their position relative to the location where parents predictably deliver food in the nest and the intensity of postural and vocal begging (e.g. Kacelnik et al. 1995; Leonard et al. 2003). Research should thus focus on the interplay between the multiple components of begging within and across sensory modalities to understand their relative function.

Assuming that the number of vocalisations suffices to deter siblings, why do barn owl nestlings modulate call duration even if it is *a priori* a redundant and apparently weaker signal of need than call rate? This issue is particularly interesting given that we did not detect any interactive effect of variations in playback call rate and call duration on the vocal response of bystander nestlings, but rather the effect of these two components was additive. A first possibility is that if the cost of increasing call number is too high, nestlings may instead lengthen the duration of their calls. In this case, call duration might act as a “backup” signal allowing nestlings to “spread the load” over these two signal types (Johnstone 1996; Rowe and Guilford 1999). Since producing many long calls is likely to be costly (e.g. Clutton-Brock and Albon 1979; Vannoni 2009), another possibility is that call duration may act as a “reinforcement” signal of endurance (Payne and Pagel 1996; Rowe and Guilford 1999). Owlets may jointly adjust call rate and call duration to further signal their motivation to outcompete siblings. Because siblings challenge each other for hours, this joint modulation may vary through time depending on cues that remain to be identified. It would therefore be relevant to consider the dynamics of sib-sib interactions to identify the circumstances when call duration may be particularly important. Furthermore, investing in both call rate and call duration may be the only possibility for individuals with a low resource holding potential to compete with their stronger siblings. This proposition is consistent with the observation that the smallest individuals of a brood emit more calls of a longer duration than their older siblings, probably in an attempt to compensate for their lower physical ability to monopolise food resources (Roulin 2004a; Roulin et al. 2009). The duration of begging calls also appears to be an important component in nestling songbirds. Horn and Leonard (2008) showed that with time tree swallow nestlings converge towards similar call durations. Apparently, siblings do not synchronise their signalling behaviour to cooperatively enhance the total brood signal to further induce parents to increase provisioning rate. Thus, this convergence is more likely to be the result of siblings escalating begging relative to one another.

Interestingly, the present study also reveals that bystanders adjusted their vocal behaviour to variations in broadcasted call rates not only while hearing the playback, but also after the playback ended. Since this was independent of their own call rate during playback, this indicates that barn owls memorise the different features of their siblings' calls to optimally modulate their own vocal behaviour at least a couple of minutes later. Our findings are in line with predictions derived from the "sibling negotiation hypothesis", namely that a nestling will refrain from vocally negotiating to a larger extent if its siblings invest more effort in vocal sibling negotiation in order to save energy (Roulin et al. 2000; Roulin 2002a). Indeed, when listening to more intense playbacks bystander nestlings refrained from vocalising to a larger degree, but as soon as the playback ceased, the increase in vocal behaviour by these bystanders was proportional to the playback call rate. This suggests that in front of a highly competitive sibling, barn owl nestlings reduce their investment in vocal negotiation for later investing once the competitive sibling has eaten and hence momentarily withdraws from the competition. Previous studies in other systems also suggest that memory may be at work to optimise the energetic budget allocated to sibling competition. For instance, nestling birds are able to memorise the highest profitability zones where parents allocate food (Kölliker et al. 1998) or the competitive level they experience within a brood (Lotem 1998). Here, our playback experiments reveal that nestling birds can selectively memorise the most important acoustic components previously displayed by siblings, since we found that only the broadcasted call rate, but not call duration, still influenced the bystanders' vocal response after the playback ceased.

To conclude, our study demonstrates that sibling rivalry has promoted the evolution of complex multiple acoustic signals. The interesting issue here is that these signals are differentially related to food requirements, which raises a number of questions regarding their exact functional value. We focused on two components within the same sensory modality, i.e.

vocal signalling, but it would be worth analysing other sensory modalities (i.e. non-vocal signals). The finding that nestling barn owls listen to siblings and memorise this information to adjust their vocal behaviour later on is an original and neglected aspect of interactions taking place between family members. In particular, research should focus more deeply on how offspring encode information about their need and resource holding potential through both vocalisations and other non-vocal behaviours and actually use these signals to outcompete siblings.

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CHAPTER III

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Negotiation rules in an animal

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ABSTRACT

Animals compete over limited resources such as food or mates. Natural selection has favoured the evolution of behaviours and weapons to outcompete conspecifics or of negotiation to find a peaceful agreement about how resources should be shared. The dynamics of negotiation processes have received mainly theoretical developments in animals because of the difficulty of measuring and analysing the iterative transfer of information to reach a compromise. We show here that precise negotiation rules have evolved in the barn owl (*Tyto alba*) to vocally compete for the priority of access to food resources. Using several experimental approaches, we show that in the prolonged absence of parents siblings use organised rules to interrupt each other and finely adjust their vocal investment in relation to the temporal dynamics of their social interactions. These negotiation rules emerge repeatedly in barn owl families, are weakly sensitive to hunger and age hierarchy, and are similar in owlets interacting with a live sibling or pre-recorded playback sequences. Optimal adjustment of investment in a communication network between several negotiators is therefore not solved at a single instant but requires a dynamical relationship governed by specific rules.

Key-words: negotiation, dynamics, social interaction, communication network, *Tyto alba*

INTRODUCTION

Negotiation defines any situation that involves "a discussion or process of treaty with another (or others) aimed at reaching an agreement" (Oxford English Dictionary). This terminology is usually used for humans who bargain for resources and typically ends up with a compromise with each participant obtaining part of the resources (Nash 1950). A negotiation process, where each participant tries to obtain the largest portion of the pie as possible, requires repeated interactions since the behaviour of an individual at a given time point depends on past behaviours of the other participants. Evolutionary ecologists also use this concept to define situations where animals communicate to reach an agreement about how a resource should be shared or how to invest in a collaborative task (Johnstone and Hinde 2006; Johnstone and Roulin 2003; McNamara et al. 1999; Sirot 2012). Animals are faced with sequential decisions that can be seen as a negotiation process in many phases of their lives, during development when competing for resources with siblings (Johnstone and Roulin 2003) and during reproduction in intra-sexual competition (Patricelli et al. 2011), choice of partner or adjustment of parental care (McNamara et al. 1999). Each step of the process affects the outcome of the next, and the optimal decision at each stage depends on many factors. These tactical adjustments during sequential stages can be made via exchange of transient signals such as vocalisation or other behavioural cues. Negotiation is thus a dynamic process in which individuals have to decide with whom they interact in priority, the level of display and when to give up a contest (Patricelli et al. 2011).

Although scientists reckon the importance of the dynamic nature of communication to resolve conflicts, little is known about how animals organise their communication in a social network (Dobler and Kolliker 2009). Much remains to be done to identify the cues that stimulate an individual to start signalling and to pinpoint the factors that induce an individual to increase or decrease investment in signalling. Another neglected aspect is whether animals assess the absolute signalling level of conspecifics or the temporal dynamics of signal production to adjust their behaviour (Enquist and Leimar 1983; Payne and Pagel 1996). One of the rare studies on fitness consequences of signal dynamics has shown that around one third of the variation in male courtship success in satin bowerbirds *Ptilonorhynchus violaceus* was explained by the intensity of male displays, while another one third was explained by the male's ability to adjust its display intensity to female response during courtship (Patricelli et al. 2002). Therefore by examining only average signalling, researchers omit a major aspect of signal transfer, i.e. the temporal dynamics of signal production that could be seen as a signal in itself (Briffa et al. 1998; Van Dyk et al. 2007). Our aim here is to understand how animals modulate signals to one another to negotiate how resources are shared among them. More specifically, we aim at investigating the temporal dynamics of negotiation, since it is a central aspect of game theory (Enquist and Leimar 1983; Payne and Pagel 1996) with each individual iteratively adjusting its signalling level in relation to each other's behaviour. The idea here is to investigate how and when an individual decides to interrupt its conspecifics by producing a signal or how and when it decides to leave its conspecifics signalling on their own. Although game theory has dominated the way evolutionary biologists envision social interactions, the exact process leading animals to behave in a certain way at the end of a social interaction has hardly been investigated empirically (Briffa et al. 1998; Van Dyk et al. 2007). Studying the temporal dynamics of negotiation has therefore the potential to provide key elements about social animal behaviour. To this end we considered the barn owl as a model organism.

Barn owl nestlings compete intensely vocally at the nest during development. Each night an individual produces between 1,000 and 5,000 simple and noisy calls towards its sibling to negotiate priority access to the next indivisible mouse delivered by a parent (Roulin 2002a). Experimental and theoretical works have shown that nestling vocalisations during the expanded period of parent absence honestly signal hunger level. The hungriest and most vocal individual deters its siblings from responding and ultimately from begging at the parents arrival (Johnstone and Roulin 2003; Roulin 2002a). This sibling negotiation process adaptively reduces the intensity of sibling competition (Johnstone and Roulin 2003; Roulin 2002a). The aim of a nestling is thus to dissuade its siblings to vocalise, which maximises its chance of being fed at parental return (Johnstone and Roulin 2003). To dominate the vocal exchange and outcompete its siblings, an individual calls intensely, but it may be tempted to reduce vocal investment once competitor siblings give up the contest. However, as soon as this focal individual lowers vocal output, a challenger may start vocalising. The focal challenged individual may then resist and enter into confrontation with its challenger to keep its position. If resistance declines, the period of vocal confrontation (i.e. rapid vocal exchange) will end up with the challenger vocalising alone (i.e. monolog) until it is itself tempted to reduce vocal investment. This situation may prevail as long as a parent has not come with a food item. These predictions were proposed at the time when the “sibling negotiation hypothesis” was formulated (Roulin 2002a) but remain untested.

1. Dynamics of vocal negotiation in pairs of live siblings

To investigate when a barn owl nestling decides to interrupt its sibling by producing a call or decides to stop calling (i.e. turn-taking rules), we studied 68 isolated pairs of siblings that were both alternatively food-satiated or food-deprived in random order and which comprised an older (the senior) and a younger individual (the junior). Siblings were hosted in the laboratory in a nest-box similar to the one where they were reared under natural conditions, except that the box was divided into two areas so that they could vocally but not physically or visually interact with each other (fig.1A in Chap.I). From 19:00 to 23:40 we recorded their dyadic vocal interactions, which were similar to their natural behaviour (Roulin et al. 2009) since they were neither physiologically nor behaviourally stressed (Dreiss et al. 2010a).

Pairs of barn owl siblings communicated by alternating turns of successive periods of monologs where each individual called alone one after the other, or through frequent vocal alternation (fig. 1). Most calls (67%) were produced in the form of short to long monologs (i.e. more than 10 calls produced in a row without being interrupted by its sibling), which is 4% more often than would be expected by chance (paired *t*-test between observed and expected proportions of monologs for 68 pairs of siblings: $t_{67} = 3.96$, $P = 0.0002$). The alternation of calls between partners (i.e. vocal confrontation) may be necessary to settle the contest over which individual should become vocally dominant over its sibling (i.e. monolog).

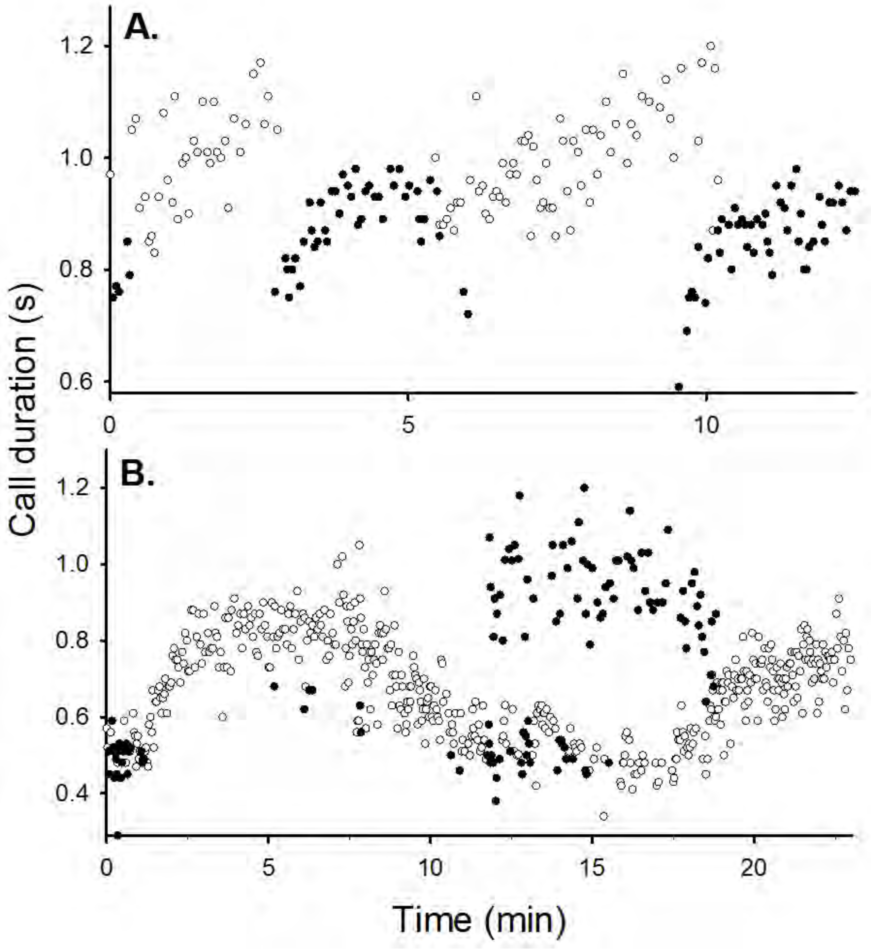


Figure 1. Two examples of vocal dyadic interactions between pairs of barn owl siblings. The symbol ● represents the senior nestling and ○ its younger junior sibling. Each data point stands for a call.

Table 1. Vocal confrontation in barn owl nestlings. Results of linear mixed models on the dynamics of call duration and pause duration between two successive calls in the course of vocal confrontation, between two barn owl siblings and between a singleton nestling and a pre-recorded playback sequence. Vocal production varies non-linearly, according to the vocal dominance of the focal nestling in the dialog, i.e. the proportion of calls this nestling produced in the preceding sequence of 10 calls. Food treatment (i.e. food-deprived *versus* food-satiated) and seniority (i.e. junior *versus* senior sibling) are also related to vocal behaviour in natural vocal interactions. For each analysis we indicate the corresponding figure. * *P* < 0.05, ** *P* < 0.001. *** *P* < 0.0001.

A. Call duration (fig. 2A)	**Natural vocal exchanges between pairs of siblings**		**Nestling responding to playback**	
Effects	**F**	**d.f.**	**F**	**d.f.**
Vocal dominance (V.d.)	**174.4*** **	**1,2215**	**89.1*** **	**1,368**
V.d.^{2}	**63.9*** **	**1,2215**	**43.0*** **	**1,368**
V.d.^{3}	**44.4*** **	**1,2215**	**27.5*** **	**1,368**
Food treatment (Food)	**62.2*** **	**1,2215**	-	-
Seniority	**11.9*** **	**1,2215**	-	-
V.d. x Food	**6.4* **	**1,2215**	-	-
V.d. x Seniority	**21.3*** **	**1,2215**	-	-
Food x Seniority	*0.8*$^{n.s.}$	*1,2215*	-	-
V.d. x Food	**14.2** **	**1,2215**	-	-
V.d.^{2} x Seniority	**16.9*** **	**1,2215**	-	-
V.d. x Seniority x Food	*0.7*$^{n.s.}$	*1,2214*	-	-

B. Duration of pause following one's own call (fig. 2B)

Effects	**F**	**d.f.**	**F**	**d.f.**
Vocal dominance (V.d.)	**22.9*** **	**1,1914**	**44.9*** **	**1,302**
V.d.^{2}	**9.7* **	**1,1914**	*0.1*$^{n.s.}$	*1,301*
V.d.^{3}	**9.9* **	**1,1914**	*0.2*$^{n.s.}$	*1,300*
Food treatment (Food)	0.1$^{n.s.}$	1,1914	-	-
Seniority	1.3$^{n.s.}$	1,1914	-	-
V.d. x Food	**13.8** **	**1,1914**	-	-
V.d. x Seniority	**5.8* **	**1,1914**	-	-
Food x Seniority	1.9$^{n.s.}$	1,1914	-	-
V.d.^{2} x Food	*1.7*$^{n.s.}$	*1,1912*	-	-
V.d.^{2} x Seniority	*1.2*$^{n.s.}$	*1,1912*	-	-
V.d. x Seniority x Food	**6.3* **	**1,1914**	-	-

C. Duration of pause following sibling's call (fig. 2C)

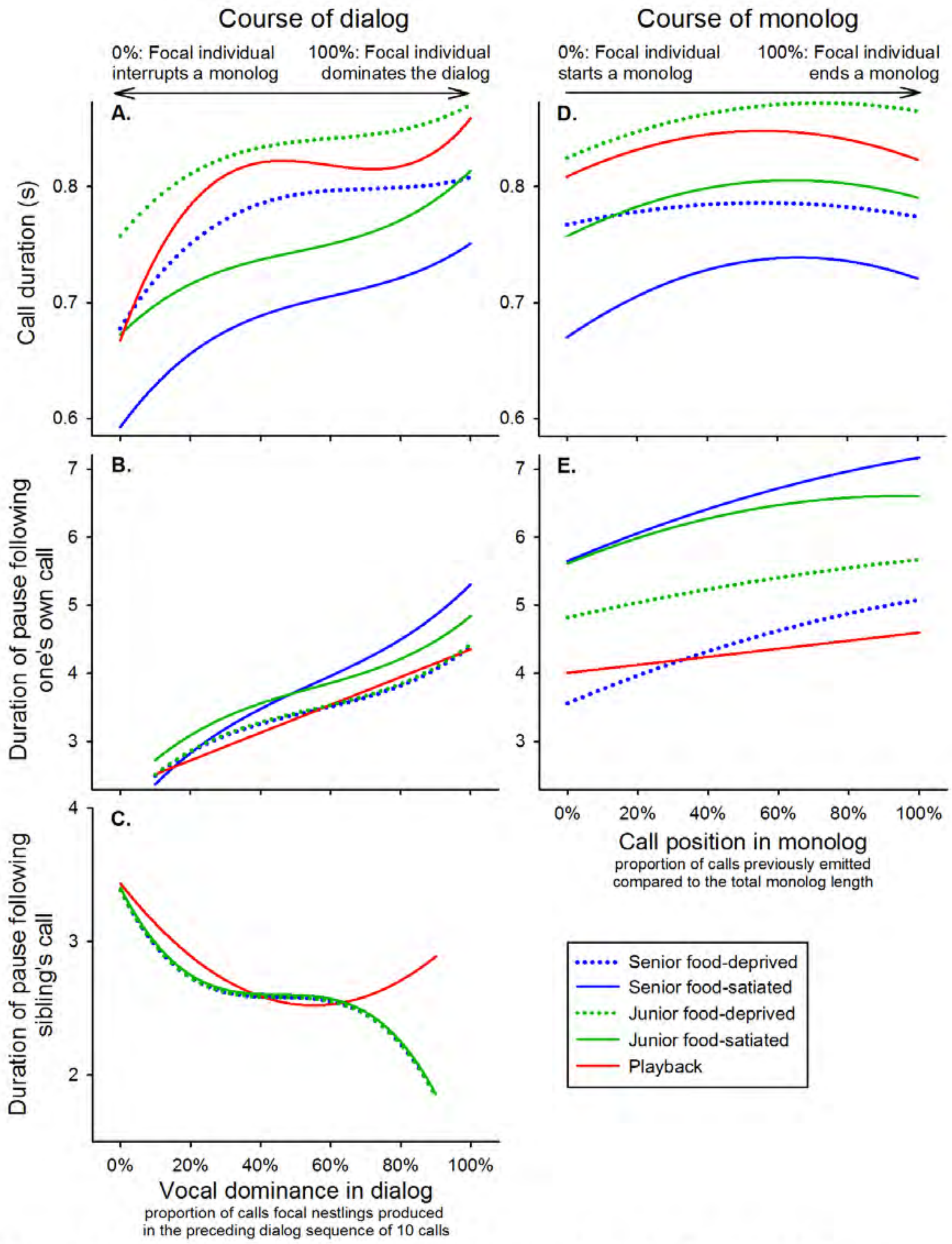
Effects	**F**	**d.f.**	**F**	**d.f.**
Vocal dominance (V.d.)	**33.8*** **	**1,1876**	**9.3** **	**1,317**
V.d.^{2}	**22.3*** **	**1,1876**	**5.4* **	**1,317**
V.d.^{3}	**22.3*** **	**1,1876**	*0.1*$^{n.s.}$	*1,316*
Food treatment (Food)	*0.1*$^{n.s.}$	*1,1876*	-	-
Seniority	*0.7*$^{n.s.}$	*1,1876*	-	-
V.d. x Food	*0.2*$^{n.s.}$	*1,1874*	-	-
V.d. x Seniority	*1.1*$^{n.s.}$	*1,1874*	-	-
Food x Seniority	*0.1*$^{n.s.}$	*1,1874*	-	-
V.d.^{2} x Food	*1.9*$^{n.s.}$	*1,1871*	-	-
V.d.^{2} x Seniority	*0.5*$^{n.s.}$	*1,1871*	-	-
V.d. x Seniority x Food	*2.0*$^{n.s.}$	*1,1871*	-	-

For the analyses of natural vocal confrontations between two live individuals, the two random variables were the order of food treatment (siblings were alternatively food-deprived and food-satiated on two successive nights in a random order) and focal nestling identity nested in both the pair of siblings (a given individual was always tested with the same sibling) and in the brood where it was raised in the field (nestlings were issued from 41 broods). For the analyses of singleton nestlings responding to playbacks, we included as random variables focal nestling identity nested in both the brood where it was raised by its parents (we tested 52 individuals from 16 broods) and the playback sequence (we broadcasted to each individual one of five different playback sequences). Statistical analyses were performed with a mean value per nestling and per degree of vocal dominance (0 to 100%, each 10%) over the entire vocal exchange. For each individual, we had a maximum of 10 mean values. Results of final models are written in bold; terms eliminated from saturated models are italicised.

How do individuals succeed in dominating the vocal confrontation before proceeding to monopolise the vocal exchange? The very first call of an individual interrupting its sibling's monolog was particularly short and was emitted with a relatively long latency after the end of the sibling's monolog. By contrast, within the course of the exchange, as an individual became more vocal than its sibling, it gradually emitted longer calls (fig. 2A; table 1A) and replied faster after a sibling's call (fig. 2C; table 1C); concomitantly it slowed down its own rhythm (fig. 2B; table 1B). These patterns were similar in seniors and juniors and weakly influenced by hunger level (fig. 2A-C; table 1).

Once an individual had monopolised the interaction, it vocalised alone in a monolog that could last up to 114 minutes and up to 1,591 calls, without being interrupted by its sibling. How did an owlet manage to keep vocalising alone over such long monologs? What cues did its sibling use to interrupt these monologs? To answer those questions, we analysed vocal behaviour from the start to the end of monologs containing more than 10 calls (mean ± s.e.m.: 39 ± 1). After having taken its turn an individual progressively produced longer (fig. 2D; table 2A) but fewer calls per minute (fig. 2E; table 2B) and just before its silent sibling vocalised again, calls became shorter (fig. 2D; table 2A). Hence, once an individual has monopolised the floor, it first increases and then progressively decreases investment in calling, until its sibling challenges it again with rapid vocal alternations. These temporal patterns in call duration and rhythm were found in seniors and juniors, and in both food-satiated and food-deprived siblings (fig. 2D-E, table 2).

(Figure 2, legend). Through the course of a vocal confrontation, as an individual became more dominant than its sibling (measured as the percentage of calls it produced out of the 10 previous calls of the dyadic interaction), it emitted longer calls (**A**), more spaced in time (**B**) and interrupted its sibling or the playback calls more rapidly (**C**). As an individual vocalised alone in a monolog, call duration (**D**) and pauses between successive calls (**E**) continued to lengthen. The silent nestling interrupted its vocal sibling or a playback's monolog once call duration started decreasing (**D**). Call features in a monolog (more than 10 calls produced in a row by a single individual without being vocally interrupted) is given in relation to the relative position of the calls between the start (0%) and the end of monologs (100%). The patterns were similar for senior (blue symbols) and junior siblings (green symbols) during natural vocal confrontations both when food-deprived (dotted lines) and food-satiated (solid lines), as well as for singleton nestlings interacting with the playback soundtrack (red symbols). Lines represent curves of the predicted fits from the mixed models presented in Tables 1 and 2.



Panels A-C: Dialogs between live individuals (green and blue curves) for 55,102 calls by 68 food-deprived seniors, 25,680 calls by 54 food-satiated seniors, 83,694 calls by 68 food-deprived juniors and 51,436 calls by 54 food-satiated juniors.
Vocalisation of 52 singleton individuals (red curves) facing a playback sequence (8,711 calls).

Panels D-E: Monologs of individuals interacting with a live sibling (green and blue curves) for 1,135 monologs (33,204 calls) by 68 food-deprived seniors, 545 monologs (21,665 calls) by 54 food-satiated seniors, 1,653 monologs (65,772 calls) by 68 food-deprived juniors and 1,005 monologs (48,324 calls) by 54 food-satiated juniors.
700 uninterrupted playback monologs (red curves) broadcasted to singleton individuals.

Figure 2. Negotiation rules in barn owl nestlings. (see legend p. 80)

Table 2. Monologs in barn owl nestlings. Results of linear mixed models on the dynamics of call and pause durations, between two successive calls, during monologs in barn owl nestlings. A monolog is a series of more than 10 calls produced in a row by a single individual without being interrupted by its sibling or produced by a pre-recorded playback sequence without being interrupted by the nestling listening to it. The duration of calls and time lapse between successive calls were analysed in relation to the position of the calls in the monolog (e.g. beginning, middle or end), food treatment (the two conversing siblings were both experimentally food-deprived or both food-satiated) and seniority (senior *versus* junior). For each analysis we indicate the corresponding figure.

	Natural vocal exchanges between pairs of siblings		**Nestling responding to playback**	
A. Call duration (fig. 2D)				
Effects	**F**	**d.f.**	**F**	**d.f.**
Call position in monolog (Position)	**220.0*** **	**1,2280**	**64.9*** **	**1,518**
Position^2	**138.8*** **	**1,2280**	**56.4*** **	**1,518**
Food treatment (Food)	**96.3*** **	**1,2280**	-	-
Seniority	**5.0* **	**1,2280**	-	-
Position x Food	**17.7*** **	**1,2280**	-	-
Position x Seniority	$0.2^{n.s.}$	1,2280	-	-
Food x Seniority	$0.1^{n.s.}$	1,2280	-	-
Position^2 x Food	**11.3** **	**1,2280**	-	-
Position^2 x Seniority	*$0.1^{n.s.}$*	*1,2279*	-	-
Position x Food x Seniority	**25.4*** **	**1,2280**	-	-

B. Pause duration between successive calls (fig. 2E)				
Effects	**F**	**d.f.**	**F**	**d.f.**
Call position in monolog (Position)	**11.2** **	**1,2248**	**25.5 *** **	**1,518**
Position^2	**1.5n.s.**	**1,2248**	*$1.1^{n.s.}$*	*1,517*
Food treatment (Food)	**60.2*** **	**1,2248**	-	-
Seniority	**3.0n.s.**	**1,2248**	-	-
Position x Food	*0.75n.s.*	*1,2247*	-	-
Position x Seniority	**4.1* **	**1,2248**	-	-
Food x Seniority	**7.8* **	**1,2248**	-	-
Position^2 x Food	*$0.5^{n.s.}$*	*1,2244*	-	-
Position^2 x Seniority	*$1.1^{n.s.}$*	*1,2244*	-	-
Position x Food x Seniority	*$0.19^{n.s}$.*	*1,2244*	-	-

* $P < 0.05$, ** $P < 0.001$. *** $P < 0.0001$. Same model construction as in table 1. Statistical analyses were performed with a mean value per nestling and per position of the calls in the monolog across all monologs (0%-beginning to 100%-end, each 10%) and for each individual we had 11 mean values. Results of final models are written in bold; terms eliminated from initial models are italicised.

2. Experimental evidence for vocal negotiation rules

To experimentally verify that sibling barn owls use these fluctuations of call features as negotiation rules to dose their vocal investment and to alternate their vocalisations, we performed a first playback test (fig.1B in Chap.I). We used five 84 minute-long recordings of vocal interactions between pairs of siblings, from which we erased the calls of one of the two individuals from the soundtrack. Such a playback experiment simulates a situation where a live nestling interacts vocally with an unfamiliar individual that does not adjust its vocal behaviour to its opponent. As expected, the 52 singleton nestlings to which we broadcasted the playbacks behaved in a similar way as individuals interacting with a live sibling (red lines in fig. 2A-C; table 1). Moreover, singleton owlets took their turn after the playback-calls became shorter again (red line in fig. 2D; table 2). As a consequence, the singleton nestling vocalised at similar time points as the individual erased from the playback (adequacy of call rate through time between the erased individual and the singleton nestling was significantly different from random permutation: Fisher's combined probability test $\chi^2 = 227$, $P < 0.0001$).

Hence the decision about the exact timing when an individual resumes vocal activities is associated with both increasing pause duration and decreasing call duration in the sibling's monolog (fig. 2D,E). To disentangle the mutual role of these two vocal cues, we performed a second playback experiment. One hundred and eight singleton nestlings could vocally answer to four two-minute-long playback sequences characterised by either increasing or decreasing call duration (final call duration is the same in the two treatments) combined with increasing or decreasing pause duration between successive calls (total number of broadcasted calls is the same in the two treatments). We recorded the vocal behaviour of singleton nestlings just after the playback ended during six minutes. This playback mimics a situation where an individual can produce a monolog after its sibling has stopped vocalising. After the end of the playback, singleton nestlings emitted more calls when the duration of the broadcasted calls had decreased along the soundtrack sequence compared to when call duration had increased, whatever the playback rhythm (table 3A, fig. 3A). Variation in pause duration between two successive broadcasted calls did not affect the number of calls produced by singleton nestlings following the end of the playback (table 3A, fig. 3A). However, nestlings vocalised sooner after the end of the playback when both duration and rate of the broadcasted calls were decreasing along the playback sequence (table 3B, fig. 3B).

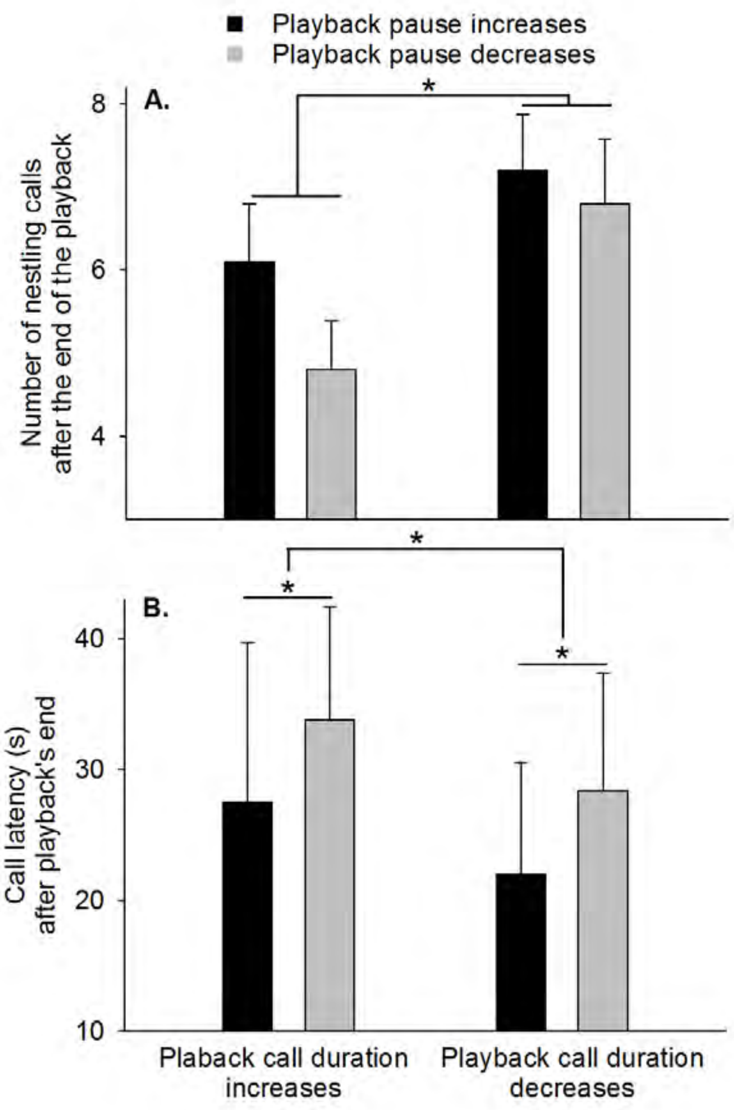


Figure 3. Calling decision after an experimental playback of varying call and pause duration. Latency (± s.e.) a nestling waited before calling after the end of a playback (**A**) and number of calls (± s.e.) produced by singleton nestlings the minute following playback's end (**B**). The two-minute-long playback sequences were composed of 20 calls of either continuously increasing duration or continuously decreasing duration separated by either continuously increasing pauses or continuously decreasing pauses. The four playback sequences were broadcasted in a random order and separated by a silence of six minutes.

Table 3. Calling decision after a playback of varying call and pause duration. Results of mixed models on the response of singleton nestlings to experimental two-minute-long sequences of calls of either continuously increasing duration or continuously decreasing duration and separated by either continuously increasing pauses or continuously decreasing pause.

A. Number of nestling calls following playback's end (fig. 3A)

Effects	**F**	**d.f.**
Order of the four playback sequences	*3.8$^{n.s.}$*	*1,126*
Number of calls produced by singleton nestling during the last minute of playback	**123.5*****	**1, 127**
Change in playback's call durations (increase or decrease) (CD)	**8.6****	**1,127**
Change in playback's pause durations (increase or decrease) (PD)	*0.3$^{n.s.}$*	*1,125*
CD x PD	*0.9$^{n.s.}$*	*1,124*

B. Call latency after playback's end (s) (fig. 3B)

Effects	**F**	**d.f.**
Order of the four playback sequences	**72.0*****	**1,175**
Change in playback's call durations (increase or decrease) (CD)	**75.2*****	**1,175**
Change in playback's pause durations (increase or decrease) (PD)	**2.9 $^{n.s.}$**	**1,175**
CD x PD	**83.9*****	**1,175**

* $P < 0.05$, ** $P < 0.001$. *** $P < 0.0001$. The number of calls produced by singleton nestlings the minute following playback's end was analysed using a linear mixed model and the latency singleton nestlings waited before calling after the end of a playback using a generalised mixed model with Poisson distribution. For both analyses, order of playback sequences was set as cofactor (the four sequences were broadcasted in a random order) and we set as random factors the individual used to build the playback sequence and the identity of singleton nestling nested in the brood where it was raised in the field (we tested 108 individuals from 33 broods). For the analyses of the number of calls, the number of calls the singleton nestling produced during the last minute of playback was set as covariate. Results of final models are written in bold; terms eliminated from saturated models are italicised.

CONCLUSION

In the present study, we have shown that the signalling of two individuals having a conflict of interest is not only determined by intrinsic quality (e.g. age) and internal state (e.g. hunger level), but also social negotiation rules. Theoreticians studying animal communication are debating how individuals settle contest and assess each other competitive ability (Enquist and Leimar 1983; Payne and Pagel 1996). In systems where opponents display signals iteratively, participants can monitor the behaviour of competitors (Briffa et al. 1998; Van Dyk et al. 2007) to decide when it is more appropriate to invest more effort in competitive interactions or to give up the contest. This decision could be based on the intensity of the most recent opponent's display, on its average level of signalling sustained over a given period of time or on the endurance of competitors in repeatedly producing costly signals (Payne and Pagel 1997). In the barn owl, where siblings exchange vocal signals over the prolonged absence of the parents, we experimentally show that it is rather the temporal dynamics in signal intensity that determine how competitors adjust signalling behaviour (fig. 3). The dynamics in the intensity of signals can thus be seen as a signal in itself. This is consistent with game theory postulating that the behaviour of a given individual at any given time depends on its past social interactions (Payne and Pagel 1997).

We show that barn owl nestlings use precise acoustic cues and rules to smoothly coordinate the temporal rotation of their vocal investment in a negotiation process. Because parents bring food at unpredictable time points and can be absent several hours (Roulin 2002b), a nestling has to dominate the vocal interaction for the longest possible period of time, to increase its chance of being fed. The nestling vocalising the most at parents’ return would most likely receive the prey (Roulin 2002a). Therefore, a nestling that has already invested substantial effort to become vocally dominant would lose this investment if vocally outcompeted by a sibling just before a parent arrives. This may explain why in the course of dominating a vocal confrontation, nestlings sharply interrupt their opponent (fig. 2C). When no more challenged, a vocally dominant barn owl nestling decreases pause duration between its own calls (fig. 2B, E), but maintains a high call duration (fig. 2A, D) to signal its willingness to compete and continue to dominate the vocal interaction. Once its call duration decreases, the opponent perceives this sharp decrease as a signal to re-enter the contest (fig. 2D, fig. 3). This dynamic of signal exchange appears adaptive for both siblings, because vocally dominant nestlings do not signal at maximal level and dominated opponents repeatedly control the honesty of the signal. We conclude that individuals constantly modulate vocal behaviour in order to optimise investment along the competitive process. The vocally dominant individual tries to minimise its investment just above the point where its opponent is willing to challenge it.

Here, we experimentally demonstrate the existence of turn-taking rules based on prosody, *i.e.* variation in rhythm, stress and pitch of vocalisation, in barn owl nestlings (*Tyto alba*). Although existence of rules coordinating vocal exchanges allowing each individual to know when to start vocalising could be *a priori* obvious, it has never been experimentally shown in competitive situations during long lasting interactions. This report is particularly interesting because it indicates that competition can promote the evolution of turn-taking rules in an animal that does not show particularly complex social interactions and in which individuals exchange simple noisy calls. In social science, conversation analysts postulate that humans adhere to implicit conversational rules that establish when to alternate turns or continue speaking (Duncan 1972; Jaffe et al. 2001; Sacks et al. 1974). Because non-human animals also exchange vocal signals that are crucial to resolve contests (Parker 1974), various species have also evolved mechanisms to maximise the efficiency of signals transfer, such as overlap avoidance (Ficken et al. 1974) and shift of frequency (Slabbekoorn and Peet 2003). Turn-taking rules, that allow each individual to know when and how to start signalling, have only been described in the particular context of duetting birds (Logue et al. 2008) and contact calls in some monkeys (Biben et al. 1986; Hauser and Fowler 1992), during exchange of very rapid phrase. As in human conversation (Stivers et al. 2009), the basic turn-taking negotiation rules demonstrated here do not depend on family, context of the interaction, age hierarchy or hunger level. As in human speech, the duration of one nestling utterance can be very long. It follows that, without turn-taking rules, competitors cannot presuppose when a monolog will end and whether and when they should interrupt it. Alternating vocalisation in competitive interactions can evolve because it increases signal efficiency (Madden et al. 2009), limits interference (Roy et al. 2011) and because individuals need to mutually and constantly assess their motivation level (Johnstone and Roulin 2003). Turn-taking rules, which predict when opponent will stop vocalising and leave an opportunity to vocalise, should thus be more widespread than previously thought in non-human animals. Such basic turn-taking negotiation rules may similarly coordinate communication in many animal species and help synchronise and alternate vocal signals.

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Supplementary material

METHODS

Study organism

We studied a wild population of barn owls breeding in nest-boxes located on barn walls located in Switzerland (46°4’N, 6°5’E). In 2008 clutches of 4 to 8 eggs were laid between 23 April and 6 August, in 2009, 2 to 10 eggs between 12 May and 16 August and in 2011, 4 to 9 eggs between 14 March and 22 July. Eggs are laid on average every 2.5 days and incubation starts after the first egg has been laid generating a pronounced age hierarchy among siblings. From 3 weeks of age onwards, nestlings are thermo-independent and able to eat prey items without maternal help. For these reasons, the mother usually stops sleeping with her offspring during the daylight hours and comes back only at night to the nest to deliver food items. We carried out our laboratory experiments on offspring that were used to their mother’s absence. Barn owls are mostly monogamous with very little extra-pair paternity: in a previous study one out of 211 offspring was not sired by the male that was feeding it (Roulin et al. 2004).

Recording of vocal interactions between pairs of food-satiated (*versus* food-deprived) siblings

In 2008, when nestlings were 22- to 45-day-old (mean ± s.e.: 35 ± 5), we brought 156 nestlings from 41 nests to the university; we always left one or several nestlings in the natural nest to make sure that parents did not abandon their brood. In the laboratory, we randomly matched siblings in pairs and housed each pair in a soundproof wooden nest-box, similar to the ones where they were reared under natural conditions. The box was divided into two equal parts by a thin wooden wall pierced with five holes at the top, so that siblings could hear each other without visually or physically interacting. Each pair of siblings comprised a senior individual and a 5-day younger junior sibling (range in age difference: 1-15 days). Nestlings were kept in these boxes during two days and three nights before being brought back to their original nest in the field. After a first night of acclimation, each pair of siblings was recorded twice from the beginning of the night until midnight, one night in a food-deprived state (no food given during the preceding 28 hours) and another night in a food-satiated (from 00:00 to 16:00 on the recording day we offered 130 g of laboratory mice, which exceeds their daily food requirement of about 67 g), with the order of the two treatments being randomly assigned across pairs. Food-deprived individuals lost on average 42 ± 1 grams over 24 hours, whereas they gained 16 ± 2 grams over 24 hours when fed *ad libitum*. Individuals that were starved on the first night were randomly chosen, since their mean body mass at the start of the experiment was similar as the mean body mass of individuals receiving the *ad libitum* treatment the first night (Student's *t*-test: $t_{202} = 0.63$, $P = 0.53$). As in natural conditions (pers. obs.), it happened that one of the two individuals did not vocalise during the 4.5 hours-recording period. This was the case in 10 of the food-deprived pairs and 24 pairs of the food-satiated pairs. Since our aim was to study vocal interactions between two individuals, we performed statistical analyses of the remaining 68 pairs of food-deprived siblings and 54 pairs of food-satiated siblings.

In a preliminary experiment with different nestlings, we recorded pairs where each sibling had an opposing treatment (food-deprived *versus* food-satiated). However, we found that in 10 out of 12 pairs, food-deprived nestlings hardly ever vocalised, because they faced a much more motivated sibling. As we are interested here in communication dynamics, we modified our experimental settings and did not cross the food treatment in each pair.

We recorded vocal interactions from the beginning of the night at 19:00 until 23:40 using two microphones (MC930, Beyerdynamic GmbH & Co KG, Heilbronn, Germany) oriented in opposite directions, each facing one nestling. The two recording soundtracks were analysed with Matlab v.7.7 (MathWorks, Natick, MA, U.S.A.) to assign calls to each individual and to measure call duration and pause duration between successive calls (see script below). To avoid superfluous disturbance we manipulated nestlings only once per day at 16:00 and opened nest-boxes again at midnight to add food. To study negotiation rules, for each call we analysed the effect of the preceding series of 10 calls. We only consider series for which pause duration between two successive calls did not exceed 20 seconds, which corresponds to 2% of all 251,047 recorded pauses. To study how nestlings initiate and finish a monolog, we analysed the series of more than 10 calls produced by a single individual without being vocally interrupted by its sibling. We chose 10 calls because it corresponds to the minimal number of calls produced in a row that was more frequent than expected by chance. Using this definition of 10 calls, 67% of the recorded calls are produced during monologs.

Playback experiments

Playback of natural half-vocal exchange

In 2009, we broadcasted natural sequences of calls produced by a single nestling, which we extracted from the vocal exchanges of sibling pairs recorded in 2008. We selected five sequences of 84 min (range 73-93) from 5 different vocal dyadic interactions, with the criterion that the more voluble nestling did not stop calling during 3 or more minutes. From these 5 soundtracks, we silenced the calls of the less voluble individual and kept intact the calls of its sibling. In these call sequences, the call rate was not related to time (number of playback-calls per minute was not significantly associated with time in a mixed model with playback identity as a random factor: $F_{1,413} = 0.11$, $P = 0.74$). In 2009, 52 nestlings of 25 to 40 days of age (mean ± s.e.: 34 ± 1), coming from 16 nests, were placed on one side of the same wooden nest-boxes as those used in 2008, while a loudspeaker was placed on the other side broadcasting one of the five sequences. Siblings were food-deprived from the morning preceding the experiment, at 08:00. The experiment started at 01:00 the following day.

Experimental playback with varying pause and call duration

In 2011, we broadcasted four two-minute-long sequences of calls to 108 singleton nestlings from 33 nests (age: 20-42 days, mean ± s.e.: 33 ± 1) placed in the same experimental boxes as those used in 2008 and 2009. Playback sequences were composed of 20 calls of either continuously increasing duration (0.60 to 0.78 s changing by 0.02 s every two calls) or continuously decreasing duration (1.00 to 0.82 s changing by 0.02 s every two calls) separated by either continuously increasing or decreasing pause (time lapse between two calls starts from 3.05 to 9.94 s, changing by 0.76 s every two pauses). The four playback sequences were broadcasted in a random order and separated by a silence of six minutes. Siblings were fed with 50 g of laboratory mice the morning preceding the experiment, at 08:00. The experiment started at 01:30.

We extracted the calls used to build playback soundtracks from the vocal interactions of five food-deprived individuals recorded in 2008, issued from five broods different from those used for the other playback experiment. For each individual, we selected 20 calls according to their duration and not modified, except for magnitude, which was standardised using Audacity v.1.3 Beta freeware (http://audacity.sourceforge.net). This manipulation did not affect other acoustic parameters. The four playback sequences broadcasted to a singleton nestling were built with the 20 calls of only one individual.

Ethical note

We brought barn owl nestlings at an age when they were able to consume food and thermoregulate without maternal help. This was convenient because to feed them at the university we deposited laboratory mice on the floor of their new experimental nest-box so that they could eat as many mice as they wanted and at any time without disturbing them. We had already observed that parents do not adjust feeding rate to short-term variations in food need (Roulin et al. 2000). Therefore, temporally removing several nestlings from a nest never induced parents to abandon their nest. Additionally, keeping owlets at the university did not negatively affect their body condition since mean body mass and survival at fledgling did not significantly differ between the recorded and non-recorded siblings (paired signed rank test on mean body mass of recorded and non-recorded siblings per brood: $S = 13.5$, $P = 0.69$ and on mean survival: $S = 8$, $P = 0.22$). Also, nestlings brought to the laboratory were not significantly different in body mass before the experiment, from nestlings left in the nest (Wilcoxon paired Signed Rank test on mean body mass of recorded and non-recorded siblings per brood: $S = 38.5$, $P = 0.48$). We already showed that in the laboratory, nestlings behave vocally in a similar way as in natural conditions (Roulin et al. 2009) and were not physiologically stressed, as shown by the absence of a rise in baseline corticosterone level compared to the situation prevailing under natural, undisturbed conditions (Dreiss et al. 2010a).

Statistical analyses

Statistical analyses were performed with SAS v.9.1 (SAS Institute Inc., Cary, NC, USA). All tests are two-tailed. Assumptions of homoscedasticity and normal distributions of variables were verified in each test (using Kolmogorov-Smirnov tests). In the following, we give more information on specific analyses.

Preferential production of monologs over rapid vocal exchanges.

For each nestling, we calculated a random distribution of the length of call-series using a geometric distribution. The probability of producing x calls in a row without being interrupted by the sibling is given by $\Pr(x) = (p)^{x-1}(1-p)$, where p is the probability of calls by focal nestlings, i.e. number of calls produced by the nestling, divided by the total number of calls of the pair. Mean values per pair of nestlings were considered in order to correct for non-independencies of nestlings within pairs. All observed distributions of length of call series were significantly different from random expectation in a Kolmogorov test.

Dynamic negotiation rules.

Dynamics of call and pause durations were analysed with linear mixed models using residual maximum likelihood method. Model selection was performed by backward elimination of the non-significant ($P > 0.05$) terms beginning with the highest order interaction terms. Elimination of non-significant terms did not significantly modify the Akaike Information Criterion (AIC). Final models only contained significant effects and when a two-way interaction term was significant, the main effects involved in the interaction were retained even if non-significant. In the analysis of vocal confrontation, the term “vocal dominance” indicates the proportion of calls produced by the focal nestling during the sequence of the 10 last calls emitted by its sibling or the playback. For each focal individual and each call, we counted the number of calls the focal individual produced in the series of the last 10 preceding calls exchanged by this individual with its sibling or the playback. Then, we computed mean values over all the calls for which the last 10 calls were all produced by its sibling (category 0%). Similarly, we computed mean values over all the calls for which only one of the 10 last calls was produced by the focal individual (category 10%) and so on, until the category 100%. Statistical analyses were performed with these mean values, and for each individual we had a maximum of 10 mean values, explaining why each individual was introduced as a random variable to control for pseudo-replication. We processed the same for the analysis of monologs (sequence of more than 10 calls produced in a row by a nestling or the playback without being interrupted). In the course of monologs, the term “call position” indicates the location (e.g. beginning, middle or end) of a given call in the course of a monolog. For each individual and each monolog, we averaged the values of a given position of calls of the monolog (e.g. first 10% of the monolog) and then averaged the values found in all monologs from this specific individual.

Experimental playback with varying pause and call duration.

The latency (in seconds) a nestling waited before calling after the end of a playback was analysed in relation to (i) the change in duration of the broadcasted calls (increase or decrease duration) and (ii) the change in the rate at which calls were broadcasted (increase or decrease rate). This was done using a generalised mixed model with Poisson distribution considering the 90 nestlings which called at least once during the six-minute-long period of silence following a playback sequence. Similarly, the number of calls produced by singleton nestlings the minute following playback’s end was analysed using linear mixed model, for the 77 nestlings which called during this interval. The number of calls produced by singleton nestlings during the last minute of playback was set as covariate. For both analyses, the order of playback sequences was set as cofactor and we incorporated as random factors the individual used to build the playback sequence and the nestling identity nested in the brood where it was raised in the field.

Acoustic analysis

The script bellow can be run under Matlab (MathWorks, Natick, MA, U.S.A.) with a sample of two soundtracks from the two microphones oriented in opposite directions, each facing one barn owl (BarnOwlSenior.wav, BarnOwlJunior.wav) as input file. It assigns calls to junior or senior siblings and estimates call timing and call duration. Call magnitude (dB) was also assessed and it was highly correlated with call duration (Pearson correlation on average values per nestling: $r_{135} = 0.41$, $P < 0.0001$), but, as it is influenced by distance to the microphone, which was not controlled for, we did not analyse this acoustic variable.

Matlab script

Input: Two wav files: BarnOwlSenior.wav, BarnOwlJunior.wav

```
% call detection parameters

ds        = 20;            % decimation factor for time analysis (for
faster execution)
minlen    = 0.3;          % minimum length of call [s]
tc_env     = 20;              % smoothing time constant for temporal
envelope [ms]
max_env   = 2;            % max increase of envelope [lin]
tc_noise  = 2000;          % smoothing time constant for noise floor
estimate [ms]
min_noise = 0.02;           % noise estimation is never smaller than
min_noise times signal

% call detail analysis

idstartoffset = 0.03; % negative offset given to detected start [s]
idstopoffset  = 0.03; % positive offset given to detected stop [s]
startstopthr  = 3.5;  % sensitivity for start/stop detection
counterlen     = 0.1;   % counter to ignore short zeros within call
[s]

% read audio files

mic1audio = 'BarnOwlJunior.wav';
mic2audio = 'BarnOwlSenior.wav';
[in1,fs,BITS]=wavread(mic1audio);
[in2,fs,BITS]=wavread(mic2audio);

% filter and decimate the signal to get less samples
% and faster time analysis

[b a] = butter(2, [8000]./fs*2,'high');
```

```
in1ds = decimate(filter(b,a,in1), ds);
in2ds = decimate(filter(b,a,in2), ds);
N = length(in1ds);
fsds = fs/ds;

% time and axis info

time = (0:(N-1)) / fsds;
mx = max(max(abs(in1ds)),max(abs(in2ds)));
in1ds = in1ds ./ mx ./ 1.1;
in2ds = in2ds ./ mx ./ 1.1;
mx = 1/1.1;
ax = [0 max(time) -1.1*mx 1.1*mx];

% compute indicator function (call on/off)

alpha1   = 1-1/(tc_env*fsds/1000);
alpha2   = 1+1/(tc_noise*fsds/1000);

in1abs  = abs(in1ds);
in1std  = mean(in1abs);
in2abs  = abs(in2ds);
in2std  = mean(in2abs);

i = 1;
env1(i)   = in1abs(i,1);
env2(i)   = in2abs(i,1);
noise1(i) = in1abs(i,1);
noise2(i) = in2abs(i,1);

env1    = zeros(size(in1ds));
env2    = zeros(size(in2ds));
noise1  = zeros(size(in2ds));
noise2  = zeros(size(in2ds));

i = 2;
counter = -1;
while i <= N,
% envelope estimate
a = env1(i-1);
env1(i) = min(max(a*alpha1, in1abs(i,1)), max_env*a+1e-10);
a = env2(i-1);
env2(i) = min(max(a*alpha1, in2abs(i,1)), max_env*a+1e-10);
% noise floor estimate
noise1(i) = min(noise1(i-1)*alpha2, env1(i));
noise1(i) = max(noise1(i), min_noise*env1(i));
noise2(i) = min(noise2(i-1)*alpha2, env2(i));
noise2(i) = max(noise2(i), min_noise*env2(i));
i = i + 1;
end
% compute call indicator function for both microphone signals
ind1 = (env1 > 8*noise1) & (env1 > mean(env1));
ind2 = (env2 > 8*noise2) & (env2 > mean(env2));
% combine both indicator functions
ind = (ind1 > 0) | (ind2 > 0);

% refine call detection
```

```
offset1  = round(idstartoffset * fsds);
offset2  = round(idstopoffset * fsds);
counter0 = round(counterlen * fsds);
ind1     = zeros(size(ind));
ind2     = zeros(size(ind));

callcount = 0;
call_id  = [];
i = 1;
counter = -1;
while i < N,
if ind(i) == 1
  start = max(i-offset1, 1);
  ind(start:i) = 1; % extend indicator at start
  while (ind(i) == 1) & (i < N),
    i = i + 1;
    if ind(i) == 0
      counter = counter - 1;
      if counter > 0
        ind(i) = 1; % extend indicator at end
      end
    else
      counter = offset2;
    end
  end
  % if call too short, don't consider it
  if (i-1-start)*ds/fs < minlen
      counter = offset2;
  end
  % detail analysis of call
  if counter == 0
    stop = i - 1; % end of call
    % compute info about call
    callcount = callcount + 1; % increase counter for bird calls
    call_start(callcount) = start * ds;
    call_stop(callcount) = stop * ds;
    % decide if bird 1 or bird 2
    if mean(env1(start:stop)) > mean(env2(start:stop))
      call_id(callcount,1) = 1; % bird 1
      ind1(start:stop) = 0.8*mx;
    else
      call_id(callcount,1) = 2; % bird 2
      ind2(start:stop) = 0.8*mx;
    end
  end
end
i = i + 1;
end

% plot time data

figure;
subplot(2,1,1);
plot(time,in1ds,'k:')
hold on
plot(time,env1,'k')
```

```
%plot(time,noise1,'g','linewidth',2)
plot(time,ind1,'k--')
hold off
axis(ax)
title('Microphone 1 (Junior)')
%xlabel('time [s]')
ylabel('amplitude')
subplot(2,1,2);
plot(time,in2ds,'k:')
hold on
plot(time,env2,'k')
%plot(time,noise2,'g','linewidth',2)
plot(time,ind2,'k--')
hold off
axis(ax)
title('Microphone 2 (Senior)')
xlabel('time [s]')
ylabel('amplitude')
legend('Waveform','Temporal Envelope','Call Detection')


% display data

disp('Detected Calls:');
for i = 1:callcount,
    disp(['ID   =   '   int2str(call_id(i))   '          Start   =   '
num2str(call_start(i)/fs,'%5.2f')    '    s             Stop    =    '
num2str(call_stop(i)/fs,'%5.2f') ' s']);
end
```

CHAPTER IV

CHAPTER IV

Big brother is watching you: eavesdropping to resolve family conflicts

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Writing of the article: Amélie N. Dreiss , personal contribution, Alexandre Roulin

ABSTRACT

Adult animals can eavesdrop on behavioural interactions between potential opponents to assess their competitive ability and motivation to contest resources without interacting directly with them. Surprisingly, eavesdropping is not yet considered as an important factor used to resolve conflicts between family members. Here, we show that nestling barn owls (*Tyto alba*) competing for food eavesdrop on nestmates' vocal interactions to assess the dominance status and food needs of opponents. During a first training playback session, we broadcasted to singleton bystander nestlings a simulated vocal interaction between two pre-recorded individuals, one relatively old (i.e. senior) and one younger nestling (i.e. junior). One playback individual, the 'responder', called systematically just after the 'initiator' playback individual, hence displaying a higher hunger level. To test whether nestlings have eavesdropped on this interaction, we broadcasted the same pre-recorded individuals separately in a subsequent playback test session. Nestlings vocalised more rapidly after former initiators' than responders' calls and they produced more calls when the broadcasted individual was formerly a junior initiator. They chiefly challenged vocally juniors and initiators against whom the likelihood of winning a vocal contest is higher. Owlets therefore identified the age hierarchy between two competitors based on their vocalizations. They also memorised the dynamics of competitors' previous vocal interactions, and used this information to optimally adjust signalling level once interacting with only one of the competitor. We conclude that siblings eavesdrop on one another to resolve conflicts over parental resources.

Key words: acoustic, communication, competition, memory, negotiation, sibling

INTRODUCTION

When animals are in conflict over limited resources such as food, territories or mates, they assess each other's resource-holding potential and motivation to compete. This is crucial to optimally adjust investment in competition (Parker 1974). A relatively cheap way to assess a competitor is to eavesdrop on its behavioural interactions with other individuals since animals can gain information about the state of their surrounding competitors without paying the costs of taking part into those interactions (McGregor 1993; Whitfield 2002). Eavesdropping occurs in a broad range of animals in many communication channels and different competitive situations (Aquiloni and Gherardi 2010; Oliveira et al. 1998). Surprisingly, the potential role of eavesdropping in resolving contests between family members has been disregarded so far in the literature.

A family can be viewed as a communication network (Horn and Leonard 2005), where the offspring signal their need to parents (Kilner and Johnstone 1997; Mas and Kolliker 2008) and siblings (Bulmer et al. 2008; Madden et al. 2009; Roulin et al. 2000) and where parents signal their willingness to provide food (Magrath et al. 2007). Staggered births often establish an age hierarchy among the siblings and parental feeding events spread over long periods of time induce pronounced asymmetry in food requirements. Before competing over limited parental resources, each offspring should therefore assess the short-term variations in hunger level of their dominant and subordinate siblings. Evolutionary biologists implicitly assume that only direct confrontation between juvenile siblings allow them to optimally modulate their behaviour to a prevailing competitive situation (Godfray 1995b; Leonard and Horn 1998; Leonard and Horn 2001; Madden et al. 2009). Provided that young animals have the cognitive ability to recognise the identity of competitor siblings and then integrate and memorise the outcome of previous interactions between them, they could assess hunger level and position in the within-brood age hierarchy by eavesdropping on their competitive interactions.

The barn owl (*Tyto alba*) is suitable species in which to test whether nestlings eavesdrop on competitor siblings' vocal interactions to adjust the level of vocal signalling. Between the staggered parental feeding visits, siblings vocally negotiate which of them will have priority access to the next delivered indivisible food item (Dreiss et al. 2010b; Johnstone and Roulin 2003; Roulin et al. 2000). Typically, hungry individuals vocalise more intensely than their siblings to induce the less hungry individuals to retreat from negotiating and ultimately from begging on the parents' arrival (Dreiss et al. 2010b). Thus, vocal negotiation while parents are away increases the likelihood of being fed (Roulin et al. 2000). Broods comprise up to nine offspring that might differ in age by three weeks. As a consequence, an individual is expected to reduce the level of vocal negotiation when facing a highly motivated and dominant senior sibling for whom the probability of obtaining the next food item is higher (Roulin 2004a).

To study eavesdropping between siblings that vocally interact, we performed playback experiments to examine whether barn owl nestlings listen to competing siblings and use this information to adjust vocalization levels when they subsequently interact with each of them separately. Preliminary experiments on vocal exchanges between siblings found that hungry individuals produce more calls and produce them more rapidly after their opponent (see Methods). In this experiment, we first played a pre-recorded vocal exchange between two unfamiliar individuals, one senior (the oldest) and one junior (the youngest), to singleton nestlings alone in a nest. In this simulated vocal exchange (so-called training playback), one playback individual, the 'responder', vocalised systematically just after the 'initiator', hence displaying a higher motivation to compete for food resource than the initiator. After having broadcasted this vocal exchange, we tested the vocal response of singleton nestlings when listening only to the calls of the initiator or only of the responder. Initiators being less motivated to compete than responders and juniors being less competitive than their senior siblings, the theoretical likelihood of obtaining the impending prey item is higher for nestlings competing with junior or with initiators than with other individuals. If owlets eavesdrop on vocal dyadic interactions, we thus expect that they invest more in vocalization when listening only to a former initiator than former responder, and when facing a junior competitor rather than a senior competitor.

METHODS

General procedure

We performed the study in 2009 between June 21 and September 23 in western Switzerland (46°4'N, 6°5'E) on a population of wild barn owls breeding in nest boxes. Fifty-four owlets, including 19 males and 35 females, from 16 broods were brought to the laboratory at around 13h00. They were aged between 26 and 45 days (mean ± s.e.: 36 ± 1), and were on average 19 days pre-fledging age (which takes place at *ca*. 55 days). Individuals were brought back to their nest after three nights of captivity. The owlets were already thermo-independent and their parents were naturally sleeping outside their nest box. In the barn owl, incubation starts as soon as the first egg has been laid and since eggs are laid every 2.5 days, the two to nine siblings can significantly differ in age. Nestling age was estimated shortly after hatching by measuring the length of the left flattened wing from a bird's wrist to the tip of the longest primary (Roulin 2004b). Nestling sex was determined using molecular markers (Py et al. 2006).

Nestlings were housed individually in an experimental nest box similar to the one in which they were reared in naturally (62 x 56 x 37 cm^3), but separated into two equal parts by a thin wooden wall pierced with holes (fig.1B in Chap. I). In these conditions they behave as in nature (Roulin et al. 2009) and are not physiologically stressed (Dreiss et al. 2010a). One owlet was placed at one side of a box, while the other side contained a loudspeaker (near05 experience, ESI Audiotechnik GmbH, Leonberg, Germany). We recorded each individual with a microphone (MC930, Beyerdynamic GmbH & Co KG, Heilbronn, Germany) oriented towards it and fixed on the inside roof of the box. The playback experiment was carried out on the third and last night of captivity starting at midnight. By that time, all nestlings had been food-deprived from the preceding morning at 8h00.

The playback experiment was based on an experiment carried out in 2008, in which we found that calling rapidly after a sibling can signal hunger level and hence the motivation to compete over parental food resources (see Chap. III). In the 2008 experiment, we recorded naturally occurring vocal interactions in 78 pairs of siblings from 41 nests. Nestlings were taken from the wild and put into similar nest-boxes as those used in 2009, but with one nestling placed in each part of the boxes. We recorded their vocal interactions from 19h00 to 23h30 on one night when both individuals were experimentally food-deprived, and on another night when both individuals were experimentally food-satiated (order of recordings was reversed for half the birds). The latency for a chick to call after its sibling ended a call was shorter for hungry individuals, after controlling for seniority (oldest or youngest sibling of the pair) and number of calls per minute which increases with hunger level (mean calling latency per minute was shorter in food-deprived than food-satiated individuals [8.20 ± 0.07 s *vs.* 8.46 ± 0.16 s]; effect of food-treatment: $F_{1,17659} = 14.50$, $P = 0.0001$ in a mixed model with individual nested in sibling pair as random factors, seniority and number of call per minute as independent terms).

Playback experiments

In 2009, we first broadcasted a training playback of a vocal exchange between two pre-recorded individuals to 54 singleton nestlings. The aim of this training playback is to allow singleton nestlings to eavesdrop on two nestlings that interact vocally. The two broadcasted individuals differed in age, the mean age difference being 10.3 ± 0.6 days (range: 2 - 19). A training playback lasted *ca*. 26 minutes and comprised 144 pairs of two calls from an 'initiator' individual always calling 1.40 ± 0.01 s before the other individual, denoted 'responder'. Pairs of two calls were separated by a silence of 7.00 ± 0.01 s (fig. 1). The 144 pairs of calls consisted in four blocks of the same 36 pairs of calls. In this way, in each training playback the two individuals had different roles - being either the initiator or the responder - but also in seniority - being either a junior or a senior individual.

After having broadcasted one of these training playback sequences to a singleton nestling, we sequentially broadcasted the initiator's and the responder's calls to each owlet in a random order. These two testing soundtracks lasted *ca*. 6 minutes and comprised the same 36 different calls from each playback individual that were placed at intervals of 10.03 ± 0.02 s in a random order, with each call played only once along the soundtrack (fig. 1).

Each of the 54 experimental nestlings heard a unique combination of playback sequences, *i.e*. either different initiator-responder pairs or the broadcasted individuals were played back in a different order in the 1st and 2nd testing playbacks (fig. 1). The experimental nestlings that listened to the four categories of playbacks as listed in the fig. 1 showed no significant difference in age and sex (ANOVA: $F_{3,50} = 2.55$, $P = 0.07$ and $F_{3,50} = 0.64$, $P = 0.59$).

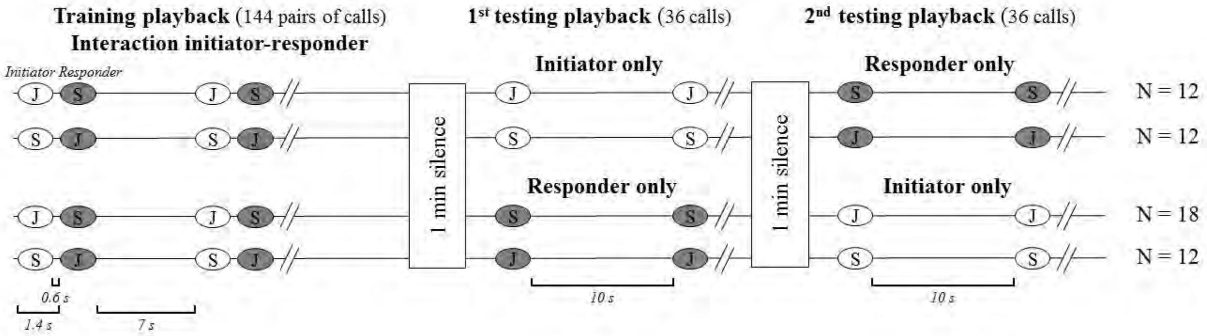

Figure 1. Design of the four possible playback combinations broadcasted to singleton nestlings and number of singleton nestlings for each combination. J stands for junior and S for senior barn owl nestlings. In the initiator-responder training playback sequence, the initiator systematically called before the responder during *ca*. 26 minutes. In the 1st and 2nd testing playback sequences, only the initiator or the responder individuals were broadcasted during 6 minutes each. N stands for the number of lively nestlings that heard each type of combination of sequence.

Construction of playback soundtracks

To build the playback soundtracks, we used natural calls we had recorded in 2008 from pairs of siblings starved for the preceding 24 hours and that could vocally interact. We selected calls from seven 26 to 45 days old individuals (five males and two females) collected from seven different broods. Each playback individual was assigned an equal number of times to the role of "initiator" and of "responder" during playbacks. The two youngest individuals used to generate playbacks were only assigned the role of junior in playbacks, the two oldest only the role of senior and the three other individuals were alternatively assigned to the "junior" and "senior" roles. We isolated 36 calls of about 0.8 s (mean ± s.e.: 0.796 ± 0.001 s) from each nestling, which corresponded to the average call duration computed from the experimentally food-deprived pairs of nestlings recorded in 2008 (mean ± s.e.: 0.81 ± 0.01 s, based on 154,503 recorded calls). We standardised the maximum amplitude of all calls using the Audacity software (http://audacity.sourceforge.net); this manipulation does not affect the other acoustic features of the calls. The chosen call latency between initiator and responder for the playback corresponds to the 1st percentile of calling latency of the food-deprived pairs of nestlings recorded in 2008. The chosen call rate corresponds to the mean call rate of two food-deprived siblings that freely interact vocally (mean ± se: 11.96 ± 0.05 calls/min, n = 68 pairs of siblings recorded in 2008).

Acoustic analyses

We used Matlab v.7.7 (MathWorks, Natick, MA, U.S.A.) to assign calls to playback or nestlings and to measure the timing and duration of the calls. In particular, we measured the latency of the responses by the nestlings to playback calls, *i.e.* the time taken for each owlet to start to call following a playback call. The calling latency could be negative when an owlet's call overlapped the broadcasted call. The analysed calling latency is the average of the owlet calls' latency.

Statistical analyses

Statistical analyses were performed with the software SAS v.9.1 (SAS Institute Inc., Cary, NC, USA). We performed two mixed models to analyse the vocal response of nestlings when hearing the initiator and the responder during the 1st and 2nd testing playback sequences. One mixed model included owlet's calling latency as dependent variable and the other model included the number of calls. As independent factors, we fitted: (1) the role of the broadcasted individual (*i.e.* initiator or responder) in the training playback sequence; (2) the seniority of the broadcasted individual (the older individual of the two broadcasted owlets was denoted 'senior' and the younger individual 'junior'); and, (3) the order in which the playback was received (i.e. whether it was played back 1st or 2nd).

Because the same playback individual was broadcasted to more than one nestling, for all models we controlled for the identity of the two broadcasted individuals as a random factor. We included as random factor the brood identity of nestlings, since we often used more than one nestling per brood. Because absolute age of the broadcasted individuals did not explain variation in calling behaviour of the focal live nestlings, we removed this fourth independent variable from the analyses. In all analyses, absolute age, age rank in their natural nest and sex of the 54 focal nestlings did not affect the way they behaved vocally when hearing the playbacks. Therefore, for clarity we did not include these variables in the models presented in the paper. Assumptions for the models used (homoscedastic and normal distributions of variables and residuals) were verified in each test. We performed backward model selection. Final models only contained significant effects ($P < 0.05$), and main effects involved in significant interactions. We verified that final models selected always presented the smallest Akaike Information Criterion (AIC) compared to initial and intermediate models.

Ethical note

The experiments were approved by the veterinary services of Canton de Vaud (Form No 2109.1). We always left one or two nestlings in the natural nest and we had already observed that parents do not adjust feeding rate to short-term variations in food need (Roulin et al. 2000). Therefore, temporally removing several nestlings from a nest never induced parents to abandon their nest. Keeping owlets at the university did not negatively affect their body condition since mean body mass and survival at fledgling did not significantly differ between nestlings brought to the university and nestlings left in their nest (Wilcoxon test on body mass at fledgling stage of recorded and non-recorded siblings in 2009: $Z = 1.9$, $P = 0.051$ [recorded: 350±5g; non-recorded: 329±6g] and on mortality: $Z = 1.7$, $P = 0.08$ [recorded: 2%; non-recorded: 10%]), while body mass at capture did not differ between the two groups ($Z = 1.2$, $P = 0.22$). In the laboratory, nestlings were not physiologically stressed, as shown by the absence of a rise in baseline corticosterone level compared to the situation prevailing under natural, undisturbed conditions (Dreiss et al. 2010a).

RESULTS

As expected, nestlings vocalised more rapidly just after the initiator produced a call compared to the responder, regardless of seniority ($F_{1,34}$ = 5.27, *P* = 0.027, table 1, fig. 2). No other main effects or interactions were significantly related to nestling's calling latency. Furthermore, the number of calls produced by singleton nestlings was significantly related to the statistical interaction between competitor individuals' role in training playback and their seniority ($F_{1,82}$ = 8.58, *P* = 0.004, table 1). To investigate this interaction, we have conducted additional analyses. When responding to an initiator alone, nestlings produced twice as many calls when listening to a junior, as opposed to a senior (4.0 ± 0.7 calls per minute *vs.* 1.9 ± 0.5, $F_{1,51}$ = 4.92, *P* = 0.031 in a mixed model with brood identity of nestlings and identity of the playback individual as random factor, fig. 3), but were similarly vocal when listening only to a junior as a senior responder (similar mixed model: $F_{1,51}$ = 2.92, P = 0.10, fig. 2; see table 1 for the full initial model including initiators and responders). Therefore, owlets vocalise differently according to the challenger role ("initiator" vs. "responder" hypothetically reflecting competitive effort) and seniority ("junior" vs. "senior" hypothetically reflecting competitive ability) previously witnessed during the training playback, even in our experimental situation where the broadcasted competitors were unfamiliar to the nestlings.

Table 1. Vocal response of bystander barn owl nestlings listening to a playback of an individual (during the so-called test session, fig. 1) that was previously heard interacting with another individual (during the so-called training session, fig. 1). Results of final models are written in bold and non-significant results eliminated from the initial full models in plain.

	Nestling's calling latency			Nestling's call number		
	F	*d.f.*	*P*	*F*	*d.f.*	*P*
Testing playback order (1^{st} *vs*. 2^{nd})	0.91	1, 37	0.35	0.61	1, 81	0.44
Role in training playback (initiator *vs*. responder)	**5.27**	**1, 34**	**0.027**	**0.06**	**1, 82**	**0.81**
Seniority (junior *vs*. senior)	0.63	1, 37	0.43	**0.06**	**1, 82**	**0.81**
Playback order x Role in training playback	0.01	1, 34	0.94	3.26	1, 79	0.07
Playback order x Seniority	0.01	1, 34	0.91	2.38	1, 79	0.13
Role in training playback x Seniority	1.94	1, 34	0.17	**8.58**	**1, 82**	**0.004**

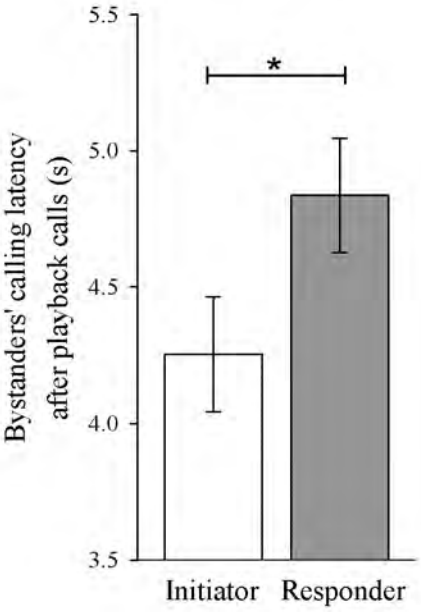

Figure 2. Nestlings' latency (s ± s.e.) to call after the calls of initiator and responder barn owl nestlings separately broadcasted during the 1st and 2nd testing playbacks. Nestlings called more rapidly after individuals that were the initiator rather the responder during the training playback sequence. The symbol * is for $P < 0.05$ in a mixed model (see table 1).

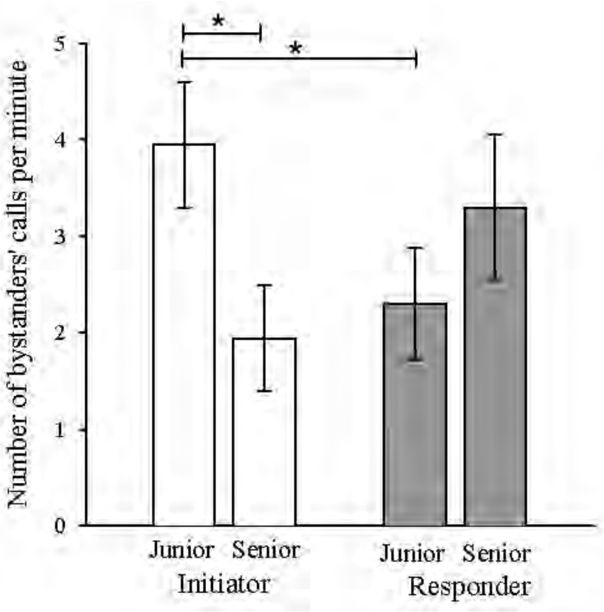

Figure 3. Eavesdropping behaviour of barn owl nestlings. After nestlings heard a vocal dyadic interaction between an initiator and a responder nestling during training playback, we tested the number of calls (± s.e.) they produced when listening only to the same initiator or responder. Nestlings produced significantly more calls when listening to a junior initiator than to a senior initiator or to a junior responder during testing playbacks. The symbol * is for $P < 0.05$ in a mixed model (see table 1).

DISCUSSION

Our results show that owlets do eavesdrop on competitive interactions between other owlets. We found that owlets adjusted their vocal behaviour according to the perceived motivation and competitiveness of nest mates in a previous interaction. This suggests that owlets are able to: (1) gather information on the competitive role played by two vocally interacting nestmates (*i.e.* which of the two brood mates was calling before the other) as well as their relative seniority (*i.e.* which of the two brood mates is the older); and, (2) remember this information for at least a few minutes before using it to adjust their vocal behaviour. To our knowledge, this is the first evidence that bird nestlings have such cognitive abilities, which could be used to resolve conflicts over the share of resources.

In many species, individual acoustic features underlie distinct signatures about its identity, such as sex, familiarity, sexual maturity, or dominance status (Blumstein and Munos 2005; Davies and Halliday 1978; Tomaszycki et al. 2001), and some studies show that receivers can discriminate individuals on the basis of these signals (Gherardi et al. 2005; Godard 1991; Hare 1998). Young animals have also shown to have the capacity to distinguish each other's familiarity and relatedness (Beecher and Beecher 1983; Maletinska et al. 2002; Palestis and Burger 1999). However, to our knowledge, the capacity for young non-humans animals to discriminate individual siblings from one another has only been experimentally demonstrated in Greylag Geese (*Anser anser*) (Scheiber et al. 2011). The adjustment of owlet behaviour following a vocal interaction supposes that they discriminate among callers using acoustic cues. This discrimination may be the result of individual recognition or of the ability to classify callers according to their relative age. We showed indeed that nestlings behaved differently according to the seniority of the two playback individuals. Interestingly, owlets adjusted their calling behaviour in relation to the age hierarchy of the two witnessed individuals (i.e. which individual is the junior vs. senior) but not in relation to their absolute age. This is probably adaptive given that age hierarchy between siblings persists throughout the 55 day-long rearing period. In the dark nocturnal conditions of a relatively closed nest, barn owl siblings appear to mainly communicate vocally (Dreiss et al. 2010b), visual cues being faint. Calls of barn owl nestlings probably provide an age-specific signature that enables nestlings to discriminate their opponent according to their age, even when facing unfamiliar and hardly visible individuals. Age hierarchy is an important factor of sibling competition in many altricial species (Drummond 2006; Roulin 2004a). In a crowded nest, estimating the position of hungry vocal competitors in the within-brood age hierarchy would allow a focal individual to estimate its chance to win the contest for the next delivered prey.

Individual discrimination in the context of sibling negotiation would thus allow bystander nestlings to adjust their investment in sibling competition for food according to the level of competitiveness and motivation of their vocal siblings. The relative hunger state of siblings is worthwhile remembering if it does not fluctuate rapidly over time. Here, we have shown that barn owl nestlings remember the state of two competitors for at least a few minutes. Two ecological factors that might influence the evolution of the social and cognitive ability to remember siblings' hunger state are parental feeding rate and the size of food items. In the barn owl, parents feed their brood on average every hour (Roulin 2002b) with relatively large food items. Thus, siblings' hunger level does not oscillate quickly, as fed individuals are satiated for a while and hungry individuals can wait several minutes up to a few hours before being fed. Eavesdropping on the communicative network within a brood can thus reinforce the efficiency of exchange of valuable information during the vocal negotiation process among siblings.

Because barn owl nestlings have up to eight competitors and because of relative low parental provisioning rate, it is possible that they could remember interactions between multiple individuals for longer periods of time. Furthermore, nestlings showed here that they discriminated between two individuals that only varied in their calling latency. In nature, hungry and satiated individuals show more contrasted vocal behaviours, as they also vary with respect to call duration and call rate (Roulin et al. 2009) and probably intensity of calls. Gaining information by eavesdropping a vocal interaction between individuals varying in hunger level must thus be easier in nature than in our experimental design. It is hence likely that barn owl nestling largely use eavesdropping in natural conditions.

To conclude, barn owl nestlings eavesdrop on each other’s competitive interactions and are able to identify and remember the role of each opponent even before directly interacting with them. This ability enables them to adjust their vocal investment once interacting with these opponents. Therefore, young animals competing over parental resources can remember which of the two interacting siblings is highly motivated to compete (in this case responders) and has the higher resource holding potential (ergo seniors) and use this information to adjust investment in sibling competition. Eavesdropping relatives’ interactions therefore allows young animals to reduce the costs involved by the resolution of intra-familial conflicts over parental resource.

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CHAPTER V

CHAPTER V

Sibling rivalry favours the ability to count competitors

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This chapter is being prepared for publication.

Experimental design: personal contribution
Data collection personal contribution, Amélie N. Dreiss
Acoustic analysis: personal contribution, Anais Edme
Statistical analysis: personal contribution
Writing of the article: personal contribution, with Alexandre Roulin

ABSTRACT

Assessing the number of rivals is crucial to optimally adjust investment into a contest. If laboratory animals show numerical abilities, little is known about the ecological and evolutionary implications of the ability of counting, particularly in young animals. Barn owl siblings (*Tyto alba*) vocally compete the priority access to food resources before parents actually deliver them. The individual that vocalises at the highest rate in the absence of parents deters its siblings from calling and ultimately from begging once parents are back with food. Here, we tested the novel hypothesis that to optimally adjust vocal investment barn owl nestlings count siblings that are currently competing. To singleton owlets we broadcasted a fixed number of calls (at three possible rates) emitted by one, two or four pre-recorded unfamiliar nestlings. As predicted singleton nestlings adjusted call rate and call duration in relation to the number of playback individuals. When we broadcasted calls at a low rate, nestlings vocalised at a higher rate when one rather than two or four individuals emitted the calls. When we broadcasted calls at higher rates, nestlings refrained from vocalising by producing fewer and shorter calls when hearing four individuals rather than two or one. Barn owl nestlings assess variations in the number of siblings currently competing based on vocalisations, they process and use this information to adjust vocal behaviour. We conclude that sibling competition can promote the evolution of numeric ability in young animals.

Key-words: numerical ability, count, vocal, begging, sibling competition, *Tyto alba*

INTRODUCTION

Animals compete for limited resources such as mates, territories or food. As the likelihood of winning a contest decreases with the motivation and the number of rivals, animals are predicted not only to assess rivals' resource holding potential (Enquist and Leimar 1983; Parker 1974), but also to count them in order to optimally adjust investment in competition. Surprisingly, despite the straightforward benefits individuals should derive from counting competitors, little is known about the extent to which wild animals use numerical competences in socio-ecologically relevant contexts. Literature on non-human animals' numerical competences shows that animals as various as insects (Dacke and Srinivasan 2008; Gross et al. 2009), fish (Agrillo et al. 2011), amphibians (Uller et al. 2003), birds (Rayburn-Reeves et al. 2010) and mammals (Brannon and Terrace 1998; Kilian et al. 2003) can sequentially distinguish between small numerosities – often up to four. However, these studies are performed in the laboratory with individuals trained to discriminate among artificial objects, lights or sounds. Only a few experiments such as choice procedures in some fish and insects (Agrillo et al. 2009; Carazo et al. 2009; Gomez-Laplaza and Gerlai 2011) and playback experiments in wild birds and mammals (e.g. Kitchen 2004; McComb et al. 1994; Seddon and Tobias 2003) report that they perceive a change in the number of conspecifics based on visual or vocal cues and accordingly dose effort in mating or competing decisions.

To our knowledge, the ecological importance of numeric ability has not been evaluated in the context of family interactions and sibling competition. In altricial species, offspring commonly compete to attract parental attention and obtain a larger than equal share of parental resources (MacNair and Parker 1979). The intensity of scramble competition among the progeny and of begging solicitations signal offspring need and determine how food resources is shared among the progeny (Kilner and Johnstone 1997; McRae et al. 1993). Studies in animals typically show that nestlings adjust begging behaviour not only in relation to their own need but also to the competitive ability (Cotton et al. 1999; Price et al. 1996; Roulin 2004), postural or vocal signals (Leonard and Horn 1998; Madden et al. 2009; Marques et al. 2011; Smith and Montgomerie 1991) and location of their siblings in the nest (Kolliker et al. 1998; Ostreiher 2001). In contrast, nothing is known about whether young animals are able to assess the number of siblings that are currently competing to adjust their investment into the competition over parental resources. Given that some nestlings can be momentarily sated, only part of the progeny is expected to compete over food. This raises the possibility that young animals that are still dependent on their parents may be selected to assess siblings' signalling level not only to evaluate their motivation to compete, but also to count how many of them are currently competing. The competitive environment experienced by an individual is certainly different if begging solicitations are produced by one sibling that is very motivated to compete for parental resources or by several mildly motivated siblings.

In the present study, we investigated whether barn owl nestlings (*Tyto alba*) adjust effort invested in the contest for parental resources to the number of nestmates that are momentarily vocally competing. In this nocturnal species, the two to nine young not only beg towards parents to solicit food, but also vocally communicate with their siblings in the prolonged absence of parents between feeding events. The function of this sib-sib communication system is to inform each other about their willingness to compete once parents are back with an indivisible small mammal. Because a single offspring is fed per parental visit, only one individual will be paid back for the effort invested in sibling competition. This sib-sib communication system, referred to as "negotiation", therefore allows each individual to optimally adjust investment into each specific contest (Roulin 2002a). Typically, the hungriest individual of a brood vocalises intensely in the absence of parents, which deters its siblings from negotiating and later from begging for the prey item delivered once parents are back. Negotiating at a high level therefore gives priority access to the impending food resource at lower costs compared to a situation where negotiation would not take place (Johnstone and Roulin 2003; Roulin 2002a).

We repeatedly showed that barn owl nestlings invest in vocal negotiation according to the level at which their siblings vocalise (Dreiss et al. 2010b; Roulin 2002a). It is however unclear whether owlets also assess the number of nestmates that are currently negotiating, although this behaviour would certainly be adaptive. The number of siblings that take part into negotiation, and thus the ambient competitive level, varies across feedings events along with the hunger level and the motivation to compete of each nestling. From a cognitive point of view, this is *a priori* plausible because nestlings can identify which of two vocalising siblings is the oldest and which is negotiating at the highest level and use this information to optimally adjust signalling level (Chapter IV). Assuming that a given number of negotiation calls are emitted by an increasing proportion of siblings, a nestling will face more, but less motivated competitors. We thus propose the novel hypothesis that nestlings determine how many siblings are currently vocalising and adjust vocal investment accordingly.

Here, we report a test of this hypothesis. To singleton nestlings we broadcasted pre-recorded negotiation calls of one, two or four unfamiliar nestlings at different rates. We predict that singleton owlets adjust their vocalisation behaviour in relation to both the rate at which negotiation calls are broadcasted *per se* and to the number of individuals contributing to the overall signal. Even if we test this hypothesis in the context of sibling negotiation, our study is of general applicability. Indeed, negotiation calls honestly signal need and they are directed to family members. Therefore, the barn owl can be considered as a prime model system to study social interactions taking place between young individuals, parents and offspring but also between mature individuals.

METHODS

Data collection

The study was performed in western Switzerland (46°49’N/06°56’E) on a population of wild barn owls. We carried out the experiment once nestlings were old enough to be thermo-independent and could consume food without maternal help. We estimated nestlings’ age shortly after hatching by measuring the length of the left flattened wing from the bird’s wrist to the tip of the longest primary (Roulin 2004)

Between May and September 2011 at ca. 12h00, we brought to the laboratory 57 male and 64 female nestlings aged 33 ± 4 days (mean ± SD), issued from 31 broods (mean brood sise in the field ± SD = 6 ± 1 nestlings). We hosted them during two nights, before bringing them back to their original nest at ca. 12h00. We kept each individual in a wooden nest-box similar to the one in which it was reared in the field (fig.1B in Chap.I). Each nest-box was separated into two equal parts, with one nestling on the left side and a loudspeaker (near05experience, ESI Audiotechnik GmbH, Leonberg, Germany) on the right side. Nest-boxes were acoustically isolated with mineral foam on the sides and the roof, and at the time of recordings they were closed. To facilitate ventilation we connected nest-boxes to the outside with a plastic pipe. The acoustic isolation was efficient since calls were not audible by a human observer standing in the room.

Owlets were acclimated to the laboratory conditions during the first 24 hours and nestlings were not physiologically stressed, as shown by the absence of a rise in baseline corticosterone level compared to the situation prevailing under natural, undisturbed conditions (Dreiss et al. 2010a). On their arrival, we offered laboratory mice to the owlets as well as on each morning at ca. 9h00 a.m. with ca. 50 g of laboratory mice, which is slightly inferior to their daily food requirement of about 67 g, in order to stimulate them to vocally compete at night during our playback experiments which started at 11h45 p.m. on the second night. We used Cubase software version 5.1 (Steinberg Media Technologies GmbH, Hamburg, Germany), set at 44.1 KHz sampling rate and 16-bit resolution, to simultaneously broadcast the playback sequences and record nestlings' vocalisations.

Design of playback sequences

To build playback sequences, we selected 24 natural calls per individual in 21 barn owl nestlings (13 males and 8 females aged 32 ± 6 days (SD)) issued from 19 broods. These individuals were recorded during free vocal dyadic interactions between pairs of starved siblings in 2008 hosted in the same laboratory conditions as in 2011, except that the sibling replaced the loudspeaker. All calls lasted ca. 0.8 sec, which corresponds to the mean and median length of calls observed in the free dyadic interactions recorded in 2008 (mean ± SEM = 0.811 ± 0.0007 sec, median = 0.800 sec, range: 0.220 - 2.310 sec, *n* = 61'332 calls from 98 owlets). We standardised call intensity using free Audacity software v.1.3 Beta (http://audacity.sourceforge.net), a procedure that does not affect call frequencies and duration. Based on these standardised calls, individuals could be statistically discriminated, which supported potential for individual recognition by experimental nestlings to which we broadcasted them (see Supplementary material).

To each of the 121 singleton nestlings we broadcasted 9 playback sequences lasting 4 minutes each and separated by 6 minutes of silence. We chose these timings because previous studies showed that owlets adjust their vocal behaviour mostly according to the 2 preceding minutes of a vocal exchange with a counterpart (unpublished results). The 9 sequences corresponded to the combinations of three different call rates: 6, 12 or 24 calls per minute, emitted by one, two or four individuals. These three call rates correspond to the natural range we observed in free vocal interactions that took place between starved owlets in 2008, during minutes when owlets produced at least one call (mean ± SEM = 7.86 ± 0.06 calls/min, median

= 7.00, range: 1-34, n = 61’332 calls from 98 owlets). In the sequences where we broadcasted two or four playback individuals, we allocated the same number of pre-recorded calls for each playback individual. For example, for playbacks of four individuals for which call rate was set to 24 calls/min, we inserted 24 calls of each of the four playback individuals in the 4 minute-long playback sequence (table 1).

To avoid pseudo-replication, we broadcasted to each singleton nestling a unique combination of calls (Kroodsma et al. 2001). Using an automatic Matlab program (version R2008b MathWorks, Natick, MA, U.S.A.), we inserted calls in a random order along the four-minute-long playback sequences and separated these calls with randomly chosen time intervals. Nevertheless, we set the minimal pause between two consecutive calls to 1 second; intervals of less than 1 second between two consecutive calls emitted by two individuals corresponded to only 0.08 % of all individual pauses (n = 250’924 pauses from 98 owlets) observed in the recordings of free dyadic interactions in 2008. We randomised the order of the 9 sequences across the 121 nestlings. In each sequence, we also randomly inserted the calls and identity of each playback individual.

Table 1. Experimental playback design to study whether barn owl nestlings are able to count how many siblings are currently vocalising.

Number of broadcasted donor nestlings	Call rate from each donor nestling (calls/min)		
1 nestling	**6**	**12**	**24**
2 nestlings	**3**	**6**	**12**
4 nestlings	**1.5**	**3**	**6**
Overall playback call rate (calls/min)	**6**	**12**	**24**

Acoustic analyses

We placed a microphone (MC930, Beyerdynamic GmbH & Co KG, Heilbronn, Germany) inside nest-boxes against the roof underside and in direction to the nestling. By comparing broadcasted soundtracks to recorded soundtracks we could easily discriminate calls produced by the owlet from those emitted by the loudspeaker using a semi-automatic program in Matlab v. R2008b. For each of the nine four-minute-long sequences, the Matlab program recorded the number of calls produced by the nestling and calculated the mean duration of its calls in seconds. Among the 121 tested nestlings, 36 of them did not vocalise at all throughout the nine playback sequences, a frequently observed situation in the wild (pers. obs.). We analysed the response of the 83 nestlings that produced at least one call (mean ± SEM = 70 ± 11 calls, range: 1-412 produced by 38 males and 45 females aged 33 ± 4 days (SD)). Results (not shown) were qualitatively similar when we restricted analyses to the 57 nestlings that produced a minimum of 10 calls.

Statistical procedure

For each of the nine four-minute-long playback sequences, we computed the number of calls and mean call duration of nestlings. We ran a generalised linear mixed model with Poisson error distribution to analyse the number of calls produced by nestlings and a linear mixed model to analyse the mean call duration. We fitted the identity of nestlings nested in brood of origin as a random intercept to control for the 9 repeated measurements per individual and the fact that several tested nestlings came from the same nest. Fixed effects comprised the number of broadcasted calls (6, 12 or 24 calls/min) and the number of playback individuals that emitted these calls (one, two or four). We also added the order at which we broadcasted the playback sequence (1 to 9) as a continuous covariate to control for the effect of time-dependent vocal behaviour (e.g. owlets become more motivated to call with time as they become hungrier). In a preliminary analysis, we included sex and age of nestlings as covariates, but they proved to have non-significant effect on vocal output and were hence removed from the final analyses. In the case of significant interaction between the terms “playback call rate” and “number of playback individuals”, for each of the three call rates we ran similar mixed models to examine the influence of the number of individuals broadcasted on the vocal behaviour of nestlings.

Analyses were performed with SAS V9.2 (SAS Institute Inc., Cary, NC, USA). Residuals of linear mixed models were checked for normality.

RESULTS

Experimental nestlings vocalised less when calls were broadcasted at a higher rate (term “Playback (PB) call rate” in table 2 and fig. 1A). They also modulated the number of calls they produced in relation to the number of playback individuals used to generate the playback sequences (term ”Number of PB individuals”), but in a way that depended on the rate at which calls were broadcasted (interaction ”PB call rate x Number of PB individuals”). Nestlings vocalised significantly more when we broadcasted a single individual compared to multiple individuals, i.e. two and four, both when we played back 6 calls/min (fig. 1A; similar GLMM as in table 2, $F_{1,163} = 7.8$, $P = 0.0006$) and 12 calls/min (similar model: $F_{1,163} = 4.0$, $P = 0.02$). When we broadcasted 24 calls/min, nestlings vocalised more when hearing calls produced by two rather than one or four playback individuals (fig. 1A; similar model: $F_{1,163} = 11.7$, $P < 0.0001$).

Independently of the rate at which we broadcasted calls, nestlings emitted shorter vocalisations when they heard four rather one or two playback individuals (fig. 1B and table 2).

The significant effect of the variable “sequence order” indicates that with time nestlings produced more and longer calls (table 2).

Table 2. Number and mean duration of calls produced by barn owl nestlings hearing one, two or four playback individuals calling at various rates (i.e. 6, 12 or 24 calls/min). A generalised linear mixed model with Poisson error distribution was used to test variation in the number of calls singletons emitted in response to the playbacks and a linear mixed model to test variation in the mean duration of bystanders' calls. Both models comprised the identity of nestlings nested in brood where they were raised in the field as random intercept. The analyses were based on 83 nestlings issued from 33 broods. For each nestling and acoustic variable, we had a maximum of nine data points corresponding to the total number of calls and to the mean call duration computed over the 9 four-minute-long playback sequences corresponding to the 9 combinations of call rates (i.e. 6, 12 or 24 calls/min) and number of playback individuals (i.e. one, two or four). Because not all nestlings called when hearing a given playback sequence, we had a larger number of observations to test variation in nestling's call number (*n* = 747) than in nestling's call duration (*n* = 417). Because each owlet experienced nine playbacks broadcasted in a random order, we controlled statistically for the order at which each playback was broadcasted (term 'Sequence order').

Dependent variable	Call number		Call Duration	
Fixed effects	F_{df}	*P*-value	F_{df}	*P*-value
Sequence order	$F_{1,655} = 29.6$	<0.0001	$F_{1,331} = 19.0$	<0.0001
Playback (PB) call rate	$F_{2,655} = 237.6$	<0.0001	$F_{2,329} = 0.2$	0.80
Number of PB individuals	$F_{2,655} = 8.3$	0.0003	$F_{2,331} = 6.6$	0.002
PB call rate X Number of PB individuals	$F_{4,655} = 9.2$	<0.0001	$F_{2,325} = 1.3$	0.28

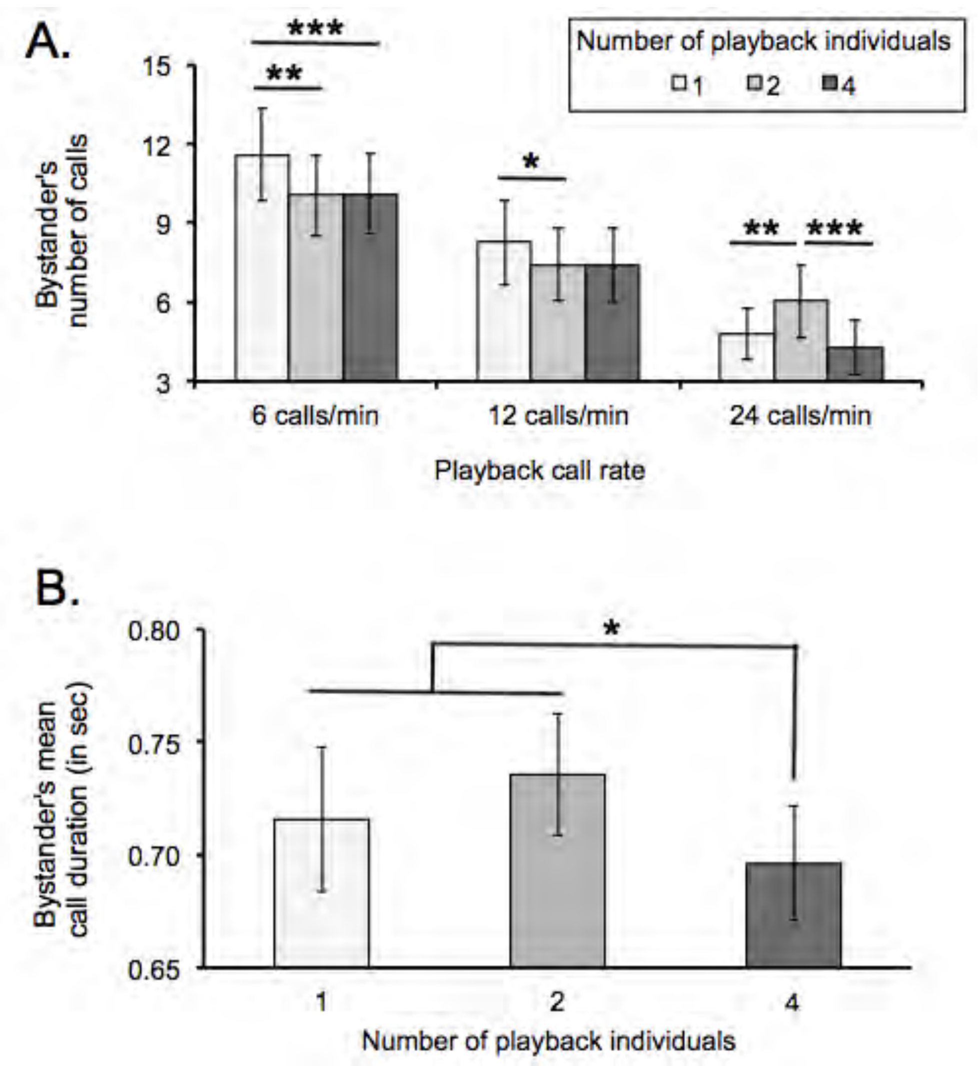

Figure 1. Number (A) and mean duration (B) of calls (± SEM) of barn owl nestlings hearing one, two or four playback individuals calling at various rates (i.e. 6, 12 or 24 calls/min). Averages are computed over the raw data of 83 nestlings from 33 broods. Levels of significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) reported above the bars are derived from mixed models examining the effect of the number of broadcasted nestlings on the nestling's call number and mean duration. Separate analyses were performed to examine the effect of the number of playback individuals for each playback call rate. The order at which calls of one, two or four individuals were broadcasted to each nestling was entered in the model as factor and nestling identity nested in brood was included as random intercept to control for the repeated measurements per nestling and per brood.

DISCUSSION

In the present paper, we experimentally tested the hypothesis that young animals still dependent on their parents evolved the ability to count the siblings that are currently competing over the same pool of parental resources, so as to dose effort invested in sibling competition. As a model system, we considered the barn owl in which nestlings vocally negotiate among them priority access to the impending indivisible food item next delivered by a parent. The individual that produces many and long calls deters siblings from vocally negotiating and ultimately begging food from parents (Dreiss et al. 2010b; Johnstone and Roulin 2003; Roulin 2002a). An individual will escalate vocal negotiation when its chance of winning the contest is higher, that is when it faces a less motivated sibling that emits few and / or short calls (Dreiss et al. 2010b). We thus predicted that owlets assess both the number and motivation of siblings that are currently competing from their calls. Accordingly, we found that owlets refrained from vocalising when hearing more calls *per se,* i.e. broadcasted at 6, 12 and 24 calls per minute. In each case they also differentially modulated the number and / or duration of their vocalisations according to whether the broadcasted calls were emitted by one, two or four playback individuals. Since we broadcasted the different playback sequences in a random order with several minutes of silence separating two sequences, owlets most likely assessed the absolute number of calls and individuals broadcasted in each sequence rather than compared the relative numbers of two adjacent sequences. Hence, we can interpret our results with confidence as experimental evidence that barn owl nestlings are able to assess variation in the number of nestmates that are competing at different levels and use this information to adjust their vocal behaviour.

Laboratory choice experiments in newborn domestic chicks previously demonstrated that even at very young stages, chicks can sequentially discriminate numbers of artificial objects (Rugani et al. 2009). Together with their study, we provide here evidence for rudimentary numerical abilities in young animals, from multiple visual and vocal cues. Without a game-theoretical approach, however, it is difficult to propose *a priori* predictions regarding how offspring should adjust effort in sibling competition according to variations in both the number and motivation of competitors. Here, we found that nestlings vocalised more often when hearing one rather than two or four broadcasted nestlings calling at a rate of 6 and 12 calls/min, whereas when the playback call rate was set to 24 calls/min nestlings emitted more calls when listening to two rather one or four nestlings. Furthermore, independently of the rate of broadcasted calls, nestlings produced longer calls when we broadcasted fewer individuals than four. Owlets thus globally invested more vocal effort when hearing fewer calls and fewer rivals. Assuming that producing many and long calls is costly (Roulin et al. 2009), by doing so, they save energy when the level of competition is too high. This energy could be reallocated once siblings are fed and hence once their own chance of obtaining the next delivered food item is higher (Roulin 2002a). This is consistent with the "sibling negotiation hypothesis", which posits that when food is indivisible, young animals inform their siblings about their willingness to compete only if the expected chance of obtaining the impending indivisible food item is relatively high (Johnstone and Roulin 2003; Roulin 2002a).

When we broadcasted calls from two or four individuals, we took care to allocate the same number of calls per individual in each playback sequence (table 1). As a consequence, when we broadcasted calls from several individuals, each emitted in total very few calls indicating a low motivation to compete over the next delivered food item. When hearing 24 calls per minute produced by a single individual, owlets refrained from calling probably because the playback individual signalled a very high motivation to compete. Hence there may be a threshold in opponents' motivation at which it becomes worth investing against more – but not too many – nestmates that are mildly motivated than against a single highly motivated sibling.

Our study mirrors works performed in territorial songbirds and mammals showing that individuals retreat from vocalising when facing two or three intruders compared to one (Kitchen 2004; McComb et al. 1994; Seddon and Tobias 2003). However, in these playback experiments, authors measured the response of groups of individuals (Seddon and Tobias 2003) or did not control for auditory cues that co-vary with number like call duration and intensity (McComb et al. 1994), which prevents disentangling the exact effect of the number of competitors from the total intensity of vocal stimuli on how individual nestlings adjust effort in competitive social interactions. In this respect, our design is conservative, since the number of playback individuals varied independently of the number of broadcasted calls and of their duration and intensity, which were fixed, and calls did not overlap. This experimental approach enables us to prove that barn owl nestlings can assess the number of competitors based on vocal signatures rather than on variation in call intensity, call rate or any other possible cues such as siblings' position in the nest. Indeed, in a single playback sequence the broadcasted individuals all emitted the same number of calls and, from a single loudspeaker mimicking the situation where all siblings are positioned at the same location. Our design therefore mimics a very difficult situation for nestling barn owls to count the number of competitors, but for three reasons, in natural conditions it may be even easier for them to assess the number and motivation of each sibling. First, in the field owlets emit calls of different durations at different rates, which may help nestlings to recognise siblings. Second, nestlings stand at different locations in their nest during relatively long periods of time implying that calls of each individual are always emitted from the same direction. This later factor is probably very important, since we already showed that an individual induces siblings to refrain from negotiating by being located close to the nest-box entrance where parents predictably deliver food (Dreiss et al. 2010b). Third, we played back calls from unfamiliar nestlings. Nestlings thus appeared to distinguish vocal characteristics of several unknown individuals.

To conclude, we propose that competition over limited resources, in particular in the case of sibling rivalry, can promote the evolution of numerical competences that help revolving conflicts. More generally, our findings reveal that to adjust optimally effort in competition animals should not only assess the overall level of competition from a group but also how many individuals are currently competing and also likely their individual competitiveness. This raises very interesting issues regarding the possibility that some individuals may form coalitions to outcompete the most motivated opponents of a group. In the case of barn owls, some nestlings may indeed vocalise not for their own benefit but to favour some particular siblings by inducing a highly vocal competitor to retreat from the contest. Historically, theoretical models of signalling to resolve conflicts are developed based on simplistic assumptions as dyadic interactions (Enquist and Leimar 1983; Parker 1974). Research should thus envisage animal communication as a complex network so as to better understand the plasticity of competing tactics and the evolution of signals. This is of particular importance while studying competitive interactions within relatively large groups of individuals who share a limited space such as begging offspring, chorusing anurans and insects, and colonial species (Horn and Leonard 2005; Schwartz and Freeberg 2008)**.**

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Supplementary material

Vocal signature of playback individuals

To verify that the pre-recorded calls we used to build playback sequences conveyed potential for individual signatures, we measured the several acoustic parameters described hereafter with Matlab v. R2008b (MathWorks, Natick, MA, U.S.A.). The signal segment containing one bird call is denoted *x(t)*, where *t* is time in seconds. The length of a bird's call is *T* seconds. The spectrum of *x(t)* is denoted *X(f)*, where *f* is frequency in Hz. The bandwidth of the signal is *F* Hz. The temporal envelope of *x(t)* is denoted *e(t)*. The considered acoustic features were the following variables (see fig. S1):

(1) Centre of Power in Time [s] (**CPT**). This point divide the call in two parts in the time axis; the parts before and after this CPT represent half of call magnitude.

$$t_c = \frac{\int_0^T t|x(t)|^2\,dt}{\int_0^T |x(t)|^2\,dt}$$

(2) Centre of Power in Frequency [Hz] (**CPF**). Frequencies above and below this CPF represent half of call magnitude.

$$f_c = \frac{\int_0^F f|X(f)|^2\,df}{\int_0^F |X(f)|^2\,df}$$

(3) Standard Deviation of Centre of Power in Frequency [Hz]: f_c is computed on short-time spectra and the standard deviation of it with respect to time is computed.

(4) Low Weighted Centre of Power in Frequency [Hz] (**CPFL**). Frequencies above this CPFL represent 75% of call magnitude.

$$f_{c,low} = \frac{\int_0^F f\left(F - f\right)\left|X(f)\right|^2 df}{\int_0^F \left(F - f\right)\left|X(f)\right|^2 df}$$

(5) High Weighted Centre of Power in Frequency [Hz] (**CPFH**). Frequencies above this CPFL represent 25% of call magnitude.

$$f_{c,high} = \frac{\int_0^F f^2\left|X(f)\right|^2 df}{\int_0^F f\left|X(f)\right|^2 df}$$

(6) Fluctuation of Temporal Envelope [1: no fluctuation, >1: fluctuation] (**FTE**).

$$e_{fluct} = \frac{\max_{0 \le t \le T} e(t)}{\frac{1}{T}\int_0^T e(t)dt}$$

(7) Absolute magnitude level [dB] (**AML**).

(8) Level ratio (**LR50**): ratio of magnitude of the first half of the call (in time) divided by the second half of the call.

We first conducted a Principal Component Analysis (PCA) over the seven acoustic parameters to disentangle the ones potentially encoding for distinct components of vocal signature. To do this, we computed a mean value for each call feature and for each call donor. According to this PCA, we identified 5 out of the 7 considered acoustic features along the two first principal components axes, which accounted for 70% of the total variance (43.0 and 27.0 % respectively), as potentially distinct components of a vocal signature. These were CPF, FTE, CPFsd, LR50 and AML.

We further ran a Discriminant Function Analysis (DFA) to verify that playback individuals could be statistically discriminated from their calls. We performed the DFA based on the CPF, FTE, CPFsd, LR50 and AML, of the 504 playback calls (i.e. 24 calls for each of the 21 playback individuals). The DFA revealed that 19 out of the 21 owlets could be statistically discriminated based on these five parameters. The mean correct assignment rate was 36.1% (± s.d. = 23.7%, range 0-75%) across individuals, which was well above chance level (4.8%). Except for two owlets, for which assignment rate was 0 %, on average, 39.9 % of the calls were correctly assigned to all of the other 19 owlets (range = 8.3 to 75.0 %).

Last, we confirmed that acoustic features of playback individuals' calls did convey a potential for individual coding (PIC), following Reers and Jacot (2011). We computed the individual coefficient of variations (CVi) for CPF, FTE, CPFsd, LR50 and AML for each playback individual, as well as the global coefficient of variation of each acoustic feature (CVb) across all calls and all individuals, following the formula: CV = 100 x SD / mean, with SD = standard deviation. For each of these five acoustic features, we then computed the potential for individual coding (PIC) following the formula PIC = CVi / CVb. We found the five PIC ratios to be greater than 1 (1.3 to 1.7), which indicated that all acoustic features were more variable across than within individuals. Hence, the individuals from which we used the calls to build our playback sequences could possibly be biologically discriminated from a vocal signature based on their acoustic features.

We used the library MASS in R freeware version 2.12.2 (R Foundation for Statistical Computing, Vienna, Austria) to run principal component and discriminant function analyses.

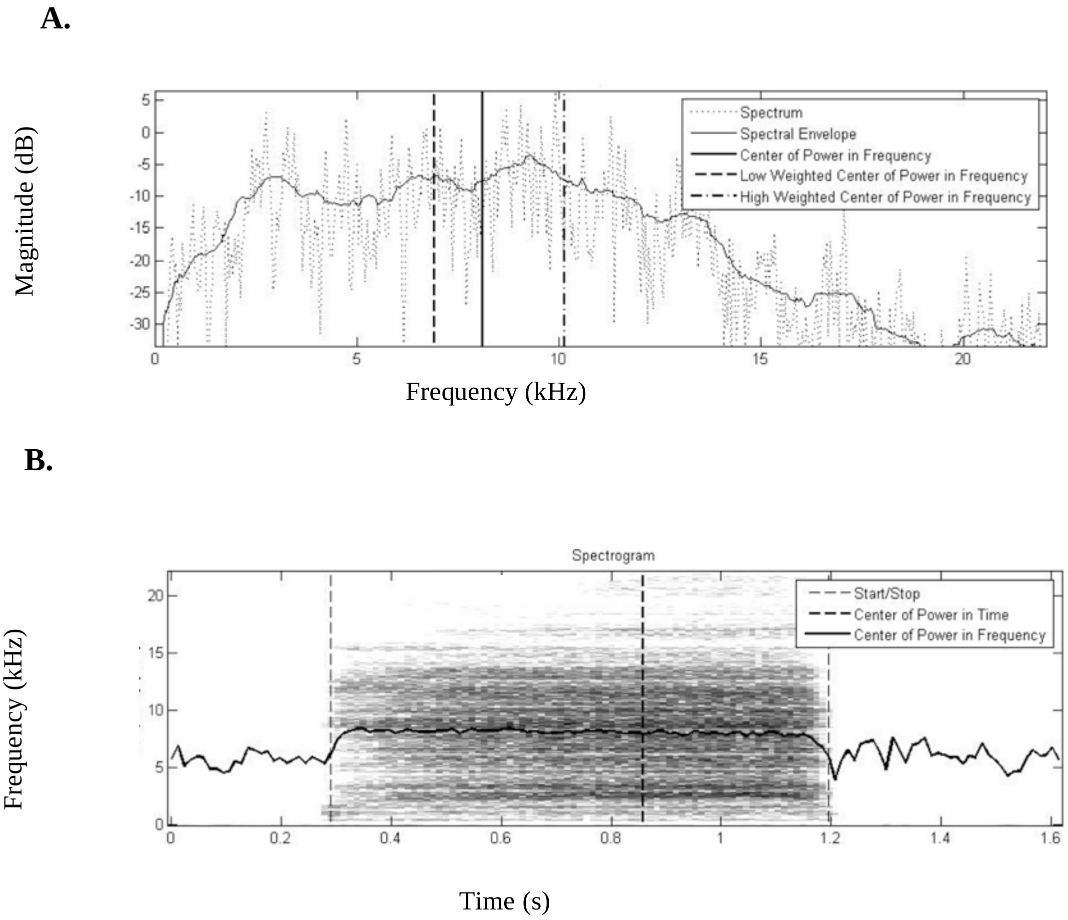


Figure S1. Frequency distribution (A) and sonogram (B) of a typical negotiation call of a barn owl nestling. The seven acoustic features first considered corresponded to the central power of frequency (CPF), its deviation (CPFsd), its lower and upper ranges (CPFL, CPFH), the spectral envelop (FTE)

GENERAL CONCLUSION

In species with parental care, young solicit care and food from their parents. Because of scramble competition, siblings not only modulate begging behaviours in function of their parents but also of each other. To date, knowledge about how and the extent to which young mutually adjust signals of need remains scant. In my thesis, I tackled this issue using the barn owl. In this species, the one to nine nestlings vocally contest priority access to indivisible food prior to parents' visits at night, by repeating a simple noisy call (fig. 2 of General Introduction), a process referred to as 'sibling negotiation' (Roulin 2002a). Using both correlative and playback procedures within dyads, I first examined how individuals mutually adjust the timing (Chap. I) and the average number and duration of their calls (Chap. II). Next, I described the acoustic rules underlying the continuous sib-sib negotiation process (Chap. III). Finally, using playbacks, I tested whether owlets socially eavesdrop on their nestmates' vocal interactions (Chap. IV) and assess their number and motivation (Chap. V) to dose competitive effort within the brood communication network.

BARN OWL SIB-SIB NEGOTIATION RULES: A SYNTHESIS

In barn owl nestlings, calling more than a sibling in parents' absence dissuades it to subsequently beg for the next indivisible prey item delivered (Roulin 2002a, Roulin et al. 2000). Correlative studies suggested that owlets also compete through call duration (Roulin et al. 2009, Dreiss et al. 2010b). Because parents' arrival is unpredictable during the hours they are left alone, we expected hungry nestlings to seek at dominating siblings constantly so as to ensure monopolising food, but at the lowest possible costs. In an iterative contest such as this sib-sib negotiation, this should translate into the use of less costly signals first to end up with more costly ones if the previous prove inefficient in deterring rivals to compete (Enquist and Leimar 1983). Models on conflict settlement also predict owlets to invest in calls (number and duration) proportionally to their own chance of winning the contest, and hence preferentially call when they face less motivated and fewer rivals (Maynard Smith 1976). Results from our playback procedures in all five chapters therefore concord with these expectations and experimentally corroborate previous correlative findings.

First, consistent with published studies (Roulin et al. 2009, Dreiss et al. 2010b), we demonstrate that owlets deter a rival to compete by emitting both more and longer calls (Chap. II & III). Moreover, we show that they enhance call discrimination by vocalising alternately, regardless of the number and rhythm of calls, their age, seniority and motivation to compete (i.e. hunger level) (Chap. I). Such antiphony seems well adapted to the fact that owlets dose vocal effort relative to subtle variations in the duration and rhythm of siblings' calls, which we referred to as 'turn-taking' cues (Chap. III). Along free dyadic exchanges, owlets alternately impose themselves through periods of " monologs", in which progressively lengthening calls dissuades a sibling to compete while reducing ones' own call rate. As previously suggested (Dreiss et al. 2010b), owlets adopt the most economic option of inducing each other to withdraw from the contest rather than desperately calling. Our findings are also in agreement with owlets minimising vocal costs, using cheaper signals first (i.e. short calls). Yet this seems tricky to experimentally test, lengthening calls could also be cheaper than emitting more calls since we found that it is less tightly linked to hunger level (Chap. II).

Second, our results verify the prediction that individuals invest in competition proportionally to their chance of winning a contest, when facing both a single and several opponents. Notably, owlets linearly reduced vocal effort in response to longer calls broadcasted at higher rates, which mimicked increased motivation from the playback (Chap. II). When interrupted by a sibling, owlets responded all the quicker with all the longer calls than they had previously vocalised much, likely so as not to waste the energy already spent (Chap. III). Also as predicted, in Chapter IV, singleton bystanders memorised which of two playback rivals dominated a vocal exchange (leader-follower) and was more competitive (i.e. senior) and behaved less aggressively once they faced it alone a few minutes later. In Chapter V, when hearing one, two or four broadcasted nestlings, bystanders preferentially vocalised towards fewer, generally – and likely individually - less motivated competitors.

To sum up, despite an apparent lack of social organisation, the several barn owl siblings communicate according to well-structured acoustic and behavioural rules that seem adaptive in that they reduce competition costs. This is strongly supported by the fact that the acoustic rules are universal regardless to owlets' hunger level, seniority, relatedness and familiarity (Chap. III). Across chapters, individuals responded to playback nestmates with which they were not born or raised as expected and similarly to when paired with a live sibling. Indeed, extra-pair copulations being rare (Roulin et al. 2004), owlets should not have had the need to evolve or use kin recognition mechanisms during development.

INSIGHTS INTO SIBLING RIVALRY OVER PARENTAL RESOURCES

Dependent young not only modulate solicitation signals as a function of their own level of need, but also of siblings' needs and resource holding potentials (e.g. Price et al. 1996). Although this so-called "scramble competition" partly determines within-brood food allocation (Royle et al. 2002), how and to what extent siblings process this information and accordingly compete is barely known. In particular, although some studies in mammals and birds suggest that young mutually adjust the timing (Blanc et al. 2010; Chaiken 1990; Madden et al. 2009), and acoustic features (Horn and Leonard 2008) of their vocalisations, only one playback experiment had been conducted to date in tree swallows (Marques et al. 2011). Taken together, our repeated playback procedures thus provide the first and solid experimental proof that, in multiple-offspring broods, young siblings can 1) mutually adjust the timing and multiple components of their calls and 2) honestly signal their need to each other. Sib-sib negotiation requires specific conditions such as indivisible food and pronounced competitive asymmetries in siblings (Johnstone and Roulin 2003). However, it is not unique to barn owls, as shown in the spotless starling (Bulmer et al. 2008), and may be more widespread, possibly occurring also in parents' presence, provided that feedings last long enough for siblings to communicate with each other (Horn and Leonard 2005). In synchronous broods, when food is divisible, or when vocalisations are only directed at parents, our results at least demonstrate that siblings can mutually process information on their need and strength from their calls to compete (i.e. so-called 'bystanding' or 'interceptive eavesdropping', Peake and McGregor 2004).

Moreover, we provide the novel findings that young modulate signalling of need as a function of the relative asymmetries in strength and motivation between two siblings (Chap. IV) and of slight variations in their number (Chap. V). Given that multiple-offspring broods are common, this should be integrated in the theoretical framework of scramble competition, which mostly focuses on pairwise sib-sib interactions. Social eavesdropping notably implies that young retain information on asymmetries between siblings. Here, owlets also memorised a rival's vocal output (call duration, rhythm) for a few minutes (c.f. playback after-effects, Chap. II & III) and anticipated the rhythm of a playback's calls (c.f. predictable *vs.* unpredictable playbacks, Chap. I). All this extends previous findings that bird chicks modulate signalling level with respect to past experience in their brood size (Kacelnik et al. 1995) and with parents, rewarding begging postures or level (Grodzinski et al. 2007; Kedar et al. 2000) and locations in the nest (Kolliker et al. 1998). Since family members continuously interact throughout the rearing period, their behaviours are interconnected over time. The ability to memorise siblings' and parents' behaviours and adequately adjust competing tactics should therefore be taken into account (Dobler and Kolliker 2009). Depending on the system and number of siblings, offspring should face a trade-off between the cognitive costs imposed by memorisation and the fitness benefits it confers in monopolising of resources (Dukas 1999).

IMPLICATIONS ON THE STUDY OF ANIMAL COMMUNICATION

More generally, the facts that owlets socially eavesdrop each other (Chap. IV) and assess siblings' number (Chap. V) to compete support the still quite anecdotal examples of long- or middle range natural contests in unrelated adult animals (McComb et al. 1994; Peake 2005; Seddon and Tobias 2003). Our findings thus extend these phenomena to close-range agonistic interactions among family members and at early life stages. This reinforces recent claims that theoretical models should depict more complex than pairwise social interactions as our empirical knowledge on communication networks is growing (Johnstone 2001; McGregor 2005; Mesterton-Gibbons and Sherratt 2009). Indeed, as shown across my chapters, opponents may concomitantly dose competitive effort as a function of their relative motivation and strength, of such asymmetries in their rivals, as well as the number and likely the individual motivation of rivals, parameters that are usually separately tested.

It is also worth highlighting the discrepancy between our findings from Chapters II and III about the role of call duration in sib-sib negotiation. In Chapter II, measuring average responses to playbacks led us first to conclude that long calls corresponded to a "reinforcement" or "back-up signal" in more to call number. In Chapter III, we actually found that the signal conveyed by call duration was encoded through its temporal variations. In contrast to usual expectations, this signal is independent of owlets' resource holding potential (i.e. seniority) and motivation to compete (i.e. hunger level), but instead serves to regulate turn-taking in long-lasting vocal exchanges. Furthermore, unlike predictions from classical models on repeated signals owlets use temporal variations in their call duration and rhythm (i.e. number) to escalate or retreat and not the averages, cumulative sums or highest values previously heard (Payne and Pagel 1996; Payne and Pagel 1997). Because, as for begging, behavioural ecologists traditionally focus on the immediate economic results that opponents draw from their interactions, they neglect these important continuous temporal signal modulations during contests. Taken together, our findings here reveal that the usual approach consisting in measuring average values can mislead our interpretation of the function of certain signals and of competing strategies.

Interestingly, when examining how individuals continuously interact at the scale of each call, we observed conversational rules from the rhythm and length of nestlings' utterances comparable to the basic rules underlying human conversations (Duncan 1972; Sacks et al. 1974). Despite extensive literature on the evolution of language, especially in primates (Locke 2001) and songbirds (Doupe and Kuhl 1999), to my knowledge, this is the first demonstration in an animal where multiple components govern the vocal exchanges through turn-taking rules. These rules appear in early life, in a species *a priori* cognitively limited and with no particular social organisation. Moreover, call number, rhythm and call duration are common acoustic parameters vertebrates use to compete and communicate as shown in birds (Todt and Naguib 2000), frogs (Arark 1983) or non-human primates (Ey et al. 2007). Hence, the acoustic rules observed in barn owls might be broadly shared, yet in species with complex social organisations and vocal repertoires turn-taking signals may consist of additional components such as frequencies and call types (Kondo et al. 2010; Miller and Hauser 2004). Our findings therefore open an interesting window for trans-disciplinary research on animal vocal communication across a large spectrum of cooperative and competitive situations.

CONCLUSION

To conclude, the sib-sib communication of barn owls emerges through the different chapters of my thesis as a powerful model to study family conflicts and animal communication.

Negotiation “can be thought of as any interaction or behavioural process by which animals with conflicting interests can reach a compromise settlement” (Cant and Johnstone 2009). It is an iterative process by which opponents that have no *a priori* knowledge on their mutual strength and intention challenge each other. It can occur in a wider range of contests than just among kin, since individuals with opposed interests usually have a common goal that may differ from sharing genes. These can be mates that seek at reproducing (Patricelli et al. 2011), parents that optimise offspring survival through mutual provisioning (Johnstone and Hinde 2006) and cooperative breeders (Johnstone 2011) or territorial rivals that at least should avoid lethal injuries (Pereira et al. 2003). However, only recently have such theoretical models been published and empirical works are still lacking. With regard to acoustic signals, our successful playback experiments thus should stimulate empiricists working on sibling rivalry and conflict settlement in general to resort to such procedures.

PERSPECTIVES

On the roles of non-vocal acoustic signals

In my thesis I focused on vocalisations, yet animals may compete through visual or other acoustic signals. In altricial young, for instance, wing-flapping correlates with chicks’ need and is used as competitive begging in many bird species (Grim 2008). If coupled with reduced loudness of calling, such signals, possibly acoustic, would serve indicating ones’ willingness to compete at lower predation costs (Magrath et al. 2010). Noises from body movements might also help locating siblings that occupy preferential positions when the visibility is restrained in dark or crowded nests. However, whether and how both parents and siblings differentially rely on vocal and such non-vocal signals or cues to allocate food and compete, respectively, has not yet been tested.

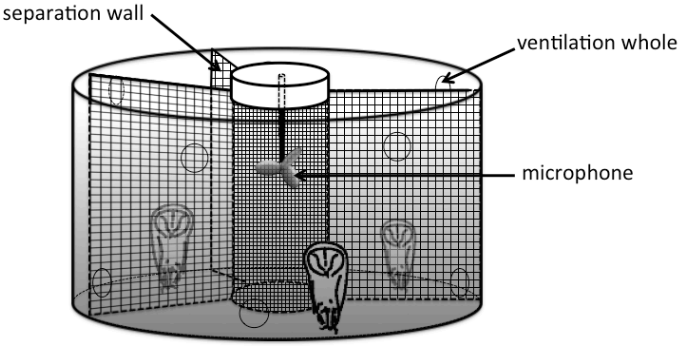
In support to this ‘non-vocal acoustic signalling’ hypothesis, detailed observation of turn-taking patterns in free dialogs (Chap. III) show that owlets happen to make noises such as leg-tapping or scratching and wing-flapping instead of vocalising at the end of a sibling’s monolog (pers. obs.). The sibling, which hears the owlet without seeing it, in turn resumes calling similarly to what it would have done if the owlet had vocalised. This leads to the exciting possibility that owlets combine both vocal and non-vocal acoustic turn-taking signals to communicate. In human conversations, gestures and noises can reflect speakers’ emotions and be also used as turn-taking cues (Duncan 1972; Wang 2009). Vertebrates, such as mammals (Randall 2001) and frogs (Lewis et al. 2001), and invertebrates, including arachnids (Gibson and Uetz 2008) and insects (Hill 2001) use non-vocal acoustic signals such as vibrations or tapping to communicate. Whether the role of such signals in communication is similar to that of humans remains, to my knowledge, remains unexplored. Hence, there is obvious opportunity for fruitful research using the barn owl, hence broadening its framework from that of sibling rivalry. Food-deprivation and playback experiments should notably help determining 1) whether, how and which of the non-vocal behaviours reflect owlets’ motivation to compete and 2) whether and how owlets indeed both vocally and non-vocally negotiate parental food resource with each other.

Negotiating within a network of several live nestmates

The second straightforward follow-up of my thesis is to examine how owlets behave among several live siblings (i.e. by contrast to playbacks used in Chap. IV & V). To this aim, based on advanced technology (i.e. triangulation, voice recognition), I already recorded in the lab the vocal interactions of (almost) complete broods during the field seasons 2009 and 2011, as described in box 1 p. 152. Upcoming data analyses will notably allow us to 1) verify how several live owlets actually use turn-taking rules (including non-vocal sounds), social eavesdropping and counting in a more natural context; 2) test the influence of brood size on those behaviours; 3) explore inter-individual differences along the dominance hierarchy, potentially in personality traits such as aggressiveness (Van den Brink et al. 2012) or vocal activity (i.e. variation in latency to negotiate, unpublished results), which can affect the outcome of sibling competition, but are still barely explored (Roulin et al. 2010); and 4) verify the consistency of individual differences in behaviours across ages. Besides, in the barn owl, young may cooperate frankly, with seniors offering extra-food to their youngest, more needy, siblings (appendix 2). This provides the opportunity to explore potential coalitions, which are known to occur in social groups (Johnstone and Dugatkin 2000), but remain an outstanding question in the context of sibling rivalry.

Box 1. Negotiation among several live siblings

In the breeding seasons 2009 and 2011, we performed two experiments in which we recorded all night long the vocal interactions of several (≥ 2) owlets raised in the same nest in the field. In both years, we recorded them on the first night of their arrival at the laboratory, all together in a circular plastic box. In 2009, we used one microphone placed at the centre of the box and at the height of nestlings and based on a triangulation process (fig. a). In 2011, we used eight microphones regularly spaced, fixed at the ceiling of the box and directed towards the inside (fig. b). To prevent siblings to stand too close and complicate call assignment to each, we hosted them in equal areas separated by a double layer of wire netting around a central tube also made of wire netting. Nestlings thus vocally and visually freely interacted with each other, but not physically. Except when we manipulated them, we closed the box with a wooden cover to limit light entrance. The box was regularly pierced with wholes for ventilation and covered inside with carpet to avoid sound reverberation.

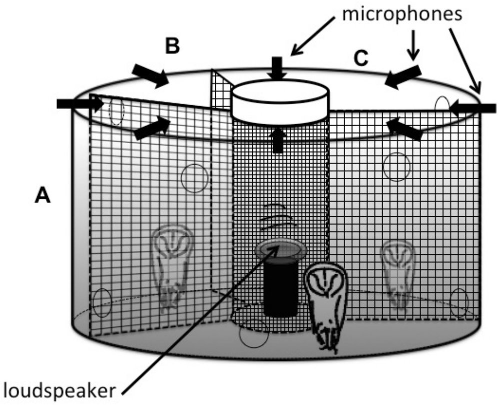


(a) Variable natural brood size & repeatability within individual and age

In 2009, at ca. 12 h, we brought to the laboratory all nestlings from each brood but one or two that we left in the field to prevent parents to abandon the nest (*n* = 16 recorded broods, brood-size in the lab = [2 – 5]). We recorded the same broodmates twice, from 18h00 to 8h00, when the first-born was aged ca. 32 days and then a week later. In between we brought back nestlings in the field.

(b) Three-chick broods: coalitions and exploratory playback

In 2011, we recorded only three nestlings (a senior, a middle-born and a junior) per natural brood and only once, when the first-born was aged ca. 37 days (*n* = 32 broods). We took care of assigning the junior, middle-born and senior nestlings to the areas A, B, C of the box in a random order across broods. From 18 to 00:30 h, we recorded free acoustic interactions among nestlings and then, using a loudspeaker placed in the centre of the box at the height of nestlings, we broadcasted a playback soundtrack. It was made of nine 4 -minute sequences, separated by 10 minutes of silence. These corresponded to the nine combinations of the pre-recorded calls of one individual (out of 7 possible ones) of ca. 0.8 sec standardised at three possible intensities (-8, -16 and -24 dB) and broadcasted at three different rates (2, 6, 10 calls / min). We randomised the order at which we broadcasted sequences across broods and at which we inserted calls within sequences.



Science & trade-offs… Our aim being to investigate individuals' vocal behaviours, we must assign the calls recorded to each brood-mate. To date, we are still developing the analysis tools required (in collaboration with C. Faller (LCAV, EPFL), H. Lissek, X. Falourd, P. Marmarolli (LEMA, EPFL)).

Perspectives

These experiments will enable us to:

- examine vocal and non-vocal acoustic behaviours among several nestlings (including turn-taking rules)
- examine (correlative data) the potential signalling role non-vocal components in acoustic interactions
- disentangle the effects of relative and absolute age on signalling strategies and examine within individual repeatability in the level and in the variations in both vocal and (presumed) non-vocal acoustic signalling
- disentangle the effect of brood size from the number of nestlings currently competing
- investigate whether call intensity is another acoustic component of vocal negotiation and its role
- investigate possible preferential interactions among nestlings that differ in age and seniority

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APPENDICES

APPENDIX 1

Corticosterone promotes scramble competition over sibling negotiation in barn owl nestlings (*Tyto alba*)

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RESEARCH ARTICLE

Corticosterone Promotes Scramble Competition Over Sibling Negotiation in Barn Owl Nestlings (*Tyto alba*)

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Abstract In species with parental care, siblings compete for access to food resources. Typically, they vocally signal their level of need to each other and to parents, and jostle for the position in the nest where parents deliver food. Although food shortage and social interactions are stressful, little is known about the effect of stress on the way siblings resolve the conflict over how food is shared among them. Because glucocorticoid hormones mediate physiological and behavioral responses to stressors, we tested whether corticosterone, the main glucocorticoid in birds, modulates physical and vocal signaling used by barn owl siblings (*Tyto alba*) to compete for food. Although corticosterone-implanted (cort-) nestlings and placebo-nestlings were similarly successful to monopolize food, they employed different behavioral strategies. Compared to placebo-nestlings, cort-individuals reduced the rate of vocally communicating with their siblings (but not with their parents) but were positioned closer to the nest-box entrance where parents predictably deliver food. Therefore, corticosterone induced nestlings to increase their effort in physical competition for the best nest position at the expense of investment in sib–sib communication without modifying vocal begging signals directed to parents. This suggests that in the barn owl stress alters nestlings' behavior and corticosterone could mediate the trade-off between scramble competition and vocal sib–sib communication. We conclude that stressful environments may prevent the evolution of sib–sib communication as a way to resolve family conflicts peacefully.

Keywords Begging · Corticosterone · Vocal signaling · Family conflict · Sib–sib communication · Physical competition · *Tyto alba*

Introduction

Parents and offspring are in conflict over the amount and duration of parental care (Trivers 1974), each offspring demanding more resources than parents and siblings are willing to concede (Godfray 1995a). In altricial birds, nestlings commonly display conspicuous visual and vocal signals to solicit food from their parents (Leonard et al. 2003; Bulmer et al. 2008; Grim 2008) and compete physically by jockeying for the nest location where parents predictably deliver food resources (Teather 1992; Kacelnik et al. 1995; Nunez-de la Mora et al. 1996; Johnstone 2004; Drummond 2006; Tanner et al. 2008). As the pattern of

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within-brood food allocation depends on the complex interplay between the parental provisioning rules, offspring solicitation and sib–sib competition (Cotton et al. 1999; Roulin 2004; Smith et al. 2005), information about how siblings adjust their vocal and physical behaviors to one another is required (Smith and Montgomerie 1991; Roulin et al. 2000).

Siblings generally differ in physiological condition and body size. At a given feeding event, the expected payoff of obtaining food varies among siblings not only according to their level of need but also in relation to their resource holding potential. The benefit of consuming a prey item is greater for the neediest offspring, while the cost of food monopolization is lower for the individuals that are intrinsically more competitive (Godfray and Parker 1991; Godfray 1995b). Although food-deprived offspring commonly increase their investment in visual and vocal begging behaviors and/or physical competition (Smith and Montgomerie 1991; Cotton et al. 1996; Leonard et al. 2003; Smiseth et al. 2003; Porkert and Spinka 2006; Williams et al. 2008), the effectiveness of these behaviors in monopolizing food depends on their own competitive ability (Kacelnik et al. 1995; Price 1996). For instance, in bird species where eggs hatch asynchronously, which generates a pronounced within-brood age hierarchy among siblings, elder and thus stronger nestlings typically reduce effort invested in begging signals in favor of scramble competition (Kilner 1995; Lichtenstein and Sealy 1998; Ostreiher 2001; Rodriguez-Girones et al. 2001a; Leonard et al. 2003; Ploger and Medeiros 2004; but see Whittingham et al. 2003; Roulin 2004). This appears to be adaptive because to monopolize food resources physical competition can be more efficient than vocal begging (McRae et al. 1993; Kacelnik et al. 1995; Budden and Wright 2005; Tanner et al. 2008). Poorly competitive nestlings (e.g. juniors) that are barely able to get access to the best nest position may compensate by producing more intense begging calls to attract the attention of their parents and thereby influence within-brood parental food allocation and induce an increase in parental feeding rate (Cotton et al. 1999; Smiseth and Amundsen 2002; Roulin 2004). Because siblings experience different cost-benefit ratios in investing in signaling and scramble competition, they are likely to employ different behavioral strategies to monopolize food resources (Smiseth and Amundsen 2002).

The behaviors each single nestling uses to get access to food resources depends on nestlings' resource holding potential, hunger level and body condition, which are all tightly linked to their physiological state. In particular, glucocorticoids may help nestlings to regulate their behavior in relation to their own need (Schwabl and Lipar 2002). When experiencing a temporary stressful situation, for instance due to food shortages or immune challenges, nestling birds release corticosterone into the blood to adopt adequate behavioral and physiological responses (Nunez-de la Mora et al. 1996; Kitaysky et al. 1999; Sockman and Schwabl 2001). Siblings often differ in their adrenocortical stress response, higher levels of baseline and stress-induced circulating corticosterone often being found in older/dominant nestlings (Schwabl 1999; Creel 2001; Love et al. 2003; Blas et al. 2006; Müller et al. 2010). Interestingly, an experimental manipulation of circulating corticosterone levels induced more intense begging behaviors in the presence of parents in both the black-legged kittiwake (*Rissa tridactyla*; Kitaysky et al. 2001) and house sparrow (*Passer domesticus*; Loiseau et al. 2008). While in kittiwakes experimental elevation of corticosterone levels did not modify the rate of sib–sib agonistic interaction, in blue-footed boobies (*Sula nebouxii*) it induced subordinate nestlings to increase spontaneous submissiveness towards their non-implanted dominant brood mates, though the authors could not disentangle the exact mutual influence of siblings on each other (Vallarino et al. 2006). The main stress hormone, corticosterone, thus appears to play a major role in how nestling birds adjust the level of vocal signaling and physical competitive behaviors. Studying this role is likely to provide key insights into how environmental and social stressful factors influence sib–sib and parent-offspring interactions.

In the present study, we investigated whether corticosterone modulates investment in vocal signaling and physical competition. To this end, we experimentally manipulated corticosterone levels in barn owl nestlings (*Tyto alba*). In the prolonged absence of parents, siblings communicate vocally among each other to indicate their motivation to compete for the indivisible food item next delivered by a parent, a process referred to as 'sibling negotiation', and they barely show physical aggressiveness. The hungriest individual vocalizes at a high rate, which deters its siblings from begging and competing once parents are back at the nest with a food item (Roulin 2004). In a free-living barn owl population, we created 34 two-chick broods by temporarily removing nestlings from nests except two randomly chosen individuals. Two or five days earlier we implanted them with either a corticosterone-releasing pellet or a placebo-pellet, a manipulation that modifies the level of circulating corticosterone within the natural range (Müller et al. 2009). We recorded the vocal behavior of the two individuals both in the absence of the parents (i.e. negotiation), during the 15 min preceding their arrival with the first prey item of the night, and in their presence (i.e. begging). We also recorded the position of the two siblings relative to the nest-box entrance where parents predictably deliver food, and which of the two individual obtained the prey item. As shown in a previous study, an experimental increase in corticosterone levels impairs humoral immunity, resistance to oxidative stress and growth rate (Stier et al. 2009). Assuming that jostling for position, vocal negotiation and begging entail substantial costs (Leech and Leonard 1996; Rodriguez-Girones et al. 2001b; Roulin 2001b; Bize and Roulin 2006; Moreno-Rueda 2010; but see Moreno-Rueda 2007), the manipulation of corticosterone levels may differentially affect these behaviors.

Methods

Study Species

The study was carried out in 2004 and 2005 using free-living barn owls breeding in nest-boxes (100 × 60 × 50 cm) located in western Switzerland (46°49′N/06°56′E). The two to eleven eggs hatch every 2.5 days since incubation starts as soon as the first egg has been laid. A pronounced within-brood age hierarchy is thus established among the siblings. Until nestlings are 3 weeks old, the female stays at the nest to provision offspring with small mammals brought by the father. Afterwards, the mother delivers one-third of the prey items to the offspring, each item being consumed by a single offspring. We thus carried out the experiments when nestlings were old enough to be thermo-independent so that when we manipulated nestlings during the daylight hours, parents were naturally sleeping outside their nest-box in another barn as our observations demonstrated. Until fledging, occurring at ca. 55 days of age, siblings compete for food vocally and physically without, however, being overly aggressive. Previous studies showed that in broods of two nestlings the individual that obtains the first prey item of the night produces on average 7.7 calls/min before a parent arrives at the nest and 45.8 in its presence; its sibling produces 3.9 calls/min in the absence and 30.4 in the presence of parents (Roulin 2001a). Nestling age was estimated shortly after hatching by measuring the length of the left flattened wing from the bird's wrist to the tip of the longest primary (Roulin 2004). Nestling sex was determined using molecular markers (Py et al. 2006).

Experimental Manipulation of Corticosterone Level

To study the effect of corticosterone on nestlings' behavior, we considered 19 nests in 2004 and 15 nests in 2005. In each nest in 2004 we implanted two individuals with a 15 mg self-degradable corticosterone-releasing pellet (cat # G-111, Innovative Research of America (Sarasota, FL, USA)) and two siblings with a placebo pellet. The pellets were placed under the skin of the flank above the knee through a small incision (see Müller et al. (2009) for further details on the implantation procedure). In 2005 we implanted one individual per nest with a similar corticosterone-releasing pellet and one sibling with a placebo pellet. In both years, at the day of implantation owlets implanted with a corticosterone-releasing pellet (hereafter 'cort-nestlings') and owlets implanted with a placebo-pellet (hereafter 'placebo-nestlings') were similarly aged (2004: 30.0 ± 0.7 days vs. 31.0 ± 0.9 days; paired t test, $P = 0.30$; 2005: 31.0 ± 0.9 days vs. 30.0 ± 1.4 days; $P = 0.70$) and had a similar weight (2004: 342 ± 10 g vs. 356 ± 9 g; $P = 0.30$; 2005: 318 ± 6 g vs. 327 ± 15 g; $P = 0.40$) on average. We did not implant younger nestmates (aged 26.0 ± 2.3 days) because the corticosterone-releasing pellets were designed for older individuals. We recorded the behavior of one cort- and one placebo-implanted nestling in each nest. To do so, we removed all owlets from their nest-box except one cort- and one placebo-individual among the four implanted individuals in each nest in 2004, and all but the two implanted individuals in 2005. Using an infrared camera with a microphone we recorded the two siblings ringed on a different leg for individual recognition from 19:00 to 24:00. At 24:00 we brought back the removed individuals that were previously placed in a large ventilated plastic box at some distance from their nest-box. This experimental design was already successfully used in a previous study (Roulin et al. 2000; Roulin 2004). We video-recorded implanted nestlings five-days post-implantation in 2004, with placebo nestlings being significantly heavier than their cort-sibling (359 ± 8 vs. 325 ± 9, $P = 0.02$) but similarly aged (paired t test, P value $= 0.71$). We chose to record five-days post implantation since the pellets were designed by Innovative Research of America to release corticosterone during 6 days. To confirm this statement, we collected blood and analyzed it in the autumn 2004. It appeared that a measurable increase in corticosterone occurred only during the first 3 days post-implantation (Müller et al. 2009). For this reason, we changed the experimental design in 2005 and recorded nestlings' behavior 2 days post-implantation and not 5 days as in 2004. In total, we implanted with a corticosterone-releasing pellet as often the younger individual, so-called junior, as the older, so-called senior ($n = 18$ vs. 16), and as often males as females (each time 17). In 2005, Cort- and placebo-siblings did not differ in age and body mass on the day when we recorded their behavior (paired t tests, both $P > 0.19$).

We measured baseline total corticosterone levels in implanted nestlings by collecting blood samples at the day of implantation, 2, 6 and 20 days after implantation in 2004, and at the day of implantation, 3 and 20 days after implantation in 2005. To determine baseline corticosterone levels we collected blood samples within 3 min after having opened the nest box (Romero and Reed 2005).

Stress-induced corticosterone levels were monitored 2 or 3 days post-implantation by collecting a blood sample on average 27 ± 0.75 min after opening the nest-box. The time until we collected the stress-induced corticosterone samples did not differ between the two treatments (paired *t* test: $P > 0.40$). Total plasma corticosterone concentration was measured with an enzyme immunoassay (Munro and Stabenfeldt 1984; Almasi et al. 2009). Before implantation, there was no difference in baseline corticosterone between placebo- and cort-nestlings in both years (Table 1). Two days after implantation cort-nestlings had significantly higher baseline corticosterone levels than placebo-nestlings (Table 1). Three, six and twenty days after implantation baseline corticosterone levels did not differ anymore between the two treatment groups (Table 1). Two and three days post-implantation the stress-induced corticosterone level of cort-nestlings was significantly lower compared to placebo-nestlings (Table 1). Thus, when we video-recorded nestlings during the second night after implantation in 2005, our experimental corticosterone treatment was likely to still have an effect on the hypothalamic–pituitary–adrenal (HPA)-axis. It was also likely the case during the fifth night after implantation in 2004 since cort-nestlings were heavier than their placebo siblings and lasting detrimental effects on body conditions were still observed several weeks later (Almasi et al. submitted).

Assessment of Nestling Behaviors

On the video footage we analyzed nestling behavior in the absence of parents (i.e. during the so-called negotiation period) during the first 14 min of the 15 min preceding the first parental visit of the night; they always brought a prey item at this visit. During this period we determined the mean negotiation call rate per individual (number of calls per minute) by counting negotiation calls produced by the placebo- and cort-siblings blindly with respect to treatment. The relative position of each owlet in the nest-box during the 14 min of observation (hereafter referred to as ‘Position in parents’ absence’ was defined as the amount of time it stood closer (but not at a similar distance) to the nest-box entrance than its sibling over the total amount of time during which one individual was closer (but not at a similar distance) to the nest-box entrance than its sibling. We analyzed 28 broods for this variable because in 6 of the 34 broods the two siblings stood at the same distance to the entrance during the 15 min preceding the arrival of a parent.

We determined the mean begging call rate per individual (number of calls per minute) in the presence of parents by counting calls between the time when nestlings detected the incoming parent and the time when this parent gave its prey item to one of the two nestlings; as soon as a parent is detected the offspring change their behavior by being more vocal and approaching the nest entrance. Because the time span during which calls were counted varied from one nest to another (mean ± SE: 14.1 ± 3.7 s), we considered only calls produced during the first 5 s after the parent was detected. We analyzed 19 broods out of 34 for this variable because in 15 broods the prey item was consumed within these 5 s. Calls were correctly assigned to one of the two siblings because nestlings open their bill while calling, and calls of different individuals are easily distinguishable by the human ear (pers. obs.). Finally, we determined the relative position of the two siblings at the exact moment when the incoming parent was detected (hereafter referred to as ‘Position in parents’ presence’). We assigned score 1 to the cort-individual when it was closer to the nest-box entrance than its placebo-sibling, score 0 when it was at the same distance, and score -1 when it was further away from

Table 1 Baseline and stress-induced corticosterone levels and body mass in corticosterone- and placebo-implanted barn owl nestlings in 2004 and 2005

Year	Day	Baseline corticosterone (ng/ml)		Welch *t* test	Stress-induced corticosterone (ng/ml)		Welch *t* test	Body mass (g)		Welch *t* test
		Cort	Placebo		Cort	Placebo		Cort	Placebo	
2004	0	8.8 ± 1.3 (6)	10.9 ± 2.3 (9)	0.4				344 ± 6 (19)	349 ± 10 (19)	0.7
	2	26.0 ± 3.1 (10)	9.5 ± 1.6 (8)	<0.001	28.1 ± 3.2 (14)	50.1 ± 5.8 (14)	<0.001	320 ± 6 (19)	341 ± 7 (19)	0.03
	3									
	6	12.6 ± 3.0 (11)	14.9 ± 2.9 (9)	0.6				327 ± 6 (19)	357 ± 8 (19)	0.02
	20	12.8 ± 2.1 (9)	11.4 ± 1.8 (8)	0.6	55.2 ± 6.6 (11)	69.5 ± 5.0 (11)	0.1	353 ± 6 (19)	356 ± 4 (19)	0.6
2005	0	8.5 ± 1.4 (14)	8.5 ± 0.9 (13)	0.1	58.1 ± 9.0 (10)	68.6 ± 14.3 (10)	0.5	313 ± 8 (15)	327 ± 14 (15)	0.4
	2									
	3	9.2 ± 1.4 (15)	11.5 ± 2.0 (9)	0.4	39.8 ± 12.1 (12)	65.7 ± 8.7 (12)	0.02	321 ± 4 (15)	333 ± 6 (15)	0.1
	6									
	20	7.8 ± 0.9 (15)	8.3 ± 2.6 (10)	0.8				334 ± 10 (15)	381 ± 9 (15)	0.002

Data were collected on the day of implantation (day 0) and 2, 3, 6 and 20 days post implantation. *P* values for two-tailed Welch *t* tests are presented. Sample sizes are given in brackets the nest-box entrance than its placebo-sibling. We also noted the identity of the nestling that obtained the first food item of the night, and in 31 broods we could determine the identity of the individual that ingested it. In all cases, the individual that ate the prey item was also the one that obtained it from its parent.

Statistical Procedure

In all our analyses we pooled the data collected in 2004 and 2005 because cort- and placebo-nestlings displayed the same trends in all behaviors in both years despite that they were recorded at different times after implantation. Nonetheless, we controlled for potential biases due to the year at which we implanted them by including the variable ‘year’ in our analyses (see further). In a first step, we investigated how often the placebo- and the cort-nestlings monopolized the first delivered prey item of the night. We also examined whether call rates and relative positions both in the absence of parents and at their arrival were associated with the probability that the individuals monopolized the prey item, by performing a nested generalized binomial mixed effect model (GLMM) with prey monopolization (1 if the individual got the prey, 0 if it did not get the prey item) as response variable. Since we had fewer data on behaviors recorded in the presence than in the absence of parents, we performed two separate analyses for the situation when nestlings were negotiating and when they were begging food from their parents. In both models, we included site identity as random intercept to control for the dependency of the data collected in the two siblings per nest. Fixed effects comprised year, nestlings’ sex, treatment (placebo- vs. cort-), negotiation (or begging) call rate and relative position in the nest in the absence (or at the arrival) of parents (i.e. position in parents’ absence/presence). We accounted for nestlings’ seniority (junior or senior) only in the model on negotiation behaviors since the model on begging behaviors did not converge when we included it and seniors and juniors displayed similar trends anyway. In both models, we also included the interaction between the corticosterone treatment and year, and the interactions between treatment and negotiation (or begging) call rate, and between treatment and relative position in the nest while negotiating (or begging). These interactions were implemented to examine whether the effect of treatment varied across the 2 years and whether the influence of nestlings’ behaviors on the success in prey monopolization depended on treatment. All fixed effects were tested using a ‘Monte Carlo simulation’ approach after Faraway (2006). Thereby, the distribution of the likelihood ratio for comparing an alternative model (containing a given term) with a null model (model without this term) was approximated using Monte Carlo simulation. We simulated 200 times a set of response values from the null model and calculated the likelihood ratio between the alternative and the null model for each set of simulated response values. From these 200 likelihood ratios an approximation of the distribution of the likelihood ratio was obtained and used instead of a Chi-square distribution to obtain the *P* value (Faraway 2006). Non-significant interactions were removed from the model before testing main effects independently from one another.

In a second step, we investigated whether negotiation and begging call rates, as well as the relative position in the nest in the absence of parents and at their arrival, were affected by treatment. For negotiation and begging call rates and the relative position in the absence of parents, we ran a nested linear mixed-effect model with normal distribution for each behavior with nestlings’ behavior as the response variable and site identity as random intercept to control for the dependency of the data collected in the two siblings per nest. We included year, nestlings’ seniority and sex, treatment and the interaction between treatment and year as covariates. The model with the negotiation call rate as dependent variable also comprised the relative position in the absence of parents and its interaction with treatment, and vice versa for the model of relative position in the absence of parents; the model of begging call rate comprised both negotiation call rate and relative position in the absence of parents plus their interaction with treatment. All fixed effects were tested using a similar approach as described above except that we could run 500 simulations since it was less time consuming than to simulate in a binomial-model. Since position in parents’ absence varied between 0 and 100% of time spent in front of the sibling relative to the nest entrance, and hence corresponded to a proportion, we arcsine-root transformed this variable to obtain normally distributed residuals.

Because of the way we measured the relative position of cort-individuals at the arrival of parents (position in relation to the nest entrance and to the location of their placebo-sibling), we did not run mixed effects models on this variable. We tested the effect of treatment on the position of cort-nestlings with a Wilcoxon signed rank test; we did not perform a paired test because there was only one value per nest (either the cort-individual was in front, behind or at the same distance to the nest entrance as its placebo sibling).

All statistical tests were done using the software package R version 2.12.1 (R Development Core Team 2010), with libraries lme4 for mixed-effect models and arm for the simulations. *P* values and estimates obtained from simulations were consistent with those of non-reduced models, indicating that our results were not biased by an inflated type-I error due to multiple testing on our small sample sizes. Means and estimates are quoted ± SE if not indicated otherwise. P values ≤ 0.05 were considered as significant.

Ethical Note

The study was carried out with the agreement of the 'Service Vétérinaire du canton de Vaud' (authorization no1736). The manipulation of corticosterone as well as implanting a pellet (corticosterone-releasing or placebo) did not alter nestlings' fledging success since 94% of all implanted nestlings in 2004 and 2005 survived until fledging (unpubl. data).

Results

Effects of Corticosterone and Nestlings' Behaviors on Prey Monopolization

Binomial mixed effect models tested the effects of the corticosterone treatment and nestlings' behaviors in the absence of parents (Table 2a) and at their arrival (Table 2b) on the success in prey monopolization. Nestlings' success in obtaining the first prey item of the night brought by the parents did not depend on treatment, year, or their interaction, and neither on sex and seniority. Indeed, a similar number of cort- and placebo-nestlings (18 vs.16), females and males (16 vs. 18), and juniors and seniors (20 vs. 14) monopolized the first prey item of the night, (all $P \geq 0.1$, Table 2a, b). Prey obtaining was associated with nestling behaviors in the absence of parents in a similar way in placebo- and cort-individuals because both interactions between behaviors and treatment were not significant (both $P \geq 0.8$, Table 2a); higher negotiation call rate (estimate: 0.20 ± 0.08) and proportion of time spent in front of their sibling relative to the nest hole before parents' arrival (estimate for 'Position in parents' absence': 2.2 ± 0.9) were associated with a higher probability of obtaining the prey item (Table 2a, Fig. 1). The probability that a nestling monopolized the prey item was 60% if it stayed always closer to the nest-box entrance than its sibling and 14% if it stayed always behind its sibling.

By contrast to behaviors in the absence of parents, nestlings' call rate and relative position in the presence of parents did not significantly affect their chance of being fed (both $P = 0.1$, Table 2b). However, the non-significance of begging call rate on the success in prey monopolization may be due to a lack of statistical power, since both placebo- and cort-nestlings begged at a higher rate just before monopolizing the first prey item than when the item was monopolized by their sibling (Student's t tests, both $P < 0.05$, Fig. 1).

Table 2 Binomial mixed-effect models on prey monopolization in the barn owl with nestlings' call rate and relative position (A) in the absence of parents and (B) in the presence of parents, as covariates

Prey monopolization			
Fixed effects	df	LR	P_{boot}
A: behaviors in parents' absence			
Year	1	0.0	0.9
Nestling seniority	1	0.6	0.5
Nestling sex	1	0.4	0.6
Cort treatment	1	3.0	0.1
Negotiation call rate	1	**10.2**	**0.005**
Position in parents' absence	1	**7.4**	**0.02**
Cort treatment × year	1	0.8	0.4
Cort treatment × negotiation call rate	1	0.0	0.9
Cort treatment × position in parents' absence	1	0.1	0.8
B: behaviors in parents' presence			
Year	1	0.0	0.9
Nestling seniority	–	–	–
Nestling sex	1	1.4	0.3
Cort treatment	1	0.9	0.4
Begging call rate	1	4.4	0.1
Position in parents' presence	2	5.9	0.1
Cort treatment × year	1	0.0	0.9
Cort treatment × begging call rate	1	0.1	0.7
Cort treatment × position in parents' presence	2	3.6	0.2

Site identity was included as a random intercept. Analyses are based on (A) 56 individuals from 28 sites and (B) 38 individuals from 19 sites

All these findings were robust despite the dependency of behaviors on treatment. Indeed, fitting treatment and behaviors as independent variables in separated analyses gave similar results (GLMM with prey monopolization as dependent variable and with treatment as fixed effect: $df = 1$, LR = 0.9, $P_{boot} = 0.4$; GLMM with negotiation behaviors as fixed effects: negotiation call rate: $df = 1$, LR = 7.3, $P_{boot} = 0.03$, position in parents' absence: $df = 1$, LR = 10.5, $P_{boot} = 0.02$; GLMM with begging behaviors as fixed effects: begging call rate: $df = 1$, LR = 4.4, $P_{boot} = 0.06$; position in parents' presence: $df = 2$, LR = 6.5, $P_{boot} = 0.06$).

Effect of Corticosterone on Behaviors and Mutual Influences Between Behaviors

Linear mixed-effect models tested whether negotiation call rate, relative position in the nest before the arrival of parents, and begging call rate were affected by treatment and how these behaviors influenced each other (Table 3). Nestlings' negotiation and begging call rates and relative position in the absence of parents were not affected by the

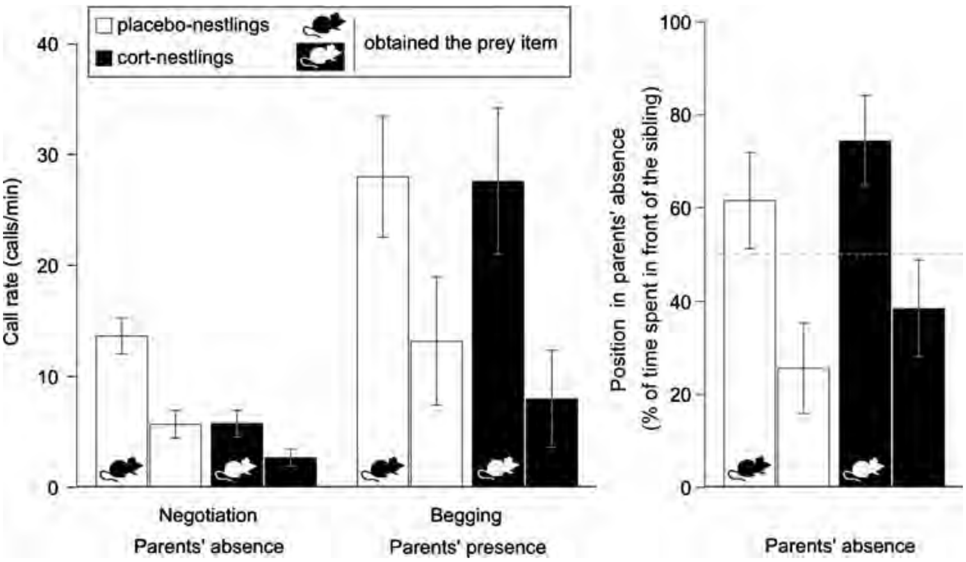

Fig. 1 Mean negotiation and begging call rates (*left*) of barn owl nestlings and proportion of time they spent closer to the nest entrance than their siblings in the absence of parents (*right*) according to whether they obtained the next prey item delivered by their parent or not. Above and below the *dotted line*, nestlings spent respectively more time in front of or behind their sibling. *Bars* represent means ± standard errors

Table 3 Mixed-effect models on negotiation call rate, relative amount of time spent closer to the nest entrance (position) in the absence of parents (arcsine-root transformed) and begging call rate

	Negotiation call rate			Position in parents' absence			Begging call rate		
Fixed effects	*df*	LR	P_{boot}	*df*	LR	P_{boot}	*df*	LR	P_{boot}
Year	1	0.3	0.6	1	0.1	0.8	1	0.05	0.8
Seniority	1	0.1	0.8	1	0.2	0.7	1	0.1	0.8
Sex	1	1.4	0.3	1	1.1	0.4	1	**5.2**	**0.05**
Cort treatment	1	**12.0**	**0.002**	1	**6.5**	**0.02**	1	0.01	0.9
Negotiation call rate	–	–		1	**5.8**	**0.04**	1	4.7	0.07
Position in parents' absence	1	**5.1**	**0.04**	–	–		1	**6.2**	**0.04**
Cort treatment × year	1	0.5	0.5	1	0.8	0.4	1	0.02	0.9
Cort treatment × negotiation call rate	–	–		1	0.04	0.9	1	0.5	0.6
Cort treatment × position in parents' absence	1	1.4	0.3	–	–		1	0.5	0.6

In each model, site identity was included as a random intercept. Analyses are based on 56 individuals from 28 sites for negotiation call rate and position in parents' absence and on 32 individuals from 16 sites for begging call rate. Dashes correspond to the fixed effects that were not included in the models

year of experiment or by seniority (all $P \geq 0.6$). Sex did not influence the behaviors either, except for begging call rate (males produced 13.3 ± 5.6 more calls/min in presence of parents than females, $P = 0.05$).

In the absence of parents, treatment had a significant effect on both negotiation call rate and relative position (Table 3), which was consistent across the 2 years of experimentation (interactions 'Cort treatment × Year', both $P \geq 0.4$). In both placebo- and cort-nestlings, the level of negotiation and the relative amount of time spent in front of their sibling were positively correlated (negotiation call rate as the response variable, estimate for position: 4.2 ± 1.8 calls/min; relative position in the absence of parents as the response variable, estimate for negotiation call rate: 3.3 ± 1.3, interactions 'Cort treatment × Position in parents' absence' and 'Cort treatment × Negotiation call rate': both $P \geq 0.3$), as illustrated in Fig. 2. Additionally, placebo-individuals produced significantly more calls on average than their cort-sibling (negotiation call rate as the response variable, estimate for treatment (placebo vs. cort): 5.6 ± 1.5 calls/min), whereas cort-individuals spent significantly more time, ca. 18% (7–33%), close to the nest-box entrance than placebo-nestlings (Table 3, relative position in the absence of parents as the response variable, estimate for treatment (placebo vs. cort): −0.4 ± 0.2).

Nestlings that spent more time close to the nest entrance in the absence of parents begged at a higher rate at the arrival of a parent (Table 3, begging call rate as the response variable, estimate for 'Position in parents' absence': 19.5 ± 7.5 calls/min). In addition, the more nestlings had negotiated the more they tended to beg at the parents, but this relationship remained marginal (begging call rate as the response variable, estimate for negotiation

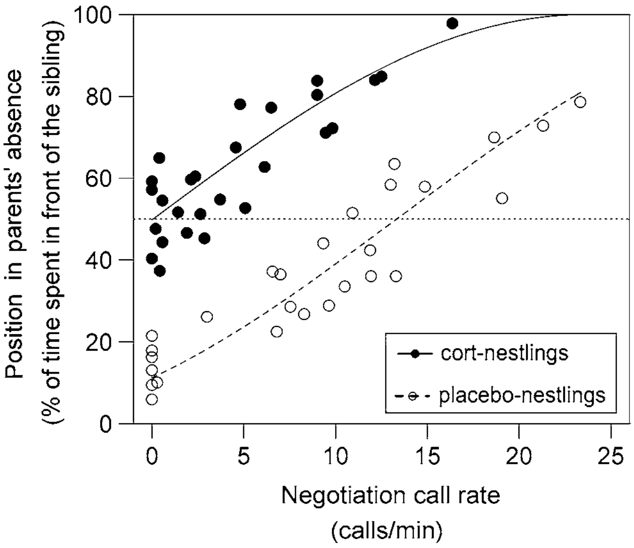

Fig. 2 Amount of time that barn owl nestlings spent closer to the nest entrance than their siblings (position in the absence of parents) in relation to negotiation call rate before the first feeding of the night. *Dots* and *curves* represent back-transformed predicted values and curves from linear mixed model of Table 3 for position in the absence of parents in relation to negotiation call rate in cort-implanted (*black dots*, *plain line*) and placebo nestlings (*white dots*, *dashed line*) respectively ($n = 28$ broods). Above and below the *dotted line*, nestlings spent respectively more time in front of or behind their sibling

call rate: 1.1 ± 0.5 calls/min, $P = 0.07$). These trends did not differ between placebo- and cort-individuals (‘Cort treatment × Position in parents’ absence’ and ‘Cort treatment × Negotiation call rate’, both $P = 0.6$). However, treatment had no effect on nestlings’ begging call rate at the parents’ visit, whatever the year of experimentation (‘Cort treatment’ and ‘Cort treatment × Year’, both $P = 0.9$). Cort-nestlings stood as often closer to the nest entrance as placebo-nestlings during parents’ presence (relative position of the cort-individual as dependent variable, Wilcoxon signed rank test: $n = 32$, $V = 0.56$, $P = 0.43$).

Discussion

In nestling barn owls, we investigated whether the hormone that mediates physiological and behavioral responses to stressors, i.e. corticosterone, regulates investment in the level of vocal signaling to both siblings (i.e. negotiation) and parents (i.e. begging), and the frequency with which siblings stand close to the nest-box entrance where parents predictably arrive. We also examined whether this hormone influences the probability of monopolizing food. Our main finding is that nestlings implanted with a corticosterone-releasing pellet, despite monopolizing the first prey item of the night as often as their placebo-siblings and maintaining an equal begging effort at the parent’s arrival, reduced investment in the level of sib–sib communication (i.e. they vocalized at a lower rate in the absence of parents) and spent more time closer to the nest entrance where parents predictably deliver food before the parent’s visit (Fig. 1). Thus, corticosterone induced nestlings to modify their strategies to compete over food resources delivered by their parents. Indeed, prey monopolization was enhanced by higher effort both in negotiating with siblings, in begging towards the parents, and in standing closer to the nest entrance before parents’ visit. Apparently, the strategies employed by cort- and placebo-nestlings were equally successful, since they monopolized food as often. Our results suggest that corticosterone induces nestlings to switch from vocal to physical competition in the absence of parents but not in their presence. This is in line with previous theoretical and empirical work showing that food supply (i.e. a potential cause of stress) affects sibling negotiation to a larger extent than begging behavior (Roulin 2001a; Johnstone and Roulin 2003).

Effect of Corticosterone on Sib–Sib Interactions

Barn owl nestlings implanted with a corticosterone-releasing pellet showed an impaired body mass gain (Table 1) and reduced humoral immunity and resistance to oxidative stress (Stier et al. 2009). Thus, cort-nestlings were in a stressful state implying that the benefit of monopolizing a food item was probably higher for them than for placebo-nestlings, while the costs per unit of investment in signaling and sibling competition were probably more detrimental to cort- than placebo-nestlings. Because cort-nestlings consumed the first prey item delivered of the night as often as placebo-nestlings, we conclude that corticosterone prevented nestlings to invest extra effort in sibling competition to an extent that would have allowed them to compensate for the negative effects of corticosterone by eating more food. Indeed, they did not beg more frequently than their sibling and they refrained from negotiating.

In the barn owl, the nestling that begs at the highest rate in the presence of parents has a higher probability of monopolizing the delivered food item, and the effort an individual invests in begging depends on complex sib–sib interactions taking place in the absence of parents. A nestling positioned close to the nest entrance will induce its siblings to reduce investment in sibling negotiation. Additionally, an individual that negotiates at a higher level than its sibling deters them to beg intensely for food from their parents (Dreiss et al. 2010). Therefore, a nestling can employ two non-mutually exclusive strategies to influence its siblings to refrain from begging: (1) it stands close to the nest entrance to induce its siblings to refrain from negotiating or (2) it negotiates at a high level to induce its siblings to refrain from begging (Dreiss et al. 2010). By reducing investment in vocalizing in the absence of parents cort-individuals probably entailed the cost of not inducing their sibling to refrain from begging once parents are back at the nest. However, by behaving in this way cort-nestlings probably saved energy (Leech and Leonard 1996; Moreno-Rueda 2010; but see Bachman and Chappell 1998) to be reallocated into begging calls directed to the parents and to be able to spend more time closer to the nest-box entrance where parents delivered food in order to induce their siblings to refrain from negotiating.

Our findings differ from similar experiments carried out in nestling house sparrows (Loiseau et al. 2008). Sparrows treated with corticosterone increased their begging rate towards the parents but were unable to obtain as much food as their control siblings. Thus, in contrast to barn owl nestlings, house sparrows treated with corticosterone apparently behaved mal-adaptively because the increase in investment in sibling competition did not lead to a higher success in food obtaining. The discrepancy between our respective studies might be due to methodological differences. While we administrated corticosterone in barn owls within the natural physiological range (Müller et al. 2009), the dose used in sparrows was pharmacological. Kitaysky et al. (2001) also found that an experimental transient elevation of corticosterone induced black legged kittiwake (*Rissa tridactyla*) nestlings to exaggerate begging, which, in that case, resulted in an increase in food monopolization by 13%. This means that in kittiwakes the higher costs of sibling competition induced by corticosterone were compensated by larger benefits. By contrast, in Nuttall's white-crowned sparrow (*Zonotrichia leucophrys nuttalli*) nestlings facing an acute transient elevation in corticosterone increased their latency to beg, hence potentially decreasing their feeding success (Wada and Breuner 2008). Clearly, more data are required in a larger range of species to evaluate the dose-dependent effect of corticosterone on sib–sib and parent-offspring interactions. This is necessary to determine under which situation and in which species corticosterone induces or reduces the costs and benefits of sibling competition.

Implications on the Evolution of Parent-Offspring and Sib–Sib Interactions

Our study was designed to investigate the role of corticosterone on sib–sib interactions and in turn on how food is shared among the progeny. Begging behavior can influence not only the within-brood food allocation (Smith and Montgomerie 1991; Whittingham et al. 2003; Rosivall et al. 2005; Porkert and Spinka 2006) but also parental overall feeding rates (e.g. Ottosson et al. 1997; Burford et al. 1998; Glassey and Forbes 2002). Unfortunately, our within-brood design did not allow us to examine whether an experimental increase in nestling corticosterone levels also influenced parental feeding rates. To examine this issue, all siblings should be implanted either with corticosterone- or placebo-pellets. This is important to consider because begging could be cooperative with siblings sharing investment in begging to a given threshold in order to ensure that parents quickly come back at the nest with food (Johnstone 2004). Hence, the question is whether corticosterone promotes or refrains siblings to behave cooperatively. Our observations on sibling negotiation, a form of cooperative behavior, suggest that corticosterone would rather refrain siblings to behave cooperatively even in species in which sibling negotiation does not occur. Thus, if only part of the nestlings are treated with corticosterone within a brood of several nestlings, these individuals may reduce investment in begging if solicitations by the placebo-siblings are sufficient to ensure higher parental feeding rate.

Our results may appear paradoxical since in spite of producing fewer negotiation calls cort-individuals reached the same success in monopolizing food as placebo-individuals. If the alternative strategy of cort-nestlings to stand closer to the nest entrance proved efficient in prey obtaining, why did placebo-individuals not behave in a similar way? A potential explanation is that standing close to the nest hole represents the most costly option with the non-negligible risk of falling out of the nest (Bize and Roulin 2006) a frequent outcome in the barn owl (pers. obs.). Additionally, reducing negotiation effort as in cort-individuals, may not be as rewarding as negotiating and may not be stable in the long-term. The primary function of sibling negotiation is to reduce the level of sibling competition, and thus reducing the level of negotiating would induce nestlings to become more aggressive among each other and to beg to higher levels; these costs may be higher than those induced by negotiation (Roulin 2002).

In conclusion, our results suggest that stressful factors that induce a transient rise in corticosterone levels may mainly promote the evolution of scramble competition rather than sibling negotiation and other forms of cooperation. Further experimental studies focusing on the adaptiveness of switching to physical competition in stressful situations would nonetheless be helpful to properly test this hypothesis. Manipulating corticosterone here rather than food need, as usually done in begging studies, thus demonstrates that diverse source of stress beyond food supply may have potent effects on the evolution of sib–sib vocal and physical interactions, and more generally on parent-offspring conflict.

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APPENDIX 2

Dominant nestlings displaying female-like melanin coloration behave altruistically in the barn owl

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Dominant nestlings displaying female-like melanin coloration behave altruistically in the barn owl

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When competing over parental resources, young animals may be typically selfish to the point of siblicide. This suggests that limited parental resources promote the evolution of sibling competition rather than altruistic or cooperative behaviours. In striking contrast, we show here that in 71% of experimental three-chick broods, nestling barn owls, *Tyto alba*, gave food to their siblings on average twice per night. This behaviour prevailed in the first-born dominant nestlings rather than the last-born subordinate nestlings. It was also more prevalent in individuals displaying a heritable dark phaeomelanin-based coloration, a typical female-specific plumage trait (owls vary from dark reddish to white, females being on average darker reddish than males). Stealing food items from siblings, which occurred in 81% of the nests, was more frequent in light than dark phaeomelanic dominant nestlings. We suggest that food sharing has evolved in the barn owl because parents store prey items in their nest that can be used by the offspring to feed their nestmates to derive indirect (kin selection) or direct benefits (pseudoreciprocity or by-product mutualism). The cost of feeding siblings may be relatively low for dominant individuals while the indirect genetic benefits could be high given that extrapair paternity is infrequent in this species. Thus, in situations in which young animals have access to more food resources than they currently need, they can altruistically share them with their siblings.

The evolution of helping where an individual increases the direct fitness of another individual is a fascinating topic. Two categories of evolutionary pathways can account for the emergence of helping behaviour. When individuals gain direct material benefits from helping they are said to behave cooperatively and when they derive indirect genetic benefits the helping behaviour is referred to as altruistic (Lehmann & Keller 2006; Bshary & Bergmüller 2007). The typical situation in which individuals derive indirect fitness benefits is when the genetic benefits of helping related individuals outweigh the cost of helping (Hamilton 1964; Hatchwell 2010). An individual may also help a conspecific (related or not) in the hope that it will reciprocate on another occasion (Trivers 1971); even if reciprocation does not occur the helper may still derive material benefits if increasing the survival of surrounding individuals is beneficial (pseudoreciprocity or by-product mutualism hypotheses, Leimar & Hammerstein 2010). For instance, helping may increase group size, which can decrease the risk of predation (Kokko et al. 2001) or induce parents to provide more food resources at the nest (Kilner et al. 2004). Helping behaviour among family members has been studied in depth in the context of so-called cooperative breeding where mature offspring help raise their parents' new offspring (Clutton-Brock 2002; Bergmüller et al. 2007). In contrast, interactions between siblings still dependent on their parents are considered as conflictual rather than harmonious (Trivers 1974).

Conflicts between siblings take their root in the mismatch between parental food supply and offspring food demand leading to intense sibling competition to monopolize the limited resources. Parents do not provide all the food requested by their offspring because reproductive activities are costly, they face a trade-off between offspring number and quality, and they often produce more offspring than they can rear to independence (Mock & Parker 1997). The evolutionary outcome of limited parental resources is therefore predicted to be sibling rivalry rather than sibling cooperation or altruism. This led biologists interested in the evolutionary implications of parental care to consider Hamilton's rule useful not only to specify the conditions promoting altruism but also the conditions that promote selfish behaviour. This so-called 'inverse Hamilton's rule' states that an allele coding for

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selfishness will spread if the benefits of being selfish exceed the costs to the victim multiplied by the coefficient of relatedness between the selfish individual and the victim (Mock & Parker 1997).

In line with the view that interactions between siblings are conflictual rather than harmonious, altricial offspring have only been anecdotally reported to help their siblings obtain parental resources (Marti 1989). Frequent observations of aggressive competition between siblings over parental attention to the point of siblicide suggest that conflicts of interests between young siblings indeed promote the evolution of selfish rather than altruistic or cooperative behaviours (Mock & Parker 1997). Sibling rivalry over parental resources may hamper the evolution of helping relatives if the indirect genetic benefits gained from helping kin are inferior to their costs (West et al. 2002). For instance, dominant offspring may behave more selfishly with their siblings to impose their physical superiority (Drummond et al. 2003). Since altruistic or cooperative interactions between young siblings that are still dependent on their parents appear to be infrequent (with the exception of humans; Kramer 2011), little is known about the factors that could induce helping behaviours among them. As sharing the same family unit is an individual's first social experience, family interactions may have facilitated the evolutionary transition from selfishness to helping, while helping behaviours may reinforce family bonds (von Bayern et al. 2007).

Sharing parental food resources with siblings may occur in species in which parents store food in their nest. If some individuals have privileged access to stored resources and are unable to utilize all of them, they may be selected to share them with hungry siblings that have less access to these resources. Such helping behaviour can evolve if individuals that help their siblings obtain parental resources derive direct or indirect fitness benefits (Lehmann & Keller 2006; Bshary & Bergmüller 2007; West et al. 2007). Reciprocity would be the most likely type of direct benefits. An individual may share food with a sibling in expectation of a future return from it that will compensate for the costs of the initial cooperative investment (e.g. Wilkinson 1992). In other words, a helper individual shares surplus parental resources with their hungry siblings in the hope that they will reciprocate once the helper is hungry. Another category of direct benefits, so-called pseudoreciprocity or by-product mutualism, is if, by feeding siblings, helper offspring release their parents from spending time distributing food among the progeny, which would allow them to invest more time in foraging and increase the total amount of food brought to the progeny. Alternatively, helping siblings may enhance their survival and thereby reduce the helper's risk of being killed by a predator or increase the total begging solicitation levels produced by progeny to stimulate parents to come back rapidly with food. Indirect genetic benefits may occur through kin selection, if the costs entailed by food sharing are compensated for by the increased survival of related individuals.

Species such as raptors, in which food is often stored in the nest (e.g. Korpimäki 1987; Bakaloudis et al. 2012), are prime candidates to examine the evolution of helping behaviour between young siblings that are still dependent on their parents. We performed a study in the barn owl, *Tyto alba*, in which sharing food between siblings has been observed in both Europe (Epple 1979; Bühler 1981; Kniprath & Stier-Kniprath 2010) and North America (Marti 1989). Parents usually bring prey items more rapidly than their offspring can consume them (Baudvin 1980; Roulin 2001, 2004a). If food-satiated nestlings do not relinquish the accumulated prey remains, they can defend access to the prey for later consumption, for instance, by sitting on them to reduce the risk of being robbed, which is relatively frequent in barn owls (Roulin et al. 2008a). Alternatively, nestlings may feed their siblings if the latter did not notice the presence of surplus prey remains in their dark nest. The barn owl is particularly interesting also because pronounced hatching asynchrony gives an edge to the first-born individuals, which have easier access to parental food resources than later-born siblings. The 2–10 eggs hatch on average every 2–3 days, which generates a pronounced age and size hierarchy between siblings. The first-born dominant individuals may face a choice between monopolizing stored food for later consumption or feeding their subordinate siblings. Furthermore, barn owl plumage varies strongly from dark reddish (phaeomelanic) to white (nonphaeomelanic) and from immaculate (noneumelanic) to heavily marked with large black spots (eumelanic), traits for which the expression is under strong genetic control and not, or weakly, sensitive to environmental factors (Roulin & Dijkstra 2003). Appetite is higher in lightly eumelanic and darker phaeomelanic individuals (Dreiss et al. 2010a), and darker phaeomelanic individuals increase their body mass more rapidly than lighter coloured ones when food is available in large quantities (Roulin et al. 2008b). Plumage traits might thus advertise the propensity to share food with siblings.

We examined whether barn owl nestlings are more likely to derive direct or indirect fitness benefits by sharing food with siblings. Under the reciprocity hypothesis, helpers share food with their siblings in the hope that they will reciprocate at a later time. If this is the case, we predicted that an individual that received a food item from a sibling would feed it on another occasion. Alternatively, dominant nestlings may feed their younger siblings to release their parents from taking care of offspring that still need assistance in the nest, thereby inducing their parents to spend more time foraging. In this case, we expected parental feeding rate to be higher in broods in which food sharing occurred. Because higher parental feeding rates will be more beneficial to hungry than satiated individuals, we also predicted food sharing would prevail mainly in nests in which nestlings were in poorer condition. Under the kin selection hypothesis (but also the pseudoreciprocity and by-product mutualism hypotheses), we expected that food sharing would be expressed by individuals for which the costs of being generous were low while the potential benefits were high. Thus, in contrast to the reciprocity hypothesis, only nestlings with privileged access to food resources would share them with the siblings that were so needy that they were unlikely to reciprocate at a later time. In the barn owl, since the first-born nestlings (so-called seniors) are better able to obtain food than their younger siblings (i.e. juniors; Roulin 2004b), sharing food with siblings should be less costly for seniors while the benefit of being fed by nestmates should be greater for juniors. Therefore, if nestling barn owls share food with siblings because they derive indirect genetic benefits (or direct benefits as predicted by the pseudoreciprocity and by-product mutualism hypotheses), we predicted that seniors would feed their junior siblings more frequently than the opposite.

To test these predictions, we recorded interactions between nestlings in the field in experimentally reduced broods of three individuals: a senior, a junior and a middle-born sibling. Observations were carried out for one night from 1900 to 0700 hours the following morning. Since short-term experimental brood reduction does not alter parental feeding rate (Roulin et al. 2000), our design ensured that food was available in large enough quantities to induce food sharing. Therefore, intrinsically altruistic individuals were expected to have the opportunity to feed siblings with prey items that accumulated in the nest. Since barn owl nestlings frequently steal food from each other (Roulin et al. 2008a), we also investigated whether the degree of generosity (i.e. food sharing) was inversely related to the degree of selfishness (i.e. food stealing).

METHODS

The study was carried out in 2007 in western Switzerland on a wild population of barn owls breeding in nestboxes located on barn walls. The age of each nestling was determined soon after hatching by frequent nest visits during this period. We recognized each chick by clipping different combinations of toenails before ringing them with one or two aluminium numbered rings. Each individual was thus easily recognizable on the video footage because one individual was ringed on the left leg, another on the right leg and the third on both legs. Also, size differences between siblings are so pronounced that we could verify that our data scoring was reliable. At 1900 hours we reduced brood sizes (mean $\pm$ SE $= 5.8 \pm 0.2$) to three individuals by removing all nestlings except the first (mean $\pm$ SE $= 42 \pm 1$ days of age), middle (38 ± 2) and last-born individuals (34 ± 2); the mean age difference between junior and senior nestmates was 9 ± 1 days. During recording nights from 1900 to 0700 hours, we placed the extra nestmates in a ventilated box located at some distance from the nest. One day before the experiment, we installed an infrared-sensitive camera in the nestboxes, and we recorded the three siblings from 1900 to 0700 hours the next morning. We never observed any sign of distress in adults and nestlings.

We scored phaeomelanin-based coloration when nestlings were 50 days of age by comparing their colour with eight chips ranging from 1 for dark reddish to 8 for white, a highly repeatable method (Roulin 1999). We measured the size of black spots with a calliper to the nearest mm, also a reliable method (Roulin 1999). We could measure plumage traits in 38 of the 43 nestlings, explaining disparities in sample size between analyses. Sex of nestlings was determined using molecular markers (Py et al. 2006). A body condition index was given by the residuals of the regression of body mass on wing length ($F_{1,52} = 18.63$, $P < 0.0001$).

When the offspring are more than 3 weeks of age, parents typically feed them by transferring food items from bill to bill. Parents appear to give priority to the offspring that begs at a high level (Dreiss et al. 2010b), and before resuming hunting activities the mother and father stay in the nest alongside their offspring for on average 29 and 15 s, respectively (Roulin & Bersier 2007). During this period the mother is frequently looking for stored prey items in order to transfer them to the offspring that continues to beg loudly (A. Roulin, A. Da Silva & C. Ruppli, personal observations).

Food sharing between siblings was defined as the transfer of a prey item from bill to bill, either actively (with the donor going towards the recipient to give its item) or passively (it was unclear whether the donor was giving the item or whether the recipient took it). Passive transfer of food was considered to be food sharing because the donor showed hardly any sign of avoiding the transfer. Food stealing was defined as any successful or unsuccessful attempt to take a food item (with the bill) from a sibling that was consuming it. Food-stealing events are easy to identify because the two siblings drag the prey in opposite directions.

Statistical Procedure

Before reporting the tests of predictions, we first describe parental feeding behaviour and nestling food consumption in order to have a better picture of family interactions taking place when nestlings handle prey items. First, to investigate whether seniors, middle-born nestlings and juniors had equal opportunities to redistribute food to their siblings, we examined differences in the number of prey items each individual received from the parents, how many items they consumed, how many items they did not entirely consume and how fast they ate. We analysed all variables using linear mixed models (LMM) when the data were normally distributed and if this was not the case we performed generalized linear mixed models (GLMM) with Poisson error distribution. To investigate the effect of the within-brood age hierarchy (i.e. senior, middle-born, junior), we fitted age rank as an independent variable. We also accounted for the effects of nestling sex, colour and 'absolute age' (in number of days) as covariates.

To test whether food sharing varied between and within broods according to variations in food supply, we examined whether food sharing was related to hunger level, that is, whether its occurrence varied during the night and was associated with the number of prey items available in the nest. To test whether nestlings differed in their propensity to share food with siblings according to age rank (seniors, middle-born nestlings and juniors), absolute age, sex and coloration, we used similar linear mixed models as described above. Similarly, we tested interbrood and interindividual variations in food stealing.

Because all statistical analyses including the size of black eumelanic spots were not significant, we present only analyses carried out on phaeomelanin-based reddish coloration. Also, the initial size of the brood out of which we created the experimental three-chick broods was not associated with the number of prey items that were shared between three isolated siblings (Spearman correlation: $r_S = -0.13$, $N = 21$ broods, $P = 0.58$) or stolen from siblings ($r_S = 0.26$, $N = 21$ broods, $P = 0.25$). For this reason, we do not consider brood size in subsequent analyses.

All (G)LMM included nest identity as random intercept because nestmates could not be considered as independent statistical units. We ran all statistical analyses using SAS v 9.2 (SAS Institute inc., Cary, NC, U.S.A.). We used the REML method for GLMM, and MIXED or GLIMMIX procedures for LMM. We performed backward model selection with final models only containing significant effects, and main effects involved in significant interactions. Residuals from linear mixed models were checked for normality. Statistical analyses are two tailed and P values lower than 0.05 are considered significant. Means are quoted $\pm$ SE.

Ethical Note

This experiment was carried out under the legal authorization of the 'Service vétérinaire du Canton de Vaud' (1508.3). A similar procedure was used as previously explained in other papers (see ethical note in Roulin & Bersier 2007). As in previous studies (Roulin et al. 2000; Dreiss et al. 2010b), we offered dead laboratory mice to nestlings that remained in the ventilated box from 1900 to 0700 hours. At 0700 hours we put them back in their nest. These individuals were old enough to consume two to three mice by themselves whenever they wanted. Our study was not detrimental to the birds because parents never abandoned broods and temporarily reducing brood size does not reduce parental feeding rate (Roulin et al. 2000). All experimental individuals fledged successfully. As also shown in another study (Dreiss et al. 2010a) keeping birds outside the nest is not stressful as measured by blood-circulating corticosterone.

RESULTS

Parental Feeding Behaviour

At 1900 hours 1.1 ± 2.6 prey remains were present per nest and overnight parents brought 16.5 ± 5.2 voles. Thus, in the 21 nests we observed in total 346 parental feeding visits and an additional 56 cases where parents transferred to an offspring the remains of prey that were already lying on the nestbox floor before their arrival.

Per night, seniors and middle-born nestlings received twice as many prey items from parents as did their juniors (LMM: age rank:

$F_{1,27} = 7.04$, $P = 0.013$; Table 1; nestling sex, absolute age and colour were not significant: all $P > 0.63$). As a consequence, first- and middle-born owlets consumed more prey items per night than their junior nestmates (LMM: age rank: $F_{1,27} = 4.30$, $P = 0.04$; Table 1; nestling sex, absolute age and colour were not significant: all $P > 0.10$). This indicates that first- and middle-born owlets had probably more opportunities to redistribute food to their subordinate siblings (see below).

Nestling Food Consumption

In total we observed 404 cases in which nestlings started to consume a prey item without finishing it; in 235 cases this item was recently given by a parent and in 169 cases this was surplus prey. We observed this behaviour more often in seniors than middle-born individuals and juniors (LMM: age rank: $F_{1,35} = 4.26$, $P = 0.047$; Table 1; nestling sex, absolute age and coloration were not significant: all $P > 0.57$). This indicates that older nestlings have easier access to food and can perhaps consume the best parts of each prey item. Juniors took on average more time to eat an entire item than did seniors and middle-born nestlings (LMM: age rank: $F_{2,34.92} = 3.49$, $P = 0.04$; Table 1).

Description of Food-sharing Events

In 71% (15 of 21) of the three-chick broods, owlets fed nestmates from bill to bill on 46 occasions in total, representing one food-sharing event every 4 h 53 min or for every 7.5 prey items. On 23 occasions (50%), food sharing was an active process with the donor clearly going towards a sibling to give it an item; in the 23 other cases the process was more passive with the donor and recipient being close to each other and it was not possible to determine whether the donor gave the prey item or simply did not react when the recipient took it. For subsequent analyses we pooled active and passive food-sharing events, as separate analyses gave qualitatively similar results. The donor transferred an item that it just obtained from a parent in 31 cases (67.4%) and in the other 15 cases (32.6%) it transferred prey remains.

Food Sharing in Relation to Food Supply

Food-sharing events occurred mainly when nestlings were hungry, that is at the beginning of the night rather than the end (Spearman correlation between the proportion of times prey items were shared and the order in which they were delivered by parents, i.e. first item of the night, the second item, and so on: $r_S = -0.54$, $N = 28$, $P = 0.003$; Fig. 1a). However, the very first prey items of the night delivered by parents were usually not shared with siblings but rather quickly consumed (Fig. 1a). Indeed, at that time individuals were probably all hungry, as indicated by the fact that nestlings swallowed prey items instead of consuming them piece by piece more frequently at the beginning of the night than at the end (logistic regression for the probability that a prey item was swallowed in relation to time: $\chi^2 = 16.58$, $P < 0.0001$), probably because of the risk of being robbed (Roulin et al. 2008a).

Table 1
Feeding behaviour in senior, middle-born and junior barn owl nestlings

	Senior	Middle-born	Junior
Prey items received from parents per night	9.2±1.3	7.7±0.8	3.69±0.6
Prey items entirely consumed per night	3.9±0.4	3.6±0.4	2.9±0.4
Prey items not entirely consumed per night	7.7	6.7	4.8
Mean time taken to consume an entire prey item (s)	36±6	37±3	59±9
Prey items shared with siblings per night	1.10	0.95	0.14
Prey items stolen from siblings per night	0.57	0.42	0.62

Behaviours were recorded from 1900 to 0700 hours in 21 three-chick broods. Means are quoted ± SE.

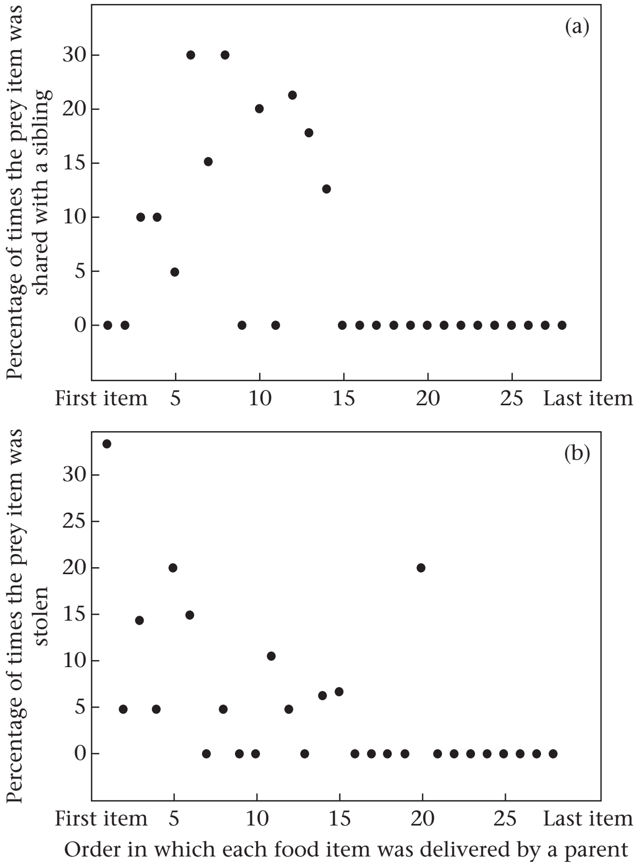

Figure 1. Probability that a food item was (a) shared with siblings or (b) stolen from a sibling in relation to the order in which this item was brought by a parent barn owl. The first item delivered during the night is represented by 1 on the X axis, the second delivered item by 2, and so on. For example, across the 21 nests, the nestling that obtained the fifth prey item delivered by a parent did not eat it but offered it to one of its siblings in 5% of cases. In 20% of cases, a nestling stole or tried to steal this fifth item that its sibling had just obtained from its parent. Data are from 21 nests.

If siblings share food to allow their parents to spend more time in foraging activities, we predicted parental feeding rate would be positively associated with the number of prey items shared between siblings. However, the total number of items transferred between nestmates in one night was not associated with the number of prey items available, that is, already present in the nests as prey remains at 1900 hours and delivered by parents during the night (GLM: number of prey remains: $F_{1,18} = 0.02$, $P = 0.90$; number of items brought by parents: $F_{1,18} = 0.03$, $P = 0.87$). Furthermore, the number of prey items that nestlings gave to their siblings was not associated with the number of items that the donors consumed throughout the night (GLMM: $F_{1,27} = 1.98$, $P = 0.17$). Therefore, variation in food sharing between and within broods was not determined by variation in food supply. Since nests with and without food sharing did not differ in mean nestlings' absolute age, body condition and mean phaeomelanin-based colour (logistic regression: all $P > 0.11$), we considered only the nests in which food sharing occurred to examine whether siblings differentially expressed this helping behaviour.

Food Sharing, Hatching Ranks and Coloration

Seniors and middle-born nestlings fed their siblings more frequently than did juniors (Tables 1, 2), as did darker compared to

Table 2
Food sharing and food stealing in relation to the position in the within-brood age hierarchy of nestling barn owls

	Food recipient/Individual from which food was stolen		
	Senior	Middle-born	Junior
Food donor			
Senior	–	0.43	0.67
Middle-born	0.38	–	0.57
Junior	0.05	0.10	–
Food stealer			
Senior	–	0.33	0.24
Middle-born	0.29	–	0.14
Junior	0.33	0.29	–

Behaviours were recorded from 1900 to 0700 hours in 21 three-chick broods. Food donors refer to individuals that gave a food item to a sibling; food recipients refer to individuals that were fed by a sibling. The oldest individual of three-chick broods is denoted 'senior', the youngest individual 'junior' and the intermediate individual 'middle-born'. Numbers indicate the mean number of items per night shared with a sibling or stolen from siblings.

light reddish phaeomelanic individuals (GLMM: age rank: $F_{2,25} = 12.05$, $P = 0.002$; colour: $F_{1,25} = 9.21$, $P = 0.006$; Fig. 2a–c; nestling sex, absolute age and the interaction between age rank and coloration were not significant: all $P > 0.09$). In fact, juniors only very rarely fed their siblings (Table 1) suggesting that they do not reciprocate.

In the present study, dark and light melanic owls consumed a similar number of prey items per night (see above). The fact that darker birds shared food more often than lighter phaeomelanic siblings therefore suggests that dark phaeomelanic nestlings are more willing to feed siblings at the potential expense of eating less. If this is the case, we should expect that darker birds would consume fewer prey items before starting to feed nestmates. To examine this proposition, we considered only nestlings that fed at least one sibling and counted the prey items they consumed before sharing food. Among 19 individuals that fed siblings, they consumed between zero and four items (1.6 ± 0.2) before sharing food. Compared to light melanic nestlings, darker reddish individuals consumed fewer prey items before starting to feed nestmates (mixed model ANCOVA: colour: $F_{1,18} = 4.43$, $P = 0.04$; Fig. 2d; nestling sex: $F_{1,18} = 11.15$, $P = 0.004$; age rank was not significant: $P = 0.60$). The factor sex was significant because nestling females started to feed nestmates earlier than nestling males.

Food Stealing

Nestlings stole ($N = 16$) or tried without success ($N = 18$) to steal a prey item from siblings on 34 occasions in 17 of the 21 nests (81%). The total number of prey items shared between siblings and stolen from siblings was not correlated within nests (Spearman correlation: $r_S = 0.07$, $N = 21$, $P = 0.76$). Stealing events primarily concerned prey items that a nestling recently received from the bill of a parent (27 cases, 79.4%); in seven cases (20.6%) it concerned prey remains that a nestling was consuming. When considering only the prey items brought by parents, stealing events happened mainly at the beginning of the night (Spearman correlation between the proportion of times items had been stolen and the order in which they were delivered: $r_S = -0.61$, $N = 28$, $P = 0.0005$; Fig. 1b). This is again consistent with the hypothesis that sibling competition is more intense at the beginning than at the end of the night.

The number of prey items stolen from siblings was related to the interaction between rank in the within-brood age hierarchy and coloration (GLMM: age rank: $F_{2,32} = 3.86$, $P = 0.03$; colour: $F_{1,32} = 0.02$, $P = 0.88$; interaction: $F_{2,32} = 4.30$, $P = 0.022$; the factor sex was not significant either alone or in interaction; Table 2). This interaction is explained by the fact that lighter-coloured seniors were more likely to steal a food item (Fig. 3a), whereas in juniors and middle-born nestlings the opposite significant relationship was found, with darker reddish birds being more likely to steal food (Fig. 3b, c).

DISCUSSION

Our correlative results suggest that by sharing food with siblings, nestling barn owls may derive indirect genetic benefits rather than direct material benefits. We are nevertheless aware that we cannot exclude the possibility that helping behaviour is also driven by pseudoreciprocity or by-product mutualism. Our observations also show how complex interactions between barn owl siblings can be. At the beginning of the night, competition over food items delivered by parents is high with most food-stealing events taking place at that time while very few food-sharing events occur. This leads nestlings to adopt specific begging behaviours to obtain food from parents (Dreiss et al. 2010b) and to avoid being robbed (Roulin et al. 2008a). This includes rapid food consumption by swallowing entire items more often at the beginning than at the end of the night. Once nestlings have consumed a few items, they start to be more generous by sharing food with siblings. This altruistic behaviour is observed mainly in the oldest dominant nestlings, particularly those displaying a female-specific plumage trait in the form of dark reddish phaeomelanin coloration. This suggests that female-like individuals of either sex behave more peacefully as further shown in female-like seniors that avoid stealing food from siblings. The opposite pattern is observed in middle-born nestlings and juniors displaying a female-specific coloration (i.e. dark reddish) since they are more likely to steal food.

The finding that barn owl nestlings frequently feed nestmates contradicts the view that, in vertebrates, competition over parental resources leads only to conflictual solutions, with siblicide as an extreme (Mock & Parker 1997). It has been suggested that cooperation between siblings is used to attract the parents' attention (Johnstone 2004), although empirical evidence remains elusive (Smale et al. 1995; Roulin & Dreiss 2012). Notwithstanding the evidence, although food sharing may not be widespread in young animals that compete over parental resources, it appears to be frequent in the barn owl. Although we observed food sharing in experimentally reduced broods, this behaviour has already been reported on several occasions in natural conditions (see Introduction). This shows that our observations are not artefacts of experimentally reducing brood size to three chicks, but is a general phenomenon in barn owl populations.

Evolution of Food Sharing

Our study raises the possibility that helping behaviour between young siblings does exist as predicted by kin selection theory (extrapair paternity is rare and hence nestmates are full-sibs; Roulin et al. 2004). A first reason why altruism between young siblings has rarely been observed in vertebrates is that altruistic behaviours may be difficult to detect. These instances may be rare (such as food sharing) requiring observations of behavioural interactions between siblings for many hours. Furthermore, if altruism involves begging behaviours as suggested by Johnstone (2004), it might not be easy to demonstrate that siblings adjust their begging level in a cooperative way (e.g. Blanc et al. 2010). Since food sharing may prevail in situations in which young offspring have access to stored food resources that can be used by offspring to feed their siblings, food sharing may be restricted to some particular groups of animals such as raptors (Baudvin 1980; Roulin 2004a). When nestlings are about 3 weeks of age, they become able to consume food by swallowing entire items or by tearing apart flesh. Once a stored item is found, nestlings do not need assistance anymore, probably explaining why we did not observe nestlings feeding their siblings by tearing apart flesh but simply by transferring items from bill to bill. Our observations should stimulate researchers who have access to species with parents also delivering a surplus of food, as is the case in many owls and raptors, to investigate whether helping behaviour in the form of food sharing occurs in other species.

Fitness Benefits of Sharing Food

Two lines of arguments plead against the hypothesis that reciprocity helps maintain the occurrence of food sharing in the barn owl. First, senior and middle-born nestlings shared food with their siblings in contrast to juniors, which rarely feed their nestmates. This suggests that individuals may not feed siblings in expectation of a future return once they are hungry. Second, provisioning siblings may be a case of pseudoreciprocity or by-product mutualism. For instance, sharing food with siblings may be a form of sib–sib and parent–offspring altruistic behaviour (Kramer 2011). In large families, parents face a trade-off between the time spent looking for food and the time spent distributing it between their progeny. Barn owls can produce very large families with up to 10 young. Staggered births imply that when the first-born offspring begin to consume food without maternal help, their younger siblings still need assistance. The feeding of younger chicks by those older may therefore allow their parents to increase hunting activities. This possibility, however, is unlikely because feeding rate was not higher in broods in which we observed food sharing compared to broods without food sharing. Furthermore, the propensity to share food with siblings was not associated with offspring body condition. Another possibility is that nestlings feed their siblings to enhance their survival in order to increase the total level of begging solicitations that would induce parents to increase their feeding rate, allowing some nestlings (i.e. the dominant and darker reddish ones) to monopolize more food resources. This possibility, however, is unlikely because parents seem to allocate a fixed budget to feeding offspring and to be weakly sensitive to begging solicitations (Roulin et al. 2000). A last possibility is that nestlings help their siblings to reduce their own risk of being killed by a predator, a possibility that we cannot discuss further owing to the lack of data.

We therefore conclude that kin selection is likely to be a valid explanation to account for the evolutionary stability of food sharing among barn owl siblings. Accordingly, the costs paid by senior chicks to feed nestmates are probably low, whereas the derived inclusive fitness benefits may be considerable given that nestmates in barn owls are usually full siblings (Roulin et al. 2004).

Food Sharing, Food Stealing and Coloration

The propensity to feed siblings was associated with the degree of reddish phaeomelanin coloration, a strongly heritable trait

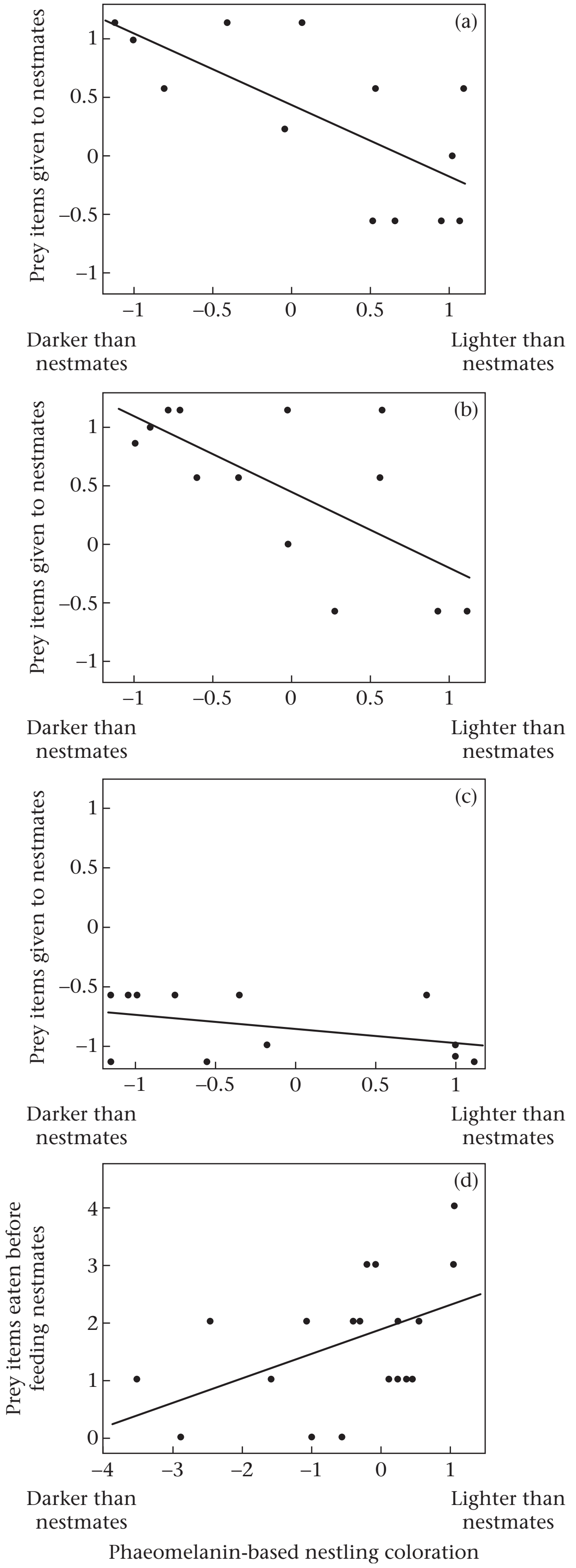

Figure 2. (a–c) Food sharing in relation to within-brood age hierarchy and phaeomelanin-based coloration in nestling barn owls. (a) Senior nestlings, (b) middle-born nestlings and (c) junior nestlings. (d) Number of prey items consumed by a nestling before it gave another item to a nestmate, in relation to nestling phaeomelanin-based coloration. We considered only 13 nests in which at least one prey item was exchanged between nestmates. The number of prey items given to nestmates was standardized, i.e. for each individual nestling we applied the following formula ([number of prey items this individual gave to siblings – mean number of items shared with siblings by the three siblings]/standard deviation of the number of items shared with siblings by the three siblings). We removed variation in phaeomelanin-based coloration explained by sex by extracting residuals from a one-way ANOVA ($F_{1,36} = 5.64$, $P = 0.023$; although the two sexes can express any coloration, females are on average darker reddish than males). Then, for each nestling we standardized the residual values by applying the following formula ([residual colour score of the focal individual – mean residual coloration of the three siblings]/standard deviation of residual coloration of the three siblings). Pearson correlations: (a) $r_{11} = -0.70$, $P = 0.007$; (b) $r_{11} = -0.66$, $P = 0.014$; (c) $r_{10} = -0.40$, $P = 0.20$. Regression lines are drawn for illustrative purposes.

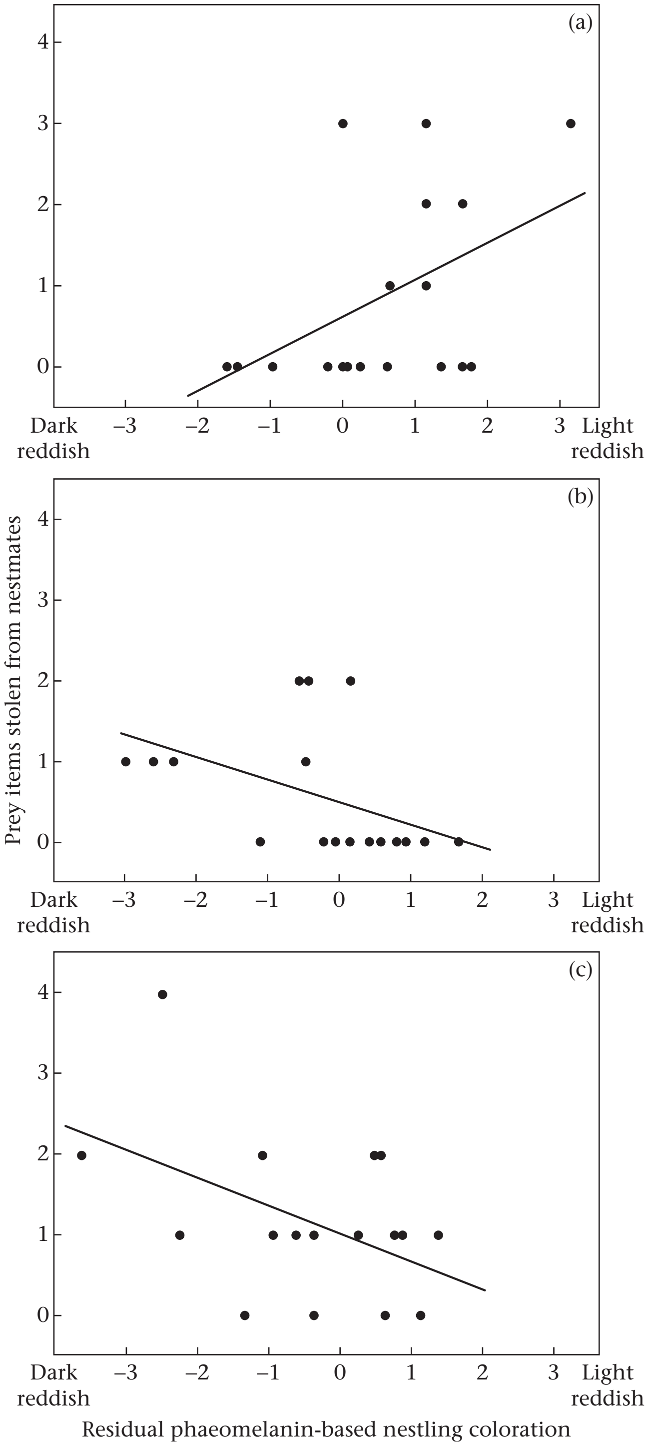

Figure 3. Food stealing in relation to within-brood age hierarchy and phaeomelanin-based coloration in nestling barn owls. (a) Senior nestlings, (b) middle-born nestlings and (c) junior nestlings. Coloration was corrected for sex by extracting residuals from a one-way ANOVA with coloration as dependent variable and sex as a factor. Pearson correlations: (a) $r_{16} = 0.49$, $P = 0.032$; (b) $r_{16} = -0.46$, $P = 0.048$; (c) $r_{16} = -0.48$, $P = 0.046$. Regression lines are drawn for illustrative purposes.

($h^2 \pm \text{SE} = 0.81 \pm 0.09$; Roulin & Dijkstra 2003). Because the expression of coloration is not or only weakly condition dependent (Roulin & Dijkstra 2003), this indicates that food sharing may not only be determined by the resource-holding potential of each individual (i.e. seniority), but also be partly genetically controlled (Keller 2009). Explaining why darker reddish owls were more likely to share food with siblings and why in seniors darker individuals were also less likely to steal food from siblings is still a matter of speculation. We can propose two nonmutually exclusive mechanisms.

First, individuals displaying a female-like reddish coloration are more likely to share food, and in seniors (but not in middle-born and junior siblings) less likely to steal it. A female-like plumage is therefore associated with helping behaviour in dominant individuals, whereas a male-like plumage (i.e. light reddish to white) is linked with agonistic behaviour. Because in animals males are commonly more aggressive than females (Lindenfors & Tullberg 2011; Senar & Domenech 2011), we propose that a dark reddish coloration could be related to female-specific behaviour. This is consistent with the observation that the motivation to feed siblings (measured in number of prey items consumed before sharing food with siblings) was not only related to phaeomelanin-based coloration but also higher in females than males. This raises the exciting possibility not only that females are more altruistic than males but also that feminine plumage traits are associated with the propensity to be generous. Second, our results could be explained by the fact that when food is available in large quantities, darker phaeomelanic individuals increase their body mass more rapidly than lighter-coloured ones (Roulin et al. 2008b). Under this scenario, dark reddish individuals need less food and hence can share it with their siblings to derive at least indirect genetic benefits.

Conclusion

Two pieces of evidence suggest that sharing food with siblings entails some fitness costs. First, food sharing occurs mainly at the beginning of the night (when nestlings are hungry) but only after food donors consumed a couple of prey items. Second, sharing food prevails in dominant individuals but not in subordinate nestlings probably because dominant nestlings have privileged access to food resources that they can redistribute among siblings. Given the potential costs of food sharing, this helping behaviour should confer some fitness advantages if it is to persist. As discussed above, nestlings may derive indirect benefits through kin selection (and eventually direct benefits through pseudoreciprocity or by-product mutualism). Our study therefore adds a new element to the potential role played by kin selection in the evolution of altruism, namely among young vertebrates that are still dependent on their parents. This system in which related individuals are confined in a limited space, and hence can develop iterative altruistic interactions, mirrors the case of cooperative breeding among mature related individuals (West et al. 2007; Cornwallis et al. 2009).

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APPENDIX 3

Darker eumelanic barn owls better withstand food depletion through resistance to food deprivation and lower appetite nestlings

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BEHAVIORAL ECOLOGY - ORIGINAL PAPER

Darker eumelanic barn owls better withstand food depletion through resistance to food deprivation and lower appetite

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Abstract The intensity of selection exerted on ornaments typically varies between environments. Reaction norms may help to identify the conditions under which ornamented individuals have a selective advantage over drab conspecifics. It has been recently hypothesized that in vertebrates eumelanin-based coloration reflects the ability to regulate the balance between energy intake and expenditure. We tested two predictions of this hypothesis in barn owl nestlings, namely that darker eumelanic individuals have a lower appetite and lose less weight when food-deprived. We found that individuals fed ad libitum during 24 h consumed less food when their plumage was marked with larger black spots. When food-deprived for 24 h nestlings displaying larger black spots lost less weight. Thus, in the barn owl the degree of eumelanin-based coloration reflects the ability to withstand periods of food depletion through lower appetite and resistance to food restriction. Eumelanic coloration may therefore be associated with adaptations to environments where the risk of food depletion is high.

Keywords Appetite · Food depletion · Energy homeostasis · Melanin · Melanocortin

Introduction

Identifying the conditions under which body condition covaries with an ornament should bring useful information to an understanding of how selection is exerted on conspicuous ornamental traits. A positive relationship between the degree of ornamental exuberance and body condition can indicate that poor-quality individuals cannot invest resources to develop a conspicuous ornament at the expanse of body maintenance (Andersson 1994). In this case, an ornament can be considered as an honest signal of absolute quality because its expression is condition dependent. As the magnitude of a covariation between an ornament and body mass can vary, selection will favor ornamented individuals mainly in environments where selection is most intense. In cases where the sign of covariation varies between environments, we can conclude that differently ornamented individuals are adapted to alternate habitats, potentially indicating that different versions of an ornament reflect adaptations to local conditions (Bussière et al. 2008; Piault et al. 2009). This situation could occur if the expression of the ornament is condition dependent (van Doorn et al. 2009) but also if it is under strong genetic control.

Studying variation in the magnitude of a covariation between body condition and an ornament is particularly convenient in species displaying melanin-based color traits. In such species, inter-individual variation in coloration is often mainly assigned to genetic factors (e.g., Roulin 2004a; but see Fargallo et al. 2007) because the expression of melanic colorations can be insensitive to variation in the environment, a phenomenon referred to as “environmental canalization” (Flatt 2005; van Buskirk and Steiner 2009). While variation in the environment will affect body condition, each genotype will still produce a single color

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phenotype, implying that a change in the magnitude of covariation between body condition and coloration is driven by variation in body condition. Assessing environmentally mediated variation in such a covariation is of interest when identifying under which conditions some genotypes outperform others, genotypes being identified with the degree of melanin-based coloration.

In vertebrates, darker individuals have been proposed to be more resistant to various sources of stress and to more efficiently regulate the balance between energy intake and expenditure (Ducrest et al. 2008). Empirical studies indeed showed that the degree of melanic colorations is associated with energetic processes. For example, in the great tit (*Parus major*) and pied flycatcher (*Ficedula hypoleuca*) the degree of plumage darkness is positively correlated with oxygen consumption (Røskaft et al. 1986). We would thus predict that dark individuals better regulate body mass than paler conspecifics mainly in stressful environments. This would indicate that dark melanic individuals or species are particularly well adapted to harsh conditions. Accordingly, an observational study in the barn owl (*Tyto alba*) showed that dark breeding females are heavier than pale conspecifics in the evening but not in the morning (Roulin 2009). As owls consume food at night and fast during daylight hours, this observation suggests that some aspects of metabolism differ between dark and pale individuals.

In the present study, we carried out two experiments to test the hypothesis that darker eumelanic barn owls better regulate body mass than pale conspecifics. First, we food-deprived nestlings for 24 h and measured body mass loss over this period of time. Second, we fed nestlings ad libitum and quantified the amount of mice consumed over 24 h. Based on properties of the melanocortin system, which is involved in melanogenesis and energy homeostasis, we predict darker eumelanic owls to lose less weight when experimentally food-deprived and to have a lower appetite (Ducrest et al. 2008). Support for these predictions would indicate that a dark melanic coloration reveals the ability to withstand periods of food depletion through low appetite and resistance to food deprivation.

Materials and methods

Model organism

The barn owl is medium sized with adult females weighing between 264 and 515 g (mean ± SD 367 ± 1.5 g) and adult males between 241 and 380 g (295 ± 1.6 g). Two to 11 eggs per clutch are incubated for 32 days and hatch asynchronously every 2–3 days. Maximal growth occurs between 17 and 40 days of age, and before fledging at 56 days nestlings spontaneously lose weight. The species is particularly convenient for the manipulation of hunger levels because from 2 to 3 weeks of age onwards nestlings consume small mammals without maternal help not only at night but also during daylight hours as parents frequently store food (Roulin 2004b). Previous studies showed that in natural conditions 36-day-old nestlings eat on average 3.4 voles per 24 h (Roulin 2001) and in laboratory conditions mean ± SD daily food intake was 67 ± 17 g between 20 and 60 days of age (Durant and Handrich 1998). Barn owls are mostly monogamous with very little extra-pair paternity (one out of 211 offspring was not sired by the male that was feeding it, Roulin et al. 2004). Food deprivation over 24 h is not rare in natural conditions as during rainy nights parents have difficulty hunting.

Assessment of melanin-based plumage traits

Nestling and adult barn owls vary in both number and size of eumelanic black spots but also in pheomelanin-based coloration from dark reddish–brown to white. These traits are genetically correlated with darker reddish owls displaying on average more and larger eumelanic spots. The expression of melanin-based traits is strongly heritable and only weakly sensitive to environmental factors ($h^2 = 0.82$; Roulin and Dijkstra 2003; Roulin et al. 2010). We measured plumage traits in 208 nestlings and in their parents (56 mothers and 52 fathers). A. Roulin compared pheomelanin-based coloration on the breast, belly, one flank and the underside of one wing with eight color chips ranging from I for reddish to VIII for white. As on each body part feathers are all similarly colored, we calculated a mean value to be used in the statistical analyses. Within a 60 × 40-mm frame placed on the same four body parts, eumelanic spots were counted and their diameter measured to the nearest 0.1 mm. Mean number of spots and mean spot diameter were calculated and used in the statistical analyses. Assessing plumage traits is reliable with repeatability values varying between 0.84 and 0.93 (Roulin 1999, 2004c). We did not consider the intensity of spot darkness because spots are lighter colored when feathers are older implying that this parameter depends on the degree of feather abrasion, which is not the case with respect to number and size of spots.

Experimental design

The study was carried out in 2008 in western Switzerland in a free-ranging population of barn owls breeding in nest-boxes. We cross-fostered approximately half of the hatchlings between pairs of nests to allocate genotypes randomly among the environments. The same number of nestlings was swapped between nests and hence brood size was left unchanged. Nestling position in the within-brood age hierarchy in the nests of origin and of rearing was not associated with nestling plumage traits (mixed-models, $P > 0.10$). Within pairs of nests biological and foster parents did not resemble each other with respect to plumage traits (Pearson's correlations, all $P > 0.15$), except that the number of spots displayed by biological and foster mothers were negatively correlated ($r = -0.33$, $n = 47$, $P = 0.022$). Among the 57 experimental nests hatching date was not correlated with plumage traits of biological parents ($r < 0.20$, $P > 0.14$) except for males displaying small black spots that bred earlier in the season than males with larger spots ($r = 0.34$, $n = 53$, $P = 0.012$).

To record body mass change in nestlings under controlled conditions, from 12 June to 1 October we brought 208 nestlings from 57 origins to the laboratory for 3 nights. Nestling age (mean ± SE 34 ± 6 days; range 18–51 days) was not correlated with plumage traits measured in their biological parents (Pearson's correlations on mean sibling values, $P > 0.09$). Nestlings were brought to the laboratory in the afternoon at 1610 hours (± 2 h 32 min; SE) and their body mass measured to the nearest gram. Nestling body mass before the experiment was not significantly correlated with plumage traits measured in the nestlings themselves or in their biological parents (mixed-model analysis of covariance with nest of origin as random variable, controlling for nestling sex and age, $P > 0.09$) but with nestling age (Pearson's correlation $r = 0.62$, $P < 0.0001$). At their arrival nestlings were assigned to a food treatment for the night: they were either starved or offered food ad libitum, i.e., 130 g of laboratory mice which exceeds their daily food requirement. Remaining food was removed the next afternoon at 1600 hours and nestling body mass change over 24 h was determined. The food treatment was inverted the second night at 0000 hours (i.e., starved nestlings were fed ad libitum). Remaining food was also removed the following afternoon at 1600 hours and body mass change determined. On the first night we food-deprived 98 nestlings and fed the 110 other nestlings ad libitum. Plumage traits of nestlings that were food-deprived or fed ad libitum on the first night were similar (Student's t test: color $t_{184} = 0.76$, $P = 0.45$; spot diameter $t_{184} = 0.46$, $P = 0.65$; number of spots $t_{184} = 0.26$, $P = 0.80$). At the end of the experiment (i.e., the morning following the third night), we brought nestlings back to their original nest-boxes, after having fed them ad libitum the third night. In the laboratory, nestlings were placed in a similar nest-box to the one where they were reared in natural conditions, but which was divided into two parts by a thin wooden wall pierced with five holes at the top. Each nestling was alone in one part of a nest-box while the other part was either empty ($n = 20$), occupied by a sibling ($n = 84$) or an unrelated individual raised in the same nest ($n = 104$).

Detailed observations on vocalizations produced by these nestlings (Roulin et al. 2009) showed that individuals behave as in natural conditions, indicating that conditions met in the laboratory were not too stressful. To further investigate the impact of keeping individuals in the laboratory, we measured baseline corticosterone levels (see Almasi et al. 2009 for details on the methods) in 20 nestlings just before bringing them in the laboratory and on average 2 days later in the laboratory just after the ad libitum food treatment was finished. This hormone is sensitive to various sources of stress (e.g., Jenni-Eiermann et al. 2008) and hence if nestlings are stressed to higher levels in the lab compared to the situation prevailing in their natural nest, baseline corticosterone levels are expected to be significantly higher in the lab. This was not the case (mean ± SD baseline corticosterone level in the field was 5.57 ± 4.2 ng/ml and in the laboratory 7.58 ± 4.4 ng/ml; paired t test on log-transformed values $t_{19} = 1.24$, $P = 0.23$).

Statistical procedure

To assess the relationship between the degree of melanin-based coloration and body mass under contrasting feeding conditions (i.e., fed ad libitum vs. food-deprived during 24 h) we performed a mixed-model ANOVA with body mass change over 24 h as the dependent variable. We included as random variables the nest of origin and the identity of each nestling nested in the nest of origin (since each individual appeared twice in the analysis, a first time when food-deprived and a second time when fed ad libitum). Covariates were nestling age, the three melanin-based plumage traits (pheomelanin-based coloration, spot diameter and spot number) measured in nestlings and in their two biological parents. We also introduced as cofactors nestling sex, food treatment, order of the food manipulation across the two experimental nights (i.e., starting with food-depriving individuals or by feeding them ad libitum) and nestling neighborhood in the laboratory nest-box (alone, in the presence of a sibling or of an unrelated but familiar individual raised in the same nest in the field). In a separate mixed-model ANOVA we introduced amount of mice consumed during 24 h in nestlings fed ad libitum. Nestling status (raised by the biological or foster parents), alone or in interaction did not account for any variation in body mass change and appetite. We thus removed this factor from the final models, which contained only significant effects and main effects involved in significant interactions. If we replace nestling age by initial body mass (the two variables are highly correlated, $r = 0.62$), we obtain qualitatively similar results. Final models always presented a smaller Akaike information criterion than previous models containing non-significant terms. Assumptions for using parametric tests (homoscedastic and normal distributions of variables or residuals) were verified for each test. *P* values smaller than 0.05 are considered significant.

Results

The final model testing whether nestling body mass change over 24 h is associated with spot diameter is given in Table 1. There were three major results from this analysis. First, nestlings lost less mass when their mother displayed larger black spots independently of the food treatment (Fig. 1). Second, the spot diameter of the father, alone or in interaction with other variables, did not explain any significant part of the variation. Third, there was a significant interaction between food treatment and nestling spot diameter. Nestlings displaying large eumelanic spots lost less body mass than those exhibiting smaller spots when food-deprived (mixed-model ANOVA with nest of origin as a random variable, $F_{1,126} = 6.31, P = 0.013$; Fig. 2) but not when fed ad libitum (similar model $F_{1,127} = 1.66, P = 0.20$). The lack of relationship between nestling spot diameter and nestling body mass could be due to differential appetite. Accordingly, when nestlings were fed ad libitum, individuals displaying large black spots consumed fewer mice than individuals displaying smaller spots (Table 2; Fig. 3a). Whitish individuals had less appetite than reddish ones, but the relationship was relatively weak (Table 2; Fig. 3b).

Table 1 Final mixed-model ANOVA testing whether body mass change over 24 h in nestling barn owls is associated with the size of eumelanic spots measured in nestlings and in their biological parents

Source of variation	*df*	*F*	*P*
Nestling pheomelanic coloration	1,171	0.85	0.36
Nestling spot number	1,171	0.01	0.97
Nestling spot diameter	1,180	0.81	**0.37**
Mother pheomelanic coloration	1,180	2.00	0.16
Mother spot number	1,171	0.64	0.42
Mother spot diameter	1,180	6.82	**0.0098**
Father pheomelanic coloration	1,171	1.95	0.16
Father spot number	1,171	0.61	0.44
Father spot diameter	1,171	0.01	0.96
Nestling sex	1,171	0.24	0.62
Nestling age	1,180	5.06	**0.026**
Food manipulation	1,180	176.36	**<0.0001**
Order of food manipulation	1,180	233.11	**<0.0001**
Food manipulation × order of food manipulation	1,180	91.49	**<0.0001**
Food manipulation × nestling spot diameter	1,180	6.95	**0.0091**

Nest of origin was incorporated as a random variable as well as nestling identity nested within the nest of origin. Independent variables were food manipulation (over 24 h individuals were either fed ad libitum or food-deprived), and the order of this manipulation (in approximately half of the cases individuals were first fed ad libitum and others were first food-deprived). Non-significant interactions were removed one after the other starting with the least significant ones

P values of the final model are in **bold** and non-significant *P* values of initial models in plain

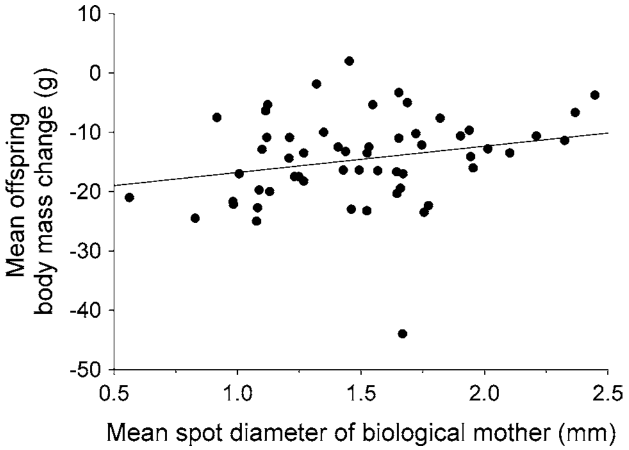

Fig. 1 Mean body mass change (in g) in nestling barn owls over 24 h in relation to the size of eumelanic spots measured in their biological mother. A mean sibling value was calculated so that each mother appears only once in this figure (Pearson's correlation is $r = 0.38$, $n = 43$, $P = 0.01$). The regression line is shown

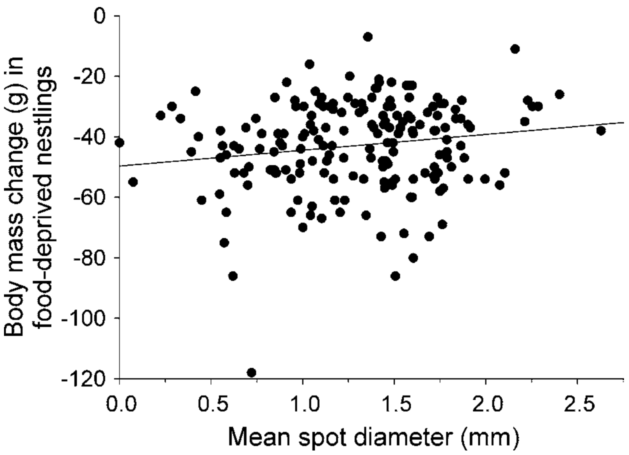

Fig. 2 Body mass change (in g) in nestling barn owls that were food-deprived over 24 h in relation to the size of their eumelanic spots. The regression line is shown

Discussion

Under laboratory conditions food-deprived nestling barn owls that displayed larger black spots lost less weight and had a lower appetite. These results shed new light on the

Table 2 Final mixed-model ANOVA testing whether appetite (expressed in g mice eaten over 24 h) in nestling barn owls fed ad libitum is associated with the size of eumelanic spots measured in nestlings and in their biological parents

Source of variation	*df*	*F*	*P*
Nestling pheomelanic coloration	1,126	5.29	**0.023**
Nestling spot number	1,117	1.46	0.23
Nestling spot diameter	1,126	7.52	**0.007**
Mother pheomelanic coloration	1,117	0.90	0.34
Mother spot number	1,117	0.18	0.67
Mother spot diameter	1,117	0.49	0.49
Father pheomelanic coloration	1,117	0.62	0.43
Father spot number	1,117	0.03	0.87
Father spot diameter	1,117	0.86	0.35
Nestling sex	1,117	0.27	0.61
Nestling age	1,117	0.01	0.99
Nestling body mass before feeding	1,126	4.69	**0.032**
Order of food manipulation	1,126	35.80	**<0.0001**

Nest of origin was incorporated as a random variable. Independent variables were melanin-based plumage traits of nestling and their biological parents, nestling age, body mass before the feeding treatment was applied, sex and the order of food manipulation (in approximately half of the cases individuals were first fed ad libitum and others were first food-deprived). Non-significant interactions were removed one after the other starting with the least significant ones

P values of the final model are in *bold* and non-significant results of initial model in *plain*

physiological adaptations associated with melanin-based color traits. A number of recent studies have indeed proposed that such colorful traits could indicate various physiological properties including for instance resistance to oxidative stress and parasites as well as energy homeostasis (Ducrest et al. 2008). These studies were based on knowledge of the physico-chemical effects of melanin pigments (Mackintosh 2001; McGraw 2005) or of physiological effects of biochemical molecules involved in melanogenesis. Interestingly, our results are consistent with pleiotropic effects of the melanocortin system suggesting that mutations located in the proopiomelanocortin gene or differential expression of this gene are responsible for the link between melanin-based coloration and energy homeostasis (Ducrest et al. 2008).

Our study has several implications for an understanding of the potential adaptive function of melanin-based color traits. Several studies suggest that darker eumelanic individuals are adapted to stressful conditions. For example, darker feral pigeons (*Columba livia*) better survived after the Chernobyl catastrophe (Johnston and Janiga 1995), offspring of darker melanic Alpine swift (*Apus melba*) fathers grew more rapidly when brood size was experimentally enlarged but not when experimentally reduced (Roulin et al. 2008), in siskins (*Carduelis spinus*) darker individuals were less susceptible to stressful laboratory conditions as measured by metabolic rate (Senar et al. 2000), and in nestling common buzzards (*Buteo buteo*) intensity of infection with *Leucocytozoon* endoparasites decreased with melanization (Chakarov et al. 2008). In the barn owl, females displaying larger eumelanic spots gave birth to offspring that were more resistant to ectoparasites, produced more antibodies towards a vaccine and were developmentally more stable (Roulin 2004c), male barn owls displaying larger black spots were less sensitive to an experimental increase in corticosterone level (Almasi et al. 2008) and offspring born from heavily spotted mothers were better able to cope with a rise in corticosterone levels due to stressful situations (Almasi et al. 2010). The present study provides further information on the ability to cope with food depletion. Darker eumelanic individuals better dealt with experimental food deprivation and had a lower appetite suggesting that they need less food to sustain metabolism. Our experiment should be repeated under natural conditions to determine whether the relationships between melanin-based coloration and energetic processes are not specific to stressful conditions such as those probably met under laboratory conditions. The observation that melanin-based coloration co-varied significantly with aspects of body condition only after we manipulated food supply is consistent with the claim that the degree of pigmentation signals quality only under specific conditions

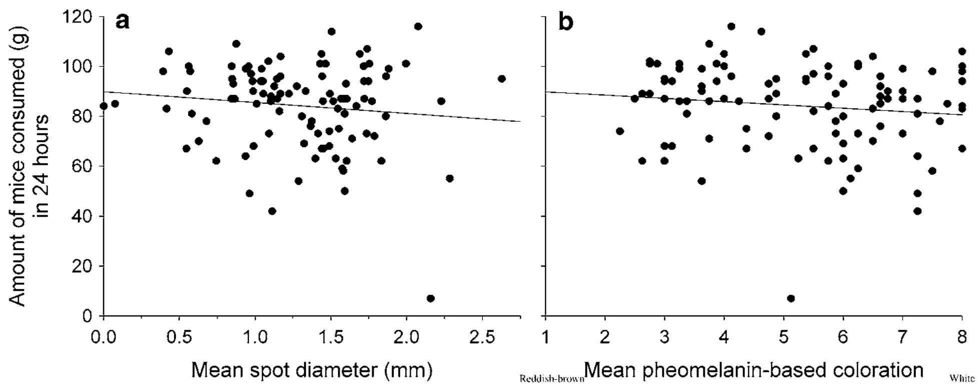

Fig. 3 Amount of mice (in g) nestling barn owls consumed in 24 h in relation to the size of their eumelanic spots (**a**) and pheomelanin-based coloration (**b**)

(Roulin 2009). Therefore, selection on melanin-based color traits is context dependent [see also Gonzales et al. (1999) for another example].

In the present experimental study and the previous observational study carried out in barn owls (Roulin 2009), the balance between energy intake and expenditure was mainly associated with the degree of eumelanin- and to a low extent with pheomelanin-based coloration. This contrasts with a similar experimental study we recently carried out in the tawny owl (*Strix aluco*), a species that varies in the degree of pheomelanin-based coloration. In the laboratory, food-deprived tawny owl nestlings lost less mass when their biological mother was pale rather than dark reddish-brown (Piault et al. 2009). This result is interesting because the intensity of melanin pigmentation is positively correlated with the ability to cope with food depletion with respect to eumelanic coloration in the barn owl but negatively with respect to pheomelanic coloration in the tawny owl. From a proximate point of view, this suggests that a molecule that triggers the production of eumelanic pigments binds to other receptors responsible for energy homeostasis which are also sensitive to an antagonistic molecule that triggers the production of pheomelanic pigments (Ducrest et al. 2008). From an ultimate point of view, this may indicate that pheomelanic and eumelanic colorations may signal similar phenotypic attributes but in opposite directions.

To conclude, if the degree of melanin-based coloration is associated with the ability to deal with poor food conditions, we could predict that dark eumelanic color traits are more prevalent in populations or in species that face a higher risk of food shortage. This is a stimulating avenue of research, which has not yet been considered.

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