

Vocal sib–sib interactions: how siblings adjust signalling level to each other

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Game theory states that iterative interactions between individuals are necessary to adjust behaviour optimally to one another. Although our understanding of the role of begging signals in the resolution of parent–offspring conflict over parental investment rests on game theory implying repeated interactions between family members, empiricists usually consider interactions at the exact moment when parents allocate food among the brood. Therefore, the mechanisms by which siblings adjust signalling level to one another remain unclear. We tackled this issue in the barn owl, *Tyto alba*. In the absence of parents, hungry nestlings signal vocally to siblings their intention to contest vigorously the next, indivisible, food item. Such behaviour deters siblings from competing and begging when parents return to the nest. In experimental two-chick broods, nestlings producing the longest calls in the absence of parents, a signal of hunger level, were more successful at monopolizing the food item at the first parental feeding visit of the night. Moreover, nestlings increased (versus decreased) call duration when their sibling produced longer (versus shorter) calls, and an individual was more likely to call again if its sibling began to vocalize before or just after it had ended its previous call. These results are in agreement with the hypothesis that siblings challenge each other vocally to reinforce the honesty of sib–sib communication and to resolve conflicts over which individual will have priority of access to the next delivered food item. Siblings challenge each other vocally to confirm that the level of signalling accurately reflects motivation.

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Parents and offspring are in conflict over parental investment, with offspring requesting more resources than parents are willing to provide (Mock & Parker 1997). This conflict has stimulated researchers to elucidate how parents adjust provisioning rate and how food is allocated among the offspring. Although evidence is growing that parents adjust feeding rate based on the intensity of offspring begging behaviour (e.g. Ottosson et al. 1997; Burford et al. 1998), the role of begging displays in how food is shared among the offspring remains poorly understood and controversial (Royle et al. 2002). Honest signalling models propose that parents actively deliver food to the individual that begs at the highest rate because the cost of begging ensures that this behaviour is an honest signal of need, the benefit of obtaining additional resources compensating for the cost of signalling only if the individual is hungry (Godfray 1991, 1995). Although models of honest signalling predict that parents should preferentially feed the most conspicuous offspring, competitive interactions among siblings may prevent parents from doing so. Indeed, begging behaviour may serve not only as an honest signal of need directed to parents but also as a competitive

signal between siblings to facilitate access to food items delivered by parents. In such a situation, referred to as scramble competition, the parental transfer of food to the offspring that begs at the highest level is not necessarily the outcome of a parental preference for the most conspicuous offspring. Rather, it is the result of sibling competition, with the individual begging at the highest level being better able to outcompete its siblings and monopolize food (Parker et al. 2002). It follows that knowledge of the extent to which begging is modulated by sibling interactions would provide important information on the reliability of begging as an honest signal of need (Royle et al. 2002).

Theoretical and experimental studies have shown that offspring adjust begging behaviour not only to their own level of need but also in relation to the number of siblings, the siblings' behaviour and their size (Smith & Montgomerie 1991; Godfray 1995; Price et al. 1996; Leonard & Horn 1998; Cotton et al. 1999; Kedar et al. 2000; Rivers 2006; Bell 2007; but see Cotton et al. 1996). This implies that nestlings may assess each other's begging and adjust their own level of signalling relative to the need and resource-holding potential of their sibling(s). If an individual derives fitness benefits by inducing siblings to modify their investment in begging behaviour, nestlings may actively transfer information about need not only to parents but also to siblings. This may be the case if the food delivered by parents at each visit is indivisible (i.e. only one

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individual consumes the food) and if there is an asymmetry between siblings in hunger levels (Johnstone & Roulin 2003). Under these conditions, the outcome of sibling competition is predictable, with the neediest individual being motivated to invest the most effort to obtain the next food item. If siblings communicate their motivation to compete to one another, each nestling could assess the likelihood of obtaining the next food item and adjust its investment in begging accordingly once the parents arrive with food. The least hungry nestlings may retreat momentarily from a competition for which the likelihood of succeeding is low, thereby saving energy to be invested later when the probability of succeeding in sibling competition increases. Hence, sib–sib communication allows the hungriest individual to monopolize food without having to compete intensely.

This process, denoted 'sibling negotiation', should take place whenever negotiation can reduce the costs of sibling competition to a larger extent than the costs imposed by negotiating and eventually delaying feeding time (Johnstone & Roulin 2003). In other words, in large broods it might be preferable to induce siblings to give up a contest momentarily by producing costly calls than to fight physically with them. Sibling negotiation has been shown to occur in the barn owl, *Tyto alba* (Roulin et al. 2000; Roulin 2002), spotless starling, *Sturnus unicolor* (Bulmer et al. 2007), and possibly in the black-headed gull, *Larus ridibundus* (Mathevon & Charrier 2004). There is presently little evidence that sibling negotiation is of general applicability. This situation may, however, be because few researchers study organisms in which nestlings vocalize in the absence of parents such as woodpeckers, bee-eaters, herons and raptors or in which one offspring can monopolize all the resources provided per parental feeding visit.

Adjustment of begging in relation to investment in sibling negotiation may be an iterative process. Siblings may challenge each other vocally over a relatively long period of time to assess whether signalling level corresponds to motivation to compete once the parents return with a food item (Roulin 2002). Vocal exchanges between siblings may last a long time because a less motivated nestling may increase investment in sibling negotiation if its more motivated sibling stops vocalizing, a behaviour that may be considered a sign that its motivation was not so high. Nestlings therefore have to vocalize over a prolonged period of time, as the risk of prey theft is relatively high once the parents have brought a food item and left the nest (Roulin et al. 2008). Thus, in the absence of parents, production of an intense call by an individual A may induce a change in calling behaviour by its sibling B which may in turn trigger a vocal response in individual A. This dynamic process is predicted by game theory, with two individuals adjusting their behaviour to each other until they behave optimally at equilibrium (Maynard Smith 1982; McNamara et al. 1999). Although models of signalling use game theory as an analytical approach, empirical studies usually concentrate on the end point of any family interaction by measuring begging intensity at the point at which parents transfer food among the offspring, thereby neglecting previous vocal and physical sib–sib interactions. However, considering the dynamics of sib–sib interactions would provide important insight into the mechanism that induces siblings to adjust vocalization behaviour in relation to competitive dynamics in the nest.

We investigated the dynamics of sib–sib communication in the absence of parents in two-chick barn owl broods, with the aim of highlighting how nestlings adjust calling behaviour relative to one another. Investment in sibling negotiation influences which individual will beg at the highest level once parents are back at the nest, with parents delivering food to the most conspicuous offspring (Roulin 2004b). To this end, we measured call duration and time interval between two successive calls produced, on average, 20 min before the first parental feeding visit of the night. First, we

confirmed that call duration signals hunger level. Then, we investigated whether call duration predicts which individual obtains the next prey item. Finally, we examined the following three non-mutually exclusive possibilities of how siblings may adjust calling behaviour relative to one another. These adjustments in calling are consistent with predictions of both the sibling negotiation (Roulin 2002) and scramble competition models (Parker et al. 2002) and hence are relevant to the study of family interactions in a broad context.

(1) Siblings adjust call duration with time and this influences prey monopolization. At the beginning of the night, siblings have little information about each other's need for food. Nestlings that are hungry may increase the duration of their calls as the time to the parent's appearance decreases. This behaviour may indicate increasing hunger level or high motivation for contesting the next food item. As siblings challenge one another vocally, the hungriest individual escalates the contest by producing longer calls. Because this behaviour may depend on the resource-holding potential of each individual, we examined whether adjustment of call duration with time differs between senior and junior siblings, and whether it is related to which individual obtained the first delivered prey item of the night.

(2) Siblings adjust call duration in the short term in response to the other sibling's behaviour. If the duration of calls produced in the absence of parents conveys information on the motivation of nestlings to compete for the next delivered prey item, hungry siblings may challenge each other by producing long calls. Thus, siblings may adjust the duration of their calls in response to one another. The production of long calls by one individual may trigger its sibling to respond by lengthening call duration to match that of the first individual. Production of shorter calls by one individual may indicate a decrease in the level of competition and thereby induce its sibling to reduce the length of its calls. Therefore, call duration may be matched among siblings and thus fluctuate over short periods of time.

(3) The relative timing of call production by two siblings determines who calls next. Because call overlap (i.e. an individual begins to call before its opponent ended its call) is typical of agonistic interactions (Naguib et al. 1999), the call timing may be used by siblings to challenge each other vocally. Assuming that calling just after a sibling is a competitive behaviour to reaffirm the willingness to contest the next food item, a nestling that calls immediately after its sibling may induce this sibling to produce the next call.

METHODS

Study Species

Adult barn owls weigh 241–515 g, and at night catch small mammals. Eggs are laid every 2.5 days and because females start to incubate the clutch soon after the first egg has been laid, eggs hatch asynchronously resulting in a pronounced within-brood age hierarchy, with the first-hatched nestling being up to 3 weeks older than its last-hatched sibling. As a consequence, asymmetries in short- and long-term hunger level between siblings are pronounced. Before offspring are 2 weeks of age the father gives food items to his partner who distributes them to the offspring. After about 2 weeks the mother begins to hunt and obtains about one-third of prey items. Parents deliver a single prey item at a time which is consumed by one offspring. A brood consists of one to nine nestlings ($\bar{X} \pm \text{SE}$ of 764 broods that produced at least one fledgling = 4.3 ± 1.4) which take their first flight at approximately 55 days of age. In experimental two-chick broods the individual that receives the first prey item of the night produces on average

7.7 calls/min before the arrival of a parent and 45.8 in its presence; its nestmate produces 3.9 calls in the absence and 30.4 in the presence of parents (Roulin 2001). Throughout the night each individual produces, on average, 1800 calls in the absence of parents. This represents a substantial energetic investment (Bühler & Eppler 1980; Rodriguez-Gironés et al. 2001; Roulin 2002). Thus, calling behaviour may be an honest signal of hunger level. The number of calls produced by nestlings decreases with time of night, probably because hunger levels decline (Roulin 2001, 2002). Detailed observations of two-chick broods have shown that the junior nestling is more sensitive to the sibling's vocalization rate than the senior sibling who is better at monopolizing food. When hungry, the senior produces many calls in the absence of parents, a behaviour that induces its junior sibling to beg less intensely once the parents return with a food item. Conversely, if the senior is food satiated it produces few calls in the absence of parents, informing its junior sibling that it will not contest the next prey item, which induces the junior to beg intensely once the parents return. Thus, investment in sibling negotiation by the senior influences the begging behaviour of its junior sibling, which in turn determines to whom parents will allocate food (Roulin 2004a). Even if seniors vocalize intensely in the absence of parents, junior siblings do not entirely give up vocalizing as there is always scope for juniors to obtain the next food item (Roulin et al. 2008).

General Methods

The study was carried out in western Switzerland on a population of wild barn owls breeding in nestboxes (1.0 × 0.6 m and 0.5 m high) fixed to barn walls. To study owlets' vocalization behaviour, we restricted our analyses to two-chick broods because in larger broods it is difficult to assign calls to individuals and to determine to which sibling an individual adjusts its calling behaviour. The use of two-chick broods is a biologically meaningful experimental design to study begging behaviour in the barn owl because offspring behaviour observed in two-chick broods fits predictions of a game-theoretical model (Johnstone & Roulin 2003). Furthermore, in the absence of parents one or more chicks are usually calling at any one time even in large broods (Bühler & Eppler 1980; personal observation). As a consequence, the number of calls/chick per min declines with brood size (Roulin et al. 2000; Roulin 2002). On average, in 26 unmanipulated broods of four (range 2–7), the nestlings produced, in the absence of parents, 14.2 calls/min (range 4–28), which corresponds to 9 s of calls/min. The study was carried out with the authorization of the 'Service Vétérinaire des canton de Vaud'. (For ethical details, see Roulin & Bersier 2007).

Call Duration and Hunger Level

In 2008, we performed an experiment to test whether call duration was related to hunger level. To record calls in controlled conditions, we brought 20- to 40-day-old nestlings into the laboratory in a similar nestbox to the one where they were reared in natural conditions. We created two-chick broods, with siblings being separated by a thin wooden wall pierced with five holes at the top to allow siblings to hear each other without interacting physically. Nestlings were brought into the laboratory in the afternoon. The first night, the two siblings were either not fed or fed ad libitum with 100 g of laboratory mice, *Mus musculus*. Any remaining food was removed the next afternoon at 1600 hours. The food treatment was reversed on the second night at 0000 hours so that we could record calls of each individual under two feeding situations (food deprived versus food satiated) on the second and third nights. Calls were recorded with Beyerdynamic microphones (MC 930) from dusk until midnight. We analysed calls from 30 nestlings

originating from 10 different broods. Call duration (± 1 ms) was measured over 30 min starting at a mean \pm SE of 2238 ± 0041 hours during which nestlings produced on average 286 ± 263 calls. We chose a period of 30 min to measure calls for no particular reason, the aim being to obtain enough calls per individual and to calculate a reliable mean duration value. We returned nestlings to their original nestboxes the morning after the third night, after feeding them again ad libitum from midnight onwards.

Vocalization Behaviour

To study vocalization dynamics, we created two-chick broods in natural nests between 1997 and 2001. Each evening we removed all but two randomly chosen owlets from a nestbox. These were placed in a large and ventilated plastic pail (diameter = 0.6 m; height = 0.8 m) at some distance from the nest. In total, we created 74 two-chick broods: 13 in 1997, five in 1998, 47 in 2000 and nine in 2001. In each nestbox, we had previously installed an infrared-sensitive camera and a microphone without any apparent signs of distress to either the adults or nestlings. Using VHS videotapes, we filmed the two-chick broods from sundown to midnight. We then returned the removed siblings to their respective nests. The two focal siblings were ringed on a different leg for individual recognition on the video footage. Because nestlings frequently move, it is sometimes difficult to assign calls to individuals. We analysed only those videotapes where we could easily identify callers. We therefore selected six broods in 1997 and 16 in 2000; none of the nests were used in cross-fostering experiments. The selected broods contained younger chicks than the excluded broods (mean age \pm SE of the two siblings = 28.3 ± 2.0 days versus 39.8 ± 1.3 days; Student's *t* test: $t_{72} = 4.73$, $P < 0.0001$) because old chicks frequently move and are not always visible on the video footage, thereby complicating the identification of callers. The mean age difference between the two selected siblings was 6.0 ± 1.0 days (range 1–15 days). In these 22 broods, parents were naturally absent from their nest during the day and the first parental visit of the night occurred at 2126 hours (range 2105–2344). From the video footage, we identified several time periods where both nestlings were calling prior to the first parental visit of the night (parents always came to the nest with a food item). Thus, differences in vocalization behaviour between siblings could not be influenced by whether individuals had already been fed, since the last feeding occurred about 16 h previously. Nevertheless, there was still substantial asymmetry between the two siblings in the motivation to obtain the next food item, as shown by the fact that one individual called much more than its sibling (Roulin 2002, 2004b). On average, we measured the duration of 174 calls/individual (range 15–674) starting on average 21.09 min (range 7–44 min) before the first arrival of a parent. This variation was due to nestlings starting to vocalize intensely at various times before the arrival of a parent.

We exported calls from the VHS videotapes into the computer to obtain waveforms with the Audacity program (<http://audacity.sourceforge.net/>). We measured call duration (± 1 ms) and the time interval between the end of one call and the beginning of the next call produced by the same individual or by its sibling. Because the waveforms of overlapping calls were not distinct, we were unable to measure the degree of call overlap between the calls of siblings. Overlapping calls were therefore assigned an intercall interval of 0 s. For each individual, we also counted calls produced over 14 min starting 15 min before the first arrival of a parent, that is, before nestlings noticed that a parent was close to them (we considered 15 min instead of 21.09 min because this was the method used in previous papers using the same data set). This was useful to investigate whether both call duration and call rate

determine which individual is fed first (in one nest we could not determine who was fed first). Because nestlings call at a high rate in the presence of parents, it was not possible to measure call features while parents were at the nest.

To assess whether adjustment of calling behaviour with time differed between individuals in different positions in the within-brood age hierarchy, the older individual was referred to as 'senior' while its younger sibling was denoted 'junior'. Seniority was determined soon after hatching by measuring the length of the flattened wing from the bird's wrist to the tip of the longest primary (Roulin 2004a). Size order soon after hatching and at the time of recording behaviour was the same. We collected a blood sample (20 μ l from the brachial vein) to determine sex using molecular methods (Roulin et al. 1999). We did not observe any negative effect of the blood sampling.

Statistical Procedure

For all statistical analyses we used the package JMP IN 7.1 (SAS Institute Inc., Cary, NC, U.S.A.). As we had more than one measure per individual and two individuals per nest, we controlled for the nonindependence of the data by incorporating nest site and nestling identity nested in nest site as two random factors in mixed models. When mixed models were carried out on mean nestling values we introduced only nest site as a random variable. Final models only contained significant effects, and main effects involved in significant interactions. *P* values less than 0.05 were considered significant. In mixed models the test statistics only approximate the denominator degrees of freedom. The most important part of a mixed model is the unbiased estimation of the *F* statistic and therefore is the correct test of the hypothesis. Changes in the denominator degrees of freedom have only a small influence on *F* and *P* values. Means are quoted \pm SE.

RESULTS

Call Duration Signals Hunger Level

In a mixed model with recording night (i.e. second versus third nights) and nestling identity nested in nest site as random factors, call duration was significantly related to food treatment ($F_{1,1543} = 75.38$, $P < 0.0001$). Mean call duration was longer when individuals were food deprived than when food satiated (0.80 ± 0.01 s versus 0.73 ± 0.01 s; paired *t* test: $t_{58} = 2.34$, $P = 0.02$).

Call Parameters and Food Allocation

Calls lasted 0.244–1.161 s with a mean value of 0.639 ± 0.002 s. Mean call duration was not significantly correlated with call rate in seniors and juniors (Pearson correlation: seniors: $r_{20} = -0.21$, $P = 0.35$; juniors: $r_{20} = 0.09$, $P = 0.68$). In a mixed-model ANCOVA with nest site and nestling identity nested in the nest site as two random variables, call duration was associated with hierarchy in interaction with sex (hierarchy: $F_{1,19.35} = 6.74$, $P = 0.018$; sex: $F_{1,20.65} = 0.001$, $P = 0.97$; interaction: $F_{1,29.71} = 5.10$, $P = 0.03$; in this model age and body mass were not significant and thus were excluded). The significant interaction is due to senior females producing shorter calls than junior females (0.615 ± 0.030 s versus 0.690 ± 0.028 s; similar mixed-model ANOVA: $F_{1,4.579} = 9.27$, $P = 0.032$), while no difference was observed between junior and senior males ($F_{1,1.91} = 0.07$, $P = 0.81$). At the first feeding of the night, juniors were as efficient as seniors in monopolizing the prey item delivered by a parent (in 10 versus 11 cases).

Using a logistic regression analysis on mean nestling values, we found that the individual that consumed the first prey item of the

night produced on average longer calls before the arrival of a parent than the individual that did not get the prey (0.666 ± 0.023 s versus 0.633 ± 0.023 s; $\chi^2_1 = 4.25$, $P = 0.039$) but not more calls ($\chi^2_1 = 0.10$, $P = 0.75$).

Siblings Adjust Call Duration over Time

Although each individual produced calls of a similar duration, there was sufficient intraindividual variation in call duration (repeatability \pm SE = $59.5 \pm 3.2\%$; $F_{41,5714} = 199.92$, $P < 0.0001$) for us to investigate how siblings adjusted call duration. As the time to food delivery by a parent decreased, call duration changed differently in juniors and seniors depending on which individual obtained the prey item (significant three-way interaction between time, within-brood age hierarchy and whether the junior or senior consumed the first prey item of the night; Table 1). For seniors, an individual did not receive the next food item if it decreased the duration of its call in the time interval before the parents arrived (mixed-model ANOVA with nestling identity as a random factor; time: $F_{1,1300} = 5.71$, $P = 0.017$; based on parameter estimates obtained from the model we could calculate that calls lasted on average 0.545 s at the beginning of the night and 0.523 s 30 min later). An increase in call duration resulted in successful acquisition of the next food item (similar model: $F_{1,1584} = 103.78$, $P < 0.0001$; 0.580 s versus 0.649 s). In contrast, for juniors an increase in call duration did not necessarily result in an individual obtaining the food item (when juniors obtained the item: $F_{1,1279} = 30.23$, $P < 0.0001$; 0.616 s versus 0.661 s; when juniors did not obtain the item: $F_{1,1559} = 91.71$, $P < 0.0001$; 0.655 s versus 0.721 s).

Siblings Adjust Call Duration in Response to Each Other

Siblings ($N = 22$ pairs) produced calls of a similar mean duration (Pearson correlation: $r_{20} = 0.76$, $P < 0.0001$; Fig. 1a), while the numbers of calls they produced over 14 min before the arrival of their parent were not significantly correlated ($r_{20} = -0.28$, $P = 0.21$). For calls measured for, on average, 21.09 min before the first adult arrival, the mean durations of the senior's and the junior sibling's calls produced during periods of 30 s were positively correlated (mixed-model ANCOVA with nest site as a random variable and mean duration of junior's call as the dependent variable; mean call duration of senior sibling: $F_{1,546.3} = 30.23$, $P < 0.0001$; Fig. 1b). This indicates a short-term adjustment of call duration to each other. This result cannot be explained by the possibility that siblings became hungrier with time, since in this model we statistically controlled for time and, furthermore, the relation between time and call duration was not linear but cubic (same model: time: $F_{1,528.9} = 0.65$, $P = 0.42$; time²: $F_{1,529} = 6.50$, $P = 0.011$; time³: $F_{1,528.7} = 10.02$, $P = 0.002$; interactions between

Table 1

Mixed-model ANCOVA testing variation in call duration in relation to time, within-brood age hierarchy and which of the junior or senior individuals consumed the first prey of the night ('eating success')

Source of variation	<i>F</i>	<i>df</i>	<i>P</i>
Time	100.33	1,5718	<0.0001
Eating success	5.17	1,19.02	0.035
Age hierarchy	12.14	1,19.02	0.003
Time*eating success	19.77	1,5724	<0.0001
Time*age hierarchy	16.77	1,5724	<0.0001
Eating success*age hierarchy	0.72	1,19.01	0.41
Time*eating success*age hierarchy	52.03	1,5718	<0.0001

Nest site as well as nestling identity nested in nest site were entered in the model as two random variables. 'Time' is the interval between the moment when a call was produced and when a parent arrived at the nest for the first time that night.

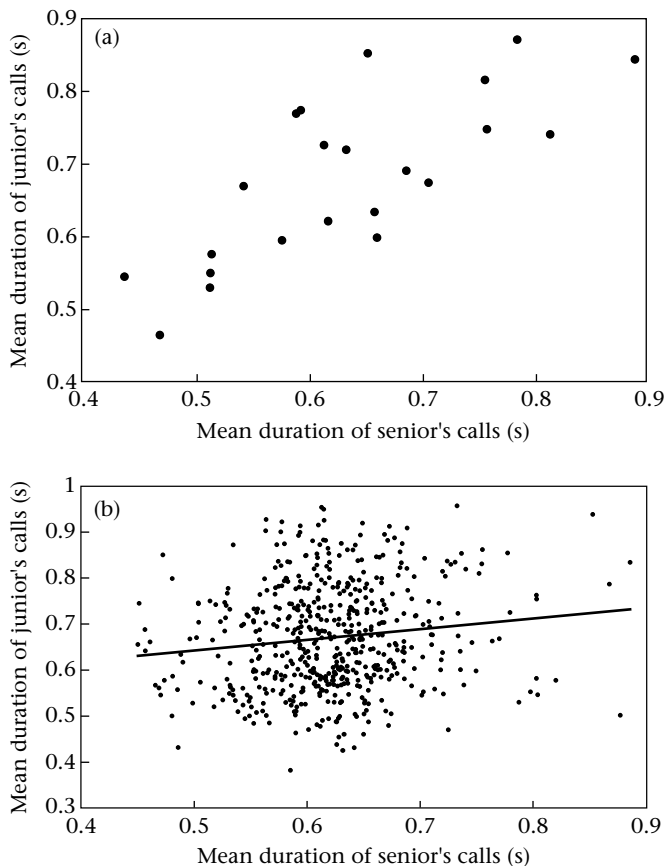


Figure 1. Relation between the mean durations of calls produced by barn owl seniors and their junior sibling before the first arrival of a parent with a food item on that night. In (a), for each individual, we calculated mean call duration over all measured calls, and thus each individual appears only once in the figure. In (b), for each individual we calculated the mean duration of calls produced in periods of 30 s, and we correlated the mean durations of calls produced by junior and senior siblings during the same periods of 30 s. We chose periods of 30 s for no particular reason. We present least-square means \pm SE extracted from the model described in the Results to remove variation explained by the time period over which the duration of calls was averaged.

timeⁿ and call duration of the senior sibling were not significant ($P > 0.26$) and were backward dropped from our final model). Results are similar if mean call duration of seniors is the dependent variable and mean call duration of juniors the independent variable ($F_{1,544.3} = 30.64$, $P < 0.0001$), except that seniors did not modify call duration in a consistent way with time (time: $F_{1,528.7} = 1.33$, $P = 0.25$; time²: $F_{1,529.4} = 1.79$, $P = 0.18$; time³: $F_{1,529.5} = 1.19$, $P = 0.28$). Figure 2 shows four examples of how siblings adjusted call duration to one another with time. These examples show that short-term adjustment of call duration to one another was independent of time, as call duration fluctuated over time.

Relative Timing of Call Production

Vocal challenges between siblings may influence not only call duration, as suggested by the above results, but also the timing of call production. We thus investigated whether call duration and/or the time interval between two calls (Fig. 3) predict which individual will call next. We selected cases where an individual produced a call after its sibling (in Fig. 4 nestling B produces a call denoted 2 after its sibling A which produced a call denoted 1). We then measured the durations of call 1 and call 2 as well as the time interval between the two calls. Using a logistic regression analysis, we determined that a short time interval between calls 1 and 2

made it more likely that A would call again while a longer time interval between calls 1 and 2 increased the likelihood that B would call again (logistic regression with nest site as a variable: time interval between call 1 and call 2: $\chi^2_1 = 19.62$, $P < 0.0001$; Fig. 4). Furthermore, individual A was more likely to call again after B if B produced a short call than when it produced a long call (same model: $\chi^2_1 = 3.81$, $P = 0.05$). In this analysis, we controlled for the duration of call 1 ($\chi^2_1 = 2.10$, $P = 0.15$) and age hierarchy (senior versus junior) as a factor ($\chi^2_1 = 1.98$, $P = 0.16$).

Because the timing of calling after a sibling was not random, we investigated whether sex, place in the within-brood age hierarchy and identity of the individual that obtained the next delivered prey item were associated with the time taken to call after its sibling. As we measured the time interval between many different pairs of calls from two siblings, we built a mixed-model ANOVA with nest site and individual nested in the nest site as two random variables, the time interval between two successive calls produced by different individuals as the dependent variable, and sex and within-brood age hierarchy as two fixed factors. Only sex of the individual producing the second call explained a significant part of the variation in the timing of calling after a sibling ($F_{1,18.08} = 5.31$, $P = 0.03$). Females responded to the calls of individual B with a shorter time interval than males (0.342 ± 0.02 s versus 0.378 ± 0.02 s). Hierarchy and identity of the individual eating the prey item did not account for the time taken by an individual to call after its sibling ($P > 0.19$), and thus these two variables were removed from the final model.

DISCUSSION

Food-deprived nestling barn owls produced longer calls than food-satiated individuals and long calls were associated with the likelihood of monopolizing the next food item delivered by parents. Call duration being a signal of hunger level, once a nestling produced long calls, its sibling took this as a challenge and responded by increasing the duration of its calls (Figs 1, 2). Conversely, when an individual reduced call duration, this was apparently the sign of a relaxation of sibling competition leading its sibling to reduce the duration of its calls. Although hungry nestlings produced on average longer calls than food-satiated individuals, call duration fluctuated over time independently of hunger level (Fig. 2). This suggests that although relatives communicate using costly signals of need, social interactions among them induce short-term variation in signalling investment that is independent of short-term variation in need. Given the correlative nature of the results, an experimental approach is now required to demonstrate that social interactions, rather than another factor not considered in the present study, account for the nonrandom statistical association of call durations, and also that similar results are obtained in larger broods. Further evidence that siblings challenge each other vocally is the observation that the more rapidly an individual B called after its sibling A, the higher the likelihood that individual A produced the next call (Fig. 4). Short-term adjustment of vocalization behaviour may reinforce the honesty of sibling negotiation to resolve conflicts over which individual will have priority of access to the next delivered food item. Because a couple of long calls may not be sufficient to obtain the next food item, nestlings that produce these few long calls are challenged to confirm that the level of signalling corresponds to motivation to compete.

Call Parameters and Food Allocation

In the absence of parents, hungry nestling barn owls vocalize intensely to inform siblings about their intention to compete vigorously once the parents return with a single food item

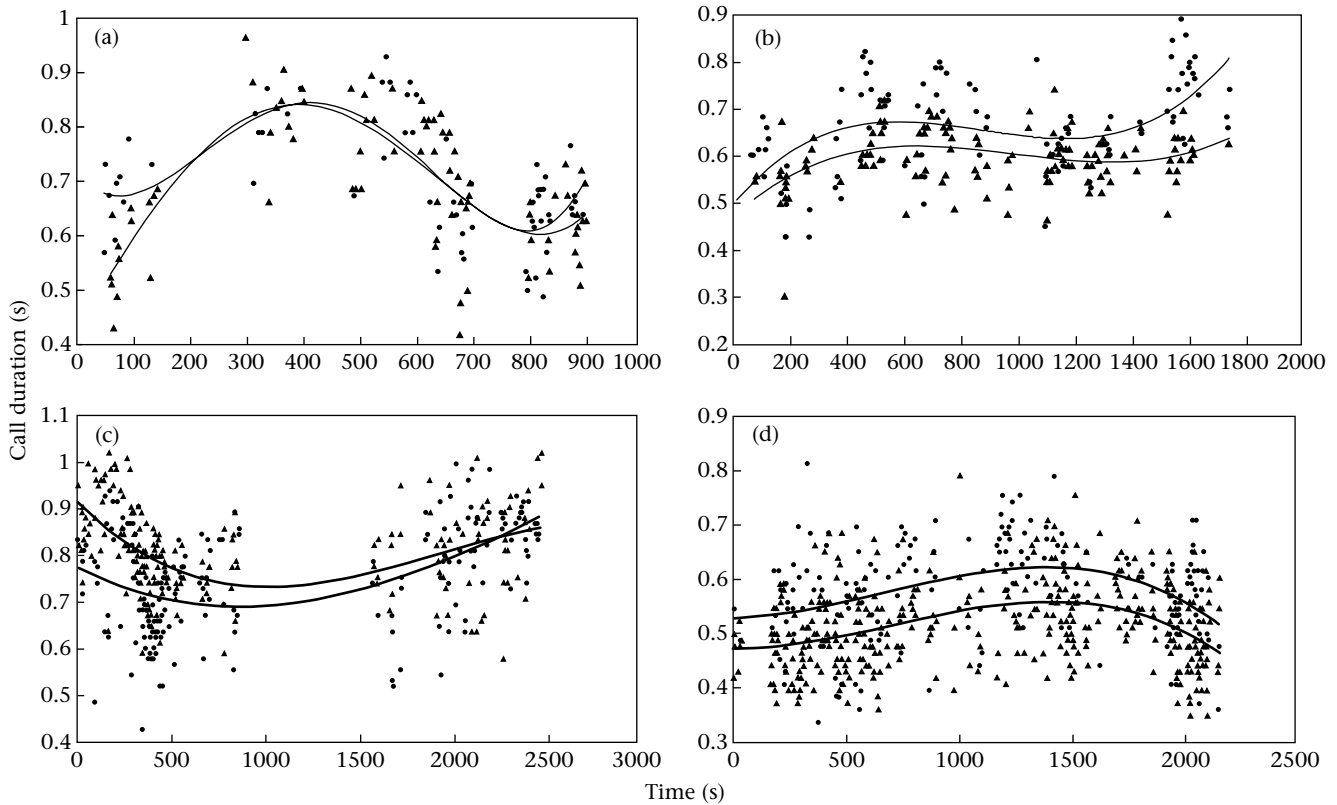


Figure 2. Duration of calls of barn owl junior (●) and senior (▲) siblings from four (a–d) two-chick broods in relation to the amount of time (s) between the moment when a given call was produced and the first arrival of a parent with a food item on that night (defined as time 0). Third-order curves were fitted for each individual (all were significant, P values < 0.003).

(Roulin et al. 2000; Roulin 2002). Being informed, less hungry siblings retreat from a contest for which the likelihood of winning is low and they thereby save energy. As a consequence, hungry nestlings gain access to food resources without having to compete

and beg intensely to attract parental attention (Johnstone & Roulin 2003; Roulin 2004b). Previous studies on the sibling negotiation hypothesis in the barn owl considered either the total number of calls produced over 14 min before the arrival of a parent

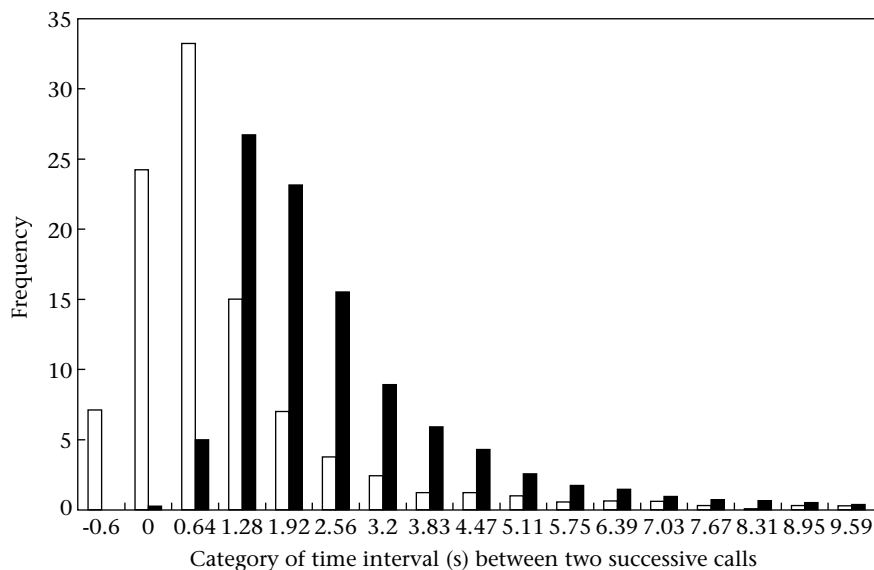


Figure 3. Distribution of time intervals (s) between two successive calls produced by two siblings (open bars: 1141 intervals; $\bar{X} \pm \text{SE} = 1.56 \pm 0.05$ s) or by the same individual (filled bars: 1836 intervals; 1.26 ± 0.03 s) before the first arrival of a parent with a food item on that night. For this figure, we considered only time intervals of less than 10 s. Time intervals were defined as the amount of time between the moment when one individual ended a call and when the same individual or its sibling started to produce the next call. Intervals were pooled in classes of 0.639 s corresponding to the mean duration of calls measured in the present study. For example, the category –0.6 indicates that calls of two individuals overlapped, while the category 0 indicates that two calls were produced within an interval of 0.639 s.

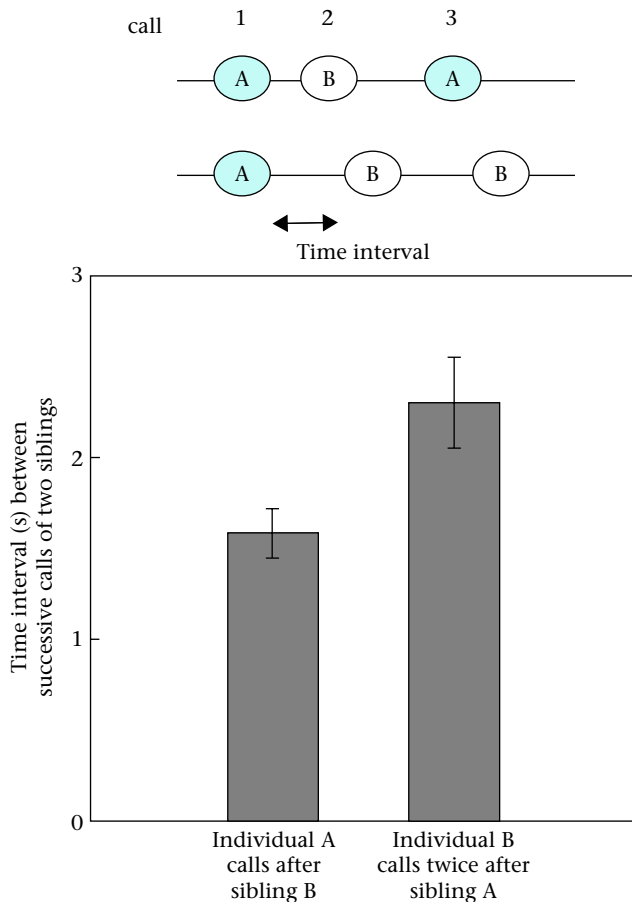


Figure 4. Mean \pm SE time interval (s) between two successive calls of two siblings (call 1 being produced by individual A and call 2 by individual B) depending on whether the next call (call 3) was produced by individual A or individual B. Time intervals were defined as the amount of time between the moment when individual A ended a call and when individual B started to produce the next call. Calls were produced in the absence of parents before the first prey item of the night was delivered.

(Roulin 2001, 2004b) or the energy contained in these calls (Roulin et al. 2000). These studies showed that individuals calling more frequently and with louder calls are more likely to be fed first because calling in the absence of parents influences begging investment in the presence of parents which ultimately determines the order in which chicks are fed. In the present study, mean call duration but not call rate predicted which individual obtained the next prey item. As we used a relatively small number of broods in the present study, we may have lacked statistical power to detect an effect of call frequency on the likelihood of monopolizing prey items. None the less, this suggests that call duration may be more important than call frequency in sibling negotiation, a proposition that should be studied further. Nestlings may thus assess the duration of each call, and thus not only the number of calls produced by their siblings, to adjust investment in sibling negotiation and in turn begging which ultimately determines within-brood food allocation (Roulin 2004b). This observation leads to two interpretations. First, to contest the next food item it may be better for nestlings to produce a small number of long calls than many short calls. This is consistent with the observation that in our study the durations but not numbers of calls produced by two siblings were correlated. To put it another way, when an individual produces long calls, the best strategy for its sibling to have a chance of succeeding in the sibling negotiation process may be to increase

the duration of its calls. Second, call duration and number of calls may be two components that facilitate individual recognition (e.g. Draganoiu et al. 2006) or that reflect different aspects of the motivation to compete for food (Träger et al. 2006; Kunc et al. 2007). Vocal individual recognition is probably an important trait in nestling barn owls because there is usually more than two siblings per brood and social interactions take place in the dark. We are currently testing whether individuals can be recognized by call structure.

Investment in Calling Behaviour

In birds and insects, although older offspring produce fewer calls in the absence (Roulin 2004b) and presence of parents (Cotton et al. 1999; Roulin 2004b; Smiseth & Moore 2007; but see Smiseth & Amundsen 2002) than their junior siblings, they are better able to monopolize food. This indicates that begging of seniors is more effective in attracting parental attention (van Heezik & Seddon 1996; Saino et al. 2001; Smiseth & Amundsen 2002; see also Ross et al. 2006 for a study in humans). Apparently, the size advantage of seniors forces younger siblings to invest substantial effort not only in vocal but also in physical competition, because even if seniors vocalize intensely in the absence of parents juniors still have a chance of obtaining the next delivered food item (Roulin et al. 2008). In the present study, we found that, before the arrival of a parent, juniors increased the duration of their calls with time but this did not necessarily result in the successful monopolization of food. In contrast, for seniors an increase in call duration resulted in the monopolization of the food item. These results are consistent with a previous study (Roulin 2004b) showing that a younger individual, whether hungry or not, is unlikely to obtain a food item unless the older sibling is satiated. Thus, if a younger, smaller owlet decides to contest a food item by increasing its call duration to match that of an older sibling, it is unlikely that the younger sibling will obtain the food, given that the older owlet has indicated its intention to monopolize food. This raises the question of why siblings match call duration to one another. A possibility is that the younger sibling requires information about how motivated the older sibling is, and so escalates by lengthening its call in response to the older individual. It may pay if these vocal exchanges are relatively cheap relative to sibling rivalry once the parents arrive. Assuming that the older individual is hungry, another possibility is that it is the older individual that increases the duration of its calls when its younger sibling produces longer calls. As stated in a previous study, 'juniors may negotiate to challenge their senior siblings, and thereby determine whether seniors are less hungry before deciding to beg for food. In contrast, seniors may negotiate to deter juniors from begging' (Roulin 2004b, page 1083).

The present study is consistent with the sibling negotiation hypothesis postulating that siblings do not have full information on each other's hunger level without producing vocal signals. Siblings interact vocally over a relatively long period of time to identify which individual is the hungriest, with hungry individuals vocalizing intensely in the absence of parents to deter their siblings from competing when a parent arrives with food. This long process may occur when siblings are all hungry, which was probably the case during our study since their last meal was on the previous night. Furthermore, if one individual reduces investment in vocalization behaviour, its siblings may take this as a sign of low motivation to contest the next food item, thereby forcing individuals to vocalize over long periods of time. Refined adjustment of call duration may be possible because call rate is relatively low in the absence of parents (on average 3.7 calls/min per nestling, Roulin 2002), and thus each individual has ample time to assess and integrate the siblings' calling behaviour to adjust its own behaviour. Even in large

broods, one or more individuals are vocalizing at any one time and thus nestlings can probably easily integrate siblings' behaviour. Constraints on the ability to integrate the behaviour of siblings may explain why a similar study on tree swallows, *Tachycineta bicolor*, did not find any adjustment of the structure of calls produced in the presence of parents, a period when nestlings are vocalizing at a high rate (Leonard & Horn 2001). Nestling barn owls can be compared to birds that match some features of song to one another as a mean of increasing the level of threat towards rivals (Naguib 2005). This behaviour is similar to avian duets where two birds coordinate their songs with a degree of temporal precision (e.g. Hall 2004). Thus, as in singing passerines, nestling barn owls challenge each other vocally in the absence of parents. A similar process occurs in humans, with speakers accommodating their speech to their addressee to win their approval (Giles & Powesland 1975).

Surprisingly, few studies have investigated whether male and female nestlings invest differentially in signalling. In barn swallows, *Hirundo rustica*, Saino et al. (2003, 2008) showed that sons and daughters can be discriminated by auditory cues and mouth coloration at some ages but not at others. In red-winged blackbirds, *Agelaius phoeniceus*, a species in which nestling males are 25% larger than females, Teather (1992) reported that males invest more effort in begging than their female nestmates. In the barn owl where nestling females are 6% heavier than males (Roulin et al. 1999), male and female offspring produce a similar number of calls in both the absence and the presence of parents suggesting that investment in signalling is not sex specific (Roulin 2004b). There was, nevertheless, a tendency for females to call more rapidly after a sibling than males, potentially indicating that female nestlings are vocally slightly more competitive than males. Moreover, juniors produced longer calls than seniors in females but not in males, further indicating that vocalization behaviour can indeed be related to sex. Further data are required on the sex-specific competitive strategies as it may be important for understanding why female nestlings are heavier than male nestlings. Indeed, it is still unclear whether females eat more food than males and/or have a different physiology. Whatever the mechanism, the implications for sex-specific competitive behaviour remain to be tackled in detail.

Alternating versus Overlapping Vocal Interactions

Studies on animal singing behaviour have shown that the timing of singing is an important component of signalling. Song alternating is a strategy with which neighbouring males take turns in delivering their song to avoid sound interference and thereby ensure that females can hear them correctly (Greenfield 1994). In other circumstances, animals overlap rather than alternate their calls or songs with nearby conspecifics, that is, an individual begins to vocalize before the opponent has ended its call. Call overlapping has been shown to function as a directed agonistic signal (Naguib et al. 1999). Our study provides important information on the potential signalling function of the timing of calling in nestling birds. When a nestling called immediately after its sibling, the probability that the latter individual produced the next call was higher. This suggests that the rapidity with which an individual calls after a sibling is taken as a challenge, which induces it to call again to contest priority of access to the next food item. This proposition should be tested experimentally by playing back calls in nests to investigate the effect of overlapping or alternating calls on vocalization behaviour.

Conclusion

The present study suggests that siblings adjust signalling level to each other in the short term. Adjustment appears to depend on

age and sex and is not entirely driven by variation in need, as signalling level fluctuated over time in the very short term. The finding that both need and social interactions influence investment in signalling opens up new avenues of research. It will be particularly interesting to examine how siblings adjust signalling level to one another in unmanipulated nests. As each individual produces about 1800 calls per night in the absence of parents, it would not be surprising if owlets are able to develop complex ways of interacting. For example, individuals may adjust signalling level in different ways when interacting with different siblings. The barn owl therefore appears to be an appropriate biological system to tackle issues associated with the evolution of social interactions in animals.

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