JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12793

Reciprocal preening and food sharing in colour-polymorphic nestling barn owls

A. ROULIN*, B. DES MONSTIERS*, E. IFRID*, A. DA SILVA*†, E. GENZONI* & A. N. DREISS*‡

*Department of Ecology and Evolution, Biophore, University of Lausanne, Lausanne, Switzerland
†Behavioural Ecology and Evolutionary Genetics, Max-Planck Institute for Ornithology, Seewiesen, Germany
‡Institute of Ecology and Evolution, University of Bern, Bern, Switzerland

Keywords:

allofeeding;
allopreening;
altruism;
cooperation;
food sharing;
food stealing;
reciprocity;
sibling competition;
social behaviour;
Tyto alba.

Abstract

Barn owl (*Tyto alba*) siblings preen and offer food items to one another, behaviours that can be considered prosocial because they benefit a conspecific by relieving distress or need. In experimental broods, we analysed whether such behaviours were reciprocated, preferentially exchanged between specific phenotypes, performed to avoid harassment and food theft or signals of hierarchy status. Three of the results are consistent with the hypothesis of direct reciprocity. First, food sharing was reciprocated in three-chick broods but not in pairs of siblings, that is when nestlings could choose a partner with whom to develop a reciprocating interaction. Second, a nestling was more likely to give a prey item to its sibling if the latter individual had preened the former. Third, siblings matched their investment in preening each other. Manipulation of age hierarchy showed that food stealing was directed towards older siblings but was not performed to compensate for a low level of cooperation received. Social behaviours were related to melanin-based coloration, suggesting that animals may signal their propensity to interact socially. The most prosocial phenotype (darker reddish) was also the phenotype that stole more food, and the effect of coloration on prosocial behaviour depended upon rank and sex, suggesting that colour-related prosociality is state dependent.

Introduction

The emergence of cooperation, along with the mechanisms underlying its evolutionary stability, has long attracted the attention of evolutionary biologists (Lehmann & Keller, 2006; Bshary & Bergmueller, 2008). Kinship is one of the primary factors that can account for the evolution of cooperation because the cost of helping a related individual is offset by the enhanced reproductive success of the recipient, which spreads genes shared with the altruistic individual (Hamilton, 1964; Breed, 2014). Therefore, kin selection may be the global explanation for the emergence of prosocial behaviours among relatives. However, numerous other benefits may contribute to the maintenance of cooperation (Queller, 2011). An individual can benefit from being cooperative because this induces conspecifics to reciprocate (Trivers, 1971) or because cooperative individuals can choose to interact together as they advertise their altruistic traits to each other (green-beard effect, Dawkins, 1976). Individuals may cooperate to avoid harassment or punishment from conspecifics (Blurton Jones, 1987) or because they signal their dominance status or 'social prestige' by performing costly altruistic activities (Zahavi, 1990). The tendency to cooperate and reciprocate may vary if the various costs and benefits of cooperating are not fixed in time or across individuals (Leimar, 1997; Dolivo & Taborsky, 2015), which explains why species and phenotypes vary in their degree of cooperativity. Identifying the costs and benefits of cooperation in different phenotypes is not trivial, in part because reciprocation can involve differing currencies, with one type of behaviour being traded against another type of behaviour.

Correspondence: Amélie N. Dreiss, Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland.
Tel.: +41 21 692 42 48; fax: +41 21 692 41 65;
e-mail: amelie.n.dreiss@gmail.com

Prosocial behaviours in bird nestlings have rarely been studied, but it is a prime model system for examining social interactions because nestlings are confined in a place where they compete for the same resources. This proximity leads to repeated interactions between individuals, promoting intense agonistic behaviours or, by contrast, altruism (Roulin & Dreiss, 2012). The study of social interactions between young siblings therefore offers the opportunity to discuss the interplay between kin selection and other mechanisms that can explain the evolutionary stability of cooperation.

In this study, we investigated food sharing and allopreening behaviour in barn owl (*Tyto alba*) nestlings. Food sharing and allogrooming/allopreening, where individuals feed and groom/preen each other, are ideal behaviours for studying the costs and benefits of engaging in complex social interactions, especially because they are short and repeated events that involve two or more individuals (e.g. Schneeberger *et al.*, 2012). Allogrooming can be considered a prosocial behaviour as it has a hygienic function for the groomee, particularly if directed to body areas that cannot be easily self-groomed (e.g. the head) and where parasites are particularly abundant (e.g. Akinyi *et al.*, 2013). Allogrooming may also reduce stress levels in both the groomee and the groomer (Kober & Gaston, 2003; Shutt *et al.*, 2007; Stoewe *et al.*, 2008; Soares *et al.*, 2011) and may thus provide mutual benefits to the groomer and the groomee. These two categories of hypotheses, hygienic function and social appeasement, are not mutually exclusive.

Our aim was to analyse food sharing and allopreening behaviours in barn owl nestlings and to test several hypotheses of the emergence of social behaviours. We studied interactions in experimental three-chick broods recorded in 2007, a sample that has been used to investigate food sharing (Roulin *et al.*, 2012), and in experimental pairs of two-chick broods in 2012. We first examined whether behavioural patterns were consistent with reciprocity. To this end, we investigated whether allofeeding and allopreening behaviours were interchanged (exchange of different commodities) or reciprocated (exchange of the same commodity). In the presence of kinship, reciprocity can have synergetic effects on the evolutionary stability of cooperation (Lehmann & Keller, 2006; McGlothlin *et al.*, 2014; Van Cleve & Akcay, 2014). Under direct reciprocity, donor individuals benefit from the fact that the receiver will reciprocate in the near future (Trivers, 1971). Cooperative individuals also derive benefits by helping conspecifics because bystanders who observed the cooperative act are more likely to cooperate with them, a situation described as ‘indirect reciprocity’ (Nowak & Sigmund, 1998). Three-chick broods allowed us to test whether giving food or preening increased the likelihood of receiving a prosocial act in return to the same sibling (direct reciprocity) or to the other nest mate (indirect reciprocity or generalized reciprocity, van Doorn & Taborsky, 2012).

Second, we determined whether variation in the propensity to allopreen and to give or steal food covaries with melanin-based coloration. Selection may favour phenotypes, such as colour traits, that allow other individuals to recognize the extent to which they are cooperative (Dawkins, 1976; McGlothlin *et al.*, 2014). Melanin is the most common pigment in the animal kingdom and has been found to be associated with social behaviour in many organisms, mainly in agonistic behaviours (Ducrest *et al.*, 2008). In the barn owl, birds vary from white to darker reddish pheomelanic, and reddish nestlings were found to give more prey items to their siblings (Roulin *et al.*, 2012). Furthermore, reddish barn owl mothers preened their offspring more often than did paler mothers (Almasi *et al.*, 2013). Therefore, if we find that reddish coloration is associated with allopreening between siblings, it could raise the possibility that pheomelanin-based coloration is used in this species, as well as potentially in other animals, to recognize which individuals are more likely to cooperate (Leimar, 1997).

Third, we investigated whether prosocial behaviours such as allofeeding and allopreening, as well as food stealing, could be interpreted as status signals and hence be determined by age hierarchy. Senior nestlings are known to give more prey items to nest mates (Roulin *et al.*, 2012), and we therefore manipulated the hierarchy by placing nestlings in junior and senior positions on different nights. In the barn owl, asynchronous hatching generates a pronounced age hierarchy (the oldest nestling can be up to 1 month older than its youngest sibling), which translates into a dominance hierarchy. This hierarchy has pronounced effects on how food is shared among the siblings (Roulin *et al.*, 1999), and vocal behaviour used in sibling competition is much more influenced by age hierarchy than by absolute age (e.g. Dreiss *et al.*, 2014). Finally, we tested whether food stealing is related to prosocial behaviours. The temptation to not reciprocally cooperate may be offset by the risk of retaliation in the form of punishment (Sigmund, 2007; El Mouden *et al.*, 2010; Raihani *et al.*, 2012) or by the end of cooperation (Axelrod & Hamilton, 1981). If prosocial behaviours are displayed in order to prevent food stealing in owlets, we expect thefts to be less numerous when food or preening is provided.

Additionally, we examined whether self-preening correlated with the allopreening received, which would indicate that allopreening is indeed a prosocial behaviour that relieves a receiver in need of being preened.

Materials and methods

We performed the study in western Switzerland on a wild population of barn owls breeding in nest boxes.

Age was determined a few days after hatching. Because of hatching asynchrony, the experimental siblings were not of the same age. Nestling sex was determined using molecular markers (Py *et al.*, 2006). Video footage was analysed by students who were blind to the scientific questions. On the video footage, it was not possible to identify nestling coloration.

Experimental three-chick broods in 2007

We used infrared-sensitive cameras installed in 21 nest boxes 1 day before the experiment to record the behaviour of three siblings during one night, from 19 : 00 until 7 : 00 the following morning. We reduced the brood sizes by placing all but the three nestlings in a ventilated box located some distance from the nest. At the time of the recording, the oldest individual was on average 42 ± 1 days (senior in the within-brood age hierarchy), the middle-aged individual was 38 ± 2 days, and the youngest individual (or junior) was 34 ± 2 days. To recognize nestlings individually on the video footage, we ringed them with one or two aluminium rings. We used the same sample of nestlings as for a previous study on allofeeding (Roulin *et al.*, 2012); hence, most of the methodological information (and ethical notes) can be found in Roulin *et al.* (2012).

Experimental pairs in 2012

In contrast to the 2007 procedure, in 2012, we recorded nestling behaviour in pairs and in nest boxes placed at the laboratory, not in their natural nests. At 13 : 00, we brought to the laboratory 118 owlets (54 males, 63 females and 1 of unknown sex) aged 30.6 ± 5.0 days (range: 16–39) from 30 broods containing 3.9 ± 1.2 nestlings (range: 2–6). We left at least one nestling in each natural nest to ensure that the parents did not abandon their brood. Nestlings were kept in nest boxes similar to those in which they were raised ($62 \times 56 \times 37$ cm^3) but that were twice as high, allowing the inclusion of an infrared-sensitive camera in the ceiling to record nestling behaviours. They were equipped with a pipe (10 cm in diameter and 20 cm long) connected to the outside, so that air and natural light could enter. Each nestling was kept for three successive experimental nights (from 14 : 00 to 13 : 00 the next day), one night alone in its nest box, another night with an older sibling and a third night with a younger sibling, with the order of the three social treatments allocated randomly. Most individuals were recorded during three nights, but some individuals were recorded during only two nights because of logistical issues (e.g. only one pair can be constituted because only two siblings were brought to the laboratory). At the beginning of each experimental night, at 14 : 00 every day, we supplied fresh laboratory mice, *Mus musculus* (120 g per individual, from the Reptile Farm, 232 Servion, Switzerland, euthanized by CO_2), corresponding to approximately twice their daily food requirement (Durant & Handrich, 1998). We placed prey items in a corridor located in the middle of the box, with one end against the long side of the box and the other end opening on the centre of the box, so that only one nestling could enter the corridor at a time. Any remaining mice offered the day before were removed. Each nestling ate an average of 1.8 ± 0.1 (range 0–4) mice per night. The number of mice eaten was not significantly associated with age (linear mixed model with nestling and nest site identities as random variables, $\chi^2 = 0.01$, $P = 0.92$), sex ($\chi^2 = 1.34$, $P = 0.51$) or social treatment ($\chi^2 = 1.62$, $P = 0.20$; junior, senior or alone). After three nights spent at the laboratory, we brought the nestlings back to their natural nests.

We recorded nestling behaviour starting 1 h after the insertion of the offspring into the laboratory nest boxes (i.e. at 15 : 00) until the end of their nocturnal activities the next morning (09:00). We marked nestlings on their heads to recognize them. We have demonstrated previously that owlets are not physiologically stressed in the laboratory (Dreiss *et al.*, 2010).

Assessment of allopreening

We defined an allopreening event as an individual preening a sibling using its bill. Allopreening consists of an unaggressive displacement of the sibling's feathers by the allopreener, whereas pecking is characterized by a swift hit with the beak. The distinction between these two behaviours was unambiguous. The occurrence and duration of an allopreening bout was defined as the period from the start of the behaviour to the cessation of the preening motion by the acting individual (a pause lasting more than one-second being considered the end of a bout).

A.S. measured the duration of allopreening events in 63 nestlings from 21 nests in 2007. B.M. recorded self- and allopreening events in 2012 for a duration of 30 s every 10 min from a subsample of 79 owlets from 20 broods gathered in 76 pairs. An individual was self-preening when it touched its feathers with its bill or when it scratched its body with its feet (Clayton & Cotgreave, 1994). For each preening or allopreening event that occurred during the 30-s time window, we measured the exact duration, even if the bout started before this specific time window or ended after it. As preening bouts never lasted more than 4.03 min, we never recorded a preening bout twice.

Assessment of food sharing and stealing

An active food sharing event between two nest mates (i.e. allofeeding) was defined as a donor individual moving towards a receiver sibling to release the item on the ground in front of its partner (from bill to foot) or from bill to bill. In 2012, food sharing was recorded in a subsample of 62 nestlings (or 60 pairs). Food stealing was defined as the nestling walking towards its sibling to take an item from the feet of the sibling that was consuming it. Stealing was recorded by E.I. for 118 nestlings (or 115 pairs).

Assessment of plumage coloration

In the field in daylight, A.R. scored pheomelanin-based coloration when nestlings were approximately 50 days of age by comparing their colour with eight chips ranging from −8 for white to −1 for darker reddish (Roulin *et al.*, 1998). Some nestlings died or could not be captured before this age and hence were not measured (10 in 2007 and 9 in 2012). Scoring was performed on the breast, belly, flank and underside of the wings, and mean values over these four body parts were used in the statistical analyses. This method is reliable as shown by measuring the same individuals twice (repeatability is 0.90, Roulin, 2004). We already showed that coloration scored by eye is strongly correlated with the reflectance in the range of 400–700 nm measured with an Ocean Optics S2000 spectrometer and a PX-2 xenon lamp (Pearson's correlation: $r = 0.78$, $n = 1107$, $P < 0.0001$; Dreiss & Roulin, 2010). Although barn owls vary not only in the degree of pheomelanin-based coloration but also in the size and number of black spots located on the tip of ventral body feathers, we analysed behaviour only in relation to pheomelanin. This decision is based on our previous finding that allofeeding and allopreening are related to pheomelanin and not to plumage spottiness (Roulin *et al.*, 2012; Almasi *et al.*, 2013).

Statistical analysis

For three-chick broods, the mean duration of allopreening bouts was Box-Cox-transformed to obtain a normal distribution. The number of allopreening bouts could not be normalized and was analysed using Poisson distribution models, but was log-transformed to approach a normal distribution when used as a covariate. For experimental pairs, duration and number of allopreening bouts were normalized using Box-Cox transformations.

Data are shown as mean values ± SE. We removed nonsignificant interactions from the models (*P*-values >0.05); otherwise no selections were performed on independent terms. Estimates ± SE. are given in the text.

Reciprocity of allopreening, rank and melanism in three-chick broods (models 1ab)

To test whether duration and number of allopreening bouts given were related to coloration, we ran, respectively, a linear mixed model (model 1a) and a generalized linear mixed model (GLMM) with Poisson distribution (model 1b) (Table 1). As dependent variables, we set the average bout duration (model 1a) and the total number of allopreening bouts (model 1b) given by the focal nestling to a sibling during the night. Donor and receiver identities nested in the nest site were set as random factors (because each individual interacted with two siblings, it appeared twice in the analysis). In the *post hoc* analyses performed separately for each sex, only donor identity nested in the nest site was set as a random factor, as the model would not converge if we added receiver identity nested in the nest site as a second random factor. To test whether an individual A was more allopreened by its sibling B, but not by C, when it allopreened this individual B, we set as independent variables the duration or number of allopreening bouts received from sibling B and the duration or number of allopreening bouts received from sibling C.

Table 1 Mean duration and number of allopreening bouts given in experimental three-chick nests. The donor was the individual that allopreened its nest mate B, the receiver 'B' was the individual that was allopreened, and the third sibling was called 'C' (models 1a–b). The covariate 'allopreening' represents either duration (model for mean duration of allopreening bouts) or number (model for number of allopreening bouts) of received allopreening bouts.

	F	*P*
Mean duration of allopreening bouts given to sibling B (d.f. = 11)		
Sex donor	0.66	0.43
Colour donor	0.12	0.73
Rank in hierarchy of donor [Junior, middle-born or senior]	1.62	0.24
Sex receiver	7.21	0.021*
Colour receiver	4.30	0.062
Rank in hierarchy of receiver	0.75	0.49
Allopreening received from B	33.11	0.0001***
Allopreening received from C	0.59	0.46
Sex donor × Colour donor	*0.80*	*0.37*
Sex receiver × Colour receiver	7.59	0.018*
Colour donor × Colour receiver	*1.21*	*0.66*
Number of allopreening bouts given to sibling B (d.f. = 12)		
Sex donor	1.22	0.29
Colour donor	0.19	0.67
Rank in hierarchy of donor	0.38	0.54
Sex receiver	0.05	0.95
Colour receiver	0.43	0.52
Rank in hierarchy of receiver	0.12	0.73
Allopreening received from B	19.67	0.0001***
Allopreening received from C	0.53	0.46
Sex donor × Colour donor	*0.36*	*0.56*
Sex receiver × Colour receiver	*0.22*	*0.64*
Colour donor × Colour receiver	*0.21*	*0.65*

Interactions excluded from the models are in italics (*$P < 0.05$; **$P < 0.005$, ***$P < 0.0005$).

Self-preening, allopreening and melanism in experimental pairs (models 2a–d)

Duration and number of self- and allopreening bouts were set as dependent variables in four linear mixed models, with experimental nights (1st, 2nd or 3rd) and the identities of the focal nestling (and of its partner for allopreening only) nested in nest site as random factors (Table 2). To test whether an individual A was more allopreened by its sibling B, but not by C, when it allopreened this individual B, we set as independent variables the duration or number of allopreening bouts received from sibling B and the duration or number of allopreening bouts received from sibling C. Because the observation sessions were longer in experimental pairs (15 : 00–9 : 00) than in three-chick broods and because allopreening changed over the course of the night, we used average values of allopreening per period of the night (15 : 00–21 : 00, 21 : 00–03 : 00 and 03:00–09:00, see results) per individual and per experimental night (we recorded each individual during 2 or 3 nights), and the period of the night was included as an independent variable. For self-preening, the average over the entire night was used.

Food sharing, rank and melanism in experimental pairs (model 3)

The probability of sharing at least one prey during an observation session (night) was set as a dependent variable in a GLMM with binomial distribution, with experimental nights (1st, 2nd or 3rd) and the identity of the focal nestling nested in nest site as random factors (Table 3).

Reciprocity between food sharing and allopreening in three-chick broods (model 4)

We tested whether the number of prey items given by individual A to its sibling B was related to whether A received a prey item from sibling B or from sibling C, as two binomial factors, in a GLMM with Poisson distribution (model 6) (Table 4). In another GLMM with Poisson

Table 2 Mean duration and number of (a) allopreening and (b) self-preening bouts given in experimental pairs of barn owl nestlings (models 2 a-d). Recordings were divided into three 'periods of night' (15:00–21:00, 21:00–03:00 and 03:00–09:00). The covariates 'allopreening' and 'self-preening' represent either duration (model for mean duration of allopreening bouts) or number (model for number of allopreening bouts). Interactions excluded from the models are in italics.

	Mean duration of allopreening bouts (d.f. = 239)		Number of allopreening bouts (d.f. = 242)	
	F	*P*	*F*	*P*
(a)				
Sex donor (Sex D)	5.50	0.020*	1.36	0.24
Colour donor (Colour D)	5.98	0.015*	0.03	0.86
Sex receiver (Sex R)	0.36	0.55	0.50	0.48
Colour receiver (Colour R)	0.96	0.33	0.12	0.73
Social treatment [junior or senior]	4.68	0.032*	0.10	0.75
Self-preening of donor	9.12	0.003**	2.09	0.15
Self-preening of receiver	2.44	0.12	7.42	0.007**
Allopreening received from partner	22.99	0.0001***	30.92	0.0001***
Period of night	7.79	0.005*	0.26	0.61
Sex D × Colour D	5.98	0.015*	*0.01*	*0.96*
Sex R × Colour R	*0.32*	*0.57*	*0.04*	*0.84*
Colour D × Colour R	*0.48*	*0.49*	*0.17*	*0.68*
Period of night × Sex D	8.67	0.004**	*0.76*	*0.38*
Period of night × Sex R	*0.01*	*0.95*	*0.99*	*0.32*
Period of night × Colour D	8.73	0.003**	*0.01*	*0.98*
Period of night × Colour R	*0.83*	*0.36*	*1.78*	*0.18*
Period of night × Sex D × Colour D	9.50	0.002**	*2.08*	*0.15*
Period of night × Sex R × Colour R	*0.07*	*0.79*	*0.67*	*0.41*

	Mean duration of self-preening bouts (d.f. = 131)		Number of self-preening bouts (d.f. = 131)	
	F	*P*	*F*	*P*
(b)				
Sex	0.58	0.45	1.16	0.28
Colour	2.79	0.10	2.27	0.13
Social treatment [junior, senior or alone]	10.19	<0.0001***	0.43	0.65

$*P < 0.05$; $**P < 0.005$, $***P < 0.0005$.

Table 3 Probability of giving a prey item during the night in experimental pairs of barn owl nestlings (model 3). Interactions excluded from the models are in italics. (d.f. = 36).

	F	*P*
Sex donor	0.27	0.60
Colour donor	4.36	0.042*
Sex receiver	0.35	0.56
Colour receiver	3.68	0.06
Social treatment [junior or senior]	0.26	0.62
Sex donor × *Colour* donor	*0.17*	*0.69*
Sex receiver × *Colour* receiver	*0.58*	*0.45*
Colour donor × *Colour* receiver	*3.90*	*0.06*
Social treatment × Colour donor	4.86	0.034*
Social treatment × *Colour* receiver	*3.51*	*0.07*

*$P < 0.05$.

Table 4 Probability of giving a prey item to a sibling B in experimental three-chick nests of barn owl nestlings (model 4). Interactions excluded from the models are in italics (d.f. = 16).

	F	*P*
Rank in hierarchy of donor [junior, middle-born or senior]	2.08	0.16
Rank in hierarchy of receiver (Rank B)	5.61	0.014
Duration of allopreening received from B	1.40	0.25
Number of allopreening received from B	7.77	0.013*
Number of prey items received from B	5.96	0. 026*
Duration of allopreening received from C	0.69	0.41
Number of allopreening received from C	1.75	0.21
Number of prey items received from C	0.10	0.76
Rank B × Duration of allopreening received from B	*0.39*	*0.68*
Rank B × Number of allopreening received from B	0.10	0.022*
Rank B × Number of prey items received from B	*0.38*	*0.69*

*$P < 0.05$.

Table 5 Probability of giving a prey item in experimental pairs of barn owl nestlings according to the allopreening received at different periods of the night (15:00–21:00, 21:00–03:00 and 03:00–09:00) **(**model 5**)**. Interactions excluded from the model are in italics (d.f. = 291).

	F	*P*
Duration of received allopreening	2.35	0.13
Number of received allopreening	0.35	0.56
Number of received prey items	1.89	0.17
Period of night of received allopreening (Period)	0.19	0.66
Period × Duration of received allopreening	5.13	0.024*
Period × Number of received allopreening	*0.14*	*0.71*

*$P < 0.05$.

Table 6 Number of stolen prey items in experimental three-chick nests of barn owl nestlings (model 6). Interactions excluded from the models are in italics. (d.f. = 92).

	F	*P*
Rank in hierarchy of nestling doing the stealing [junior, middle-born or senior]	0.15	0.86
Rank of nestling being stolen (Rank D) [youngest or oldest of the two siblings]	1.37	0.25
Origin of stolen item [food stock or received directly from a parent]	0.45	0.50
Duration of allopreening received from nestling being stolen	0.74	0.39
Number of allopreening received from nestling being stolen	0.20	0.65
Number of prey items received from nestling being stolen	0.01	0.96
Rank D × Origin of stolen item	4.92	0.029*
Rank D × Duration of allopreening received	*1.10*	*0.30*
Rank D × Number of allopreening received	*0.21*	*0.65*
Rank D × Number of prey items received	*0.06*	*0.81*

*$P < 0.05$.

distribution (model 7), we tested whether the number of preys given by A to B was related to the number and duration of allopreening bouts received from B and from C, as four independent covariates. In both models, rank in the within-brood age hierarchy of A and B was set as cofactor, and donor and receiver identities nested in the nest site were set as random factors.

Reciprocity between food sharing and allopreening in experimental pairs (model 5)

The probability that individual A gives at least one prey item to its sibling B during the entire observation session (15 : 00–9 : 00) was set as a dependent variable in a GLMM with binomial distribution (Table 5). Because the frequency of allopreening changed over the course of the night, the average values of allopreening bouts received per period of the night (15:00–21:00, 21:00–03:00 and 03:00–09:00) were set as independent variables. We set the number of prey items that individual A received from B, the period of the night and the interaction between allopreening and period of the night as independent terms. Experimental nights (1st, 2nd or 3rd) and the identity of the focal nestling nested in nest site were set as random factors.

Food stealing in three-chick broods (model 6)

We computed the number of food items stolen per individual, according to whether each item was stolen from the youngest or oldest sibling and whether the item was taken from the food stock or received from a parent (Table 6). In a GLMM with Poisson distribution, we set the number of stolen food items as a dependent variable and the identities of the nestlings doing the stealing and being stolen from nested in nest site as random factors.

Table 7 Number of stolen prey items in experimental pairs of barn owl nestlings (models 7a–b). Interactions excluded from the models are in italics.

	F	*P*
(a) (d.f. = 92)		
Sex of the nestling doing the stealing (sex)	0.51	0.48
Colour of the nestling doing the stealing (colour)	6.64	0.011*
Social treatment [junior or senior]	4.26	0.041*
Social treatment × Sex	*0.81*	*0.37*
Social treatment × Colour	*0.12*	*0.73*
(b) (d.f. = 49)		
Duration of allopreening received from nestling being stolen	0.05	0.82
Number of allopreening received from nestling being stolen	2.44	0.13
Number of prey items received from nestling being stolen	0.04	0.83

*$P < 0.05$.

Food stealing in experimental pairs (models 7a–b)

The total number of prey items stolen by an individual during an experimental night (1st, 2nd or 3rd) was set as a dependent variable in a GLMM with Poisson distribution (Table 7). Experimental nights and the identity of the focal nestling nested in nest site were set as random factors. We used two separate models to analyse social treatment, sex and colour (model 7a, Table 7a) and prosocial behaviours (model 7b, Table 7b) because prosocial behaviours were evaluated based on a smaller subset of individuals.

Results

Reciprocity of allopreening, rank and melanism

Three-chick broods

Nestling barn owls performed 0.67 ± 0.52 allopreening bouts per hour (median: 0.49; range: 0–4.13). Each allopreening bout lasted an average of 21.9 ± 2.2 s (median: 16 s; range of mean individual values: 2–152.6 s). In 78.4% of 653 cases, allopreened body regions were those that individuals cannot preen by themselves, that is the head (58.7%), the back (14.5%) and the neck (5.2%). Nestlings also preened their nest mates' wings (10.0%), flanks (2.1%), bellies (2.6%), legs (4.0%) and tails (2.9%).

The number and duration of allopreening bouts that a focal nestling gave to a sibling (B) were strongly associated with the number and duration of allopreening bouts it received from this sibling (Table 1; duration: 0.49 ± 0.08; number: 0.08 ± 0.02; Fig. 1) but not with allopreening received from the third partner (C) (Table 1; duration: −0.06 ± 0.08; number: 0.08 ± 0.11; duration of allopreening by C in a separate model without duration of allopreening by B: $F_{1,18} = 1.48$, $P = 0.24$; number of allopreening bouts by C in an additional separate model: $F_{1,17} = 2.30$, $P = 0.15$). The relationship with the duration of allopreening bouts was also significant if we considered only non-null duration (i.e. when number of bouts of given and received allopreening > 0; $F_{1,11} = 69.62$, $P < 0.0001$).

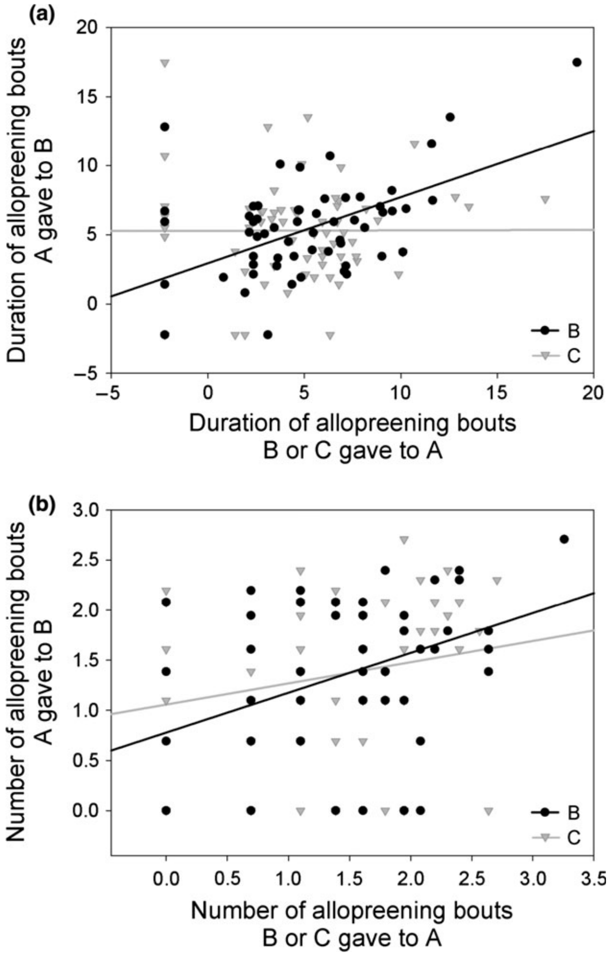

Fig. 1 Reciprocity of allopreening in barn owls in three-chick broods. Duration (a) and number (b) of allopreening bouts given by nestling A to B, in relation to the allopreening received from B or from C (transformed variables).

The mean duration of allopreening events performed by a donor individual was related to the interactions between sex and coloration of the receiver of allopreening (Table 1). To understand this interaction, we performed separate analyses for female and male receivers. In females, darker reddish individuals received longer allopreening bouts than paler ones (colour of receiver: $F_{1,8} = 18.80$, $P = 0.002$, 1.7 ± 0.4; Fig. 2). In males, the mean duration of allopreening bouts was not significantly related to the coloration of the

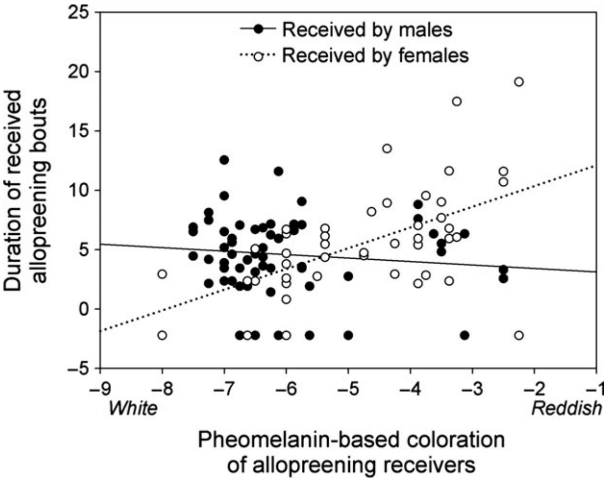

Fig. 2 Allopreening and coloration in barn owls in three-chick broods. Mean duration of allopreening bouts (transformed variable) received according to the pheomelanin-based coloration of the receiver.

receiver (colour of receiver: $F_{1,16} = 0.43$, $P = 0.52$, -0.3 ± 0.3; Fig. 2).

The number of allopreening bouts was not related to pheomelanin-based coloration, sex or position in the within-brood age hierarchy (Table 1).

Experimental pairs

At least one allopreening bout was performed during each experimental night. The number of allopreening bouts per hour was maximal before 20 : 00 (Fig. 3), whereas most prey items were eaten (88%, 239 vs. 34) and shared (allofeeding; 91%, 30 vs. 3) after 20 : 00, suggesting that allopreening may not have the same social importance during all periods of the night. We thus defined three periods of 6 h (15 : 00 to 21 : 00, 21 : 00 to 03 : 00 and 03:00 to 09:00), and we averaged preening behaviours for each period.

The mean duration of allopreening bouts performed by nestlings was positively associated with the mean duration of allopreening bouts received from their sibling (Table 2a). The numbers of allopreening bouts given and received were also positively correlated (Table 2a).

Nestlings performed longer allopreening bouts when in junior rather than senior position (Table 2a; 0.52 ± 0.24). The duration of allopreening bouts given was positively correlated with the duration of self-preening performed by the allopreener (Table 2a; 0.17 ± 0.05). The duration of allopreening bouts was also related to the interaction between the period of night, sex and coloration (Table 2a). *Post hoc* analyses showed that darker reddish females performed longer bouts than paler ones at the end of the night (effect of female coloration during the period from 03:00 to 09:00: 0.40 ± 0.19; $F_{1,30} = 4.33$, $P = 0.046$; 0.40 ± 0.19; interaction between sex and coloration at the end of the night [03:00 to 09:00]: $F_{1,10} = 5.46$, $P = 0.041$). Pheomelanin coloration was not related to the duration of allopreening bouts performed during other periods of the night or by males (all $P > 0.2$).

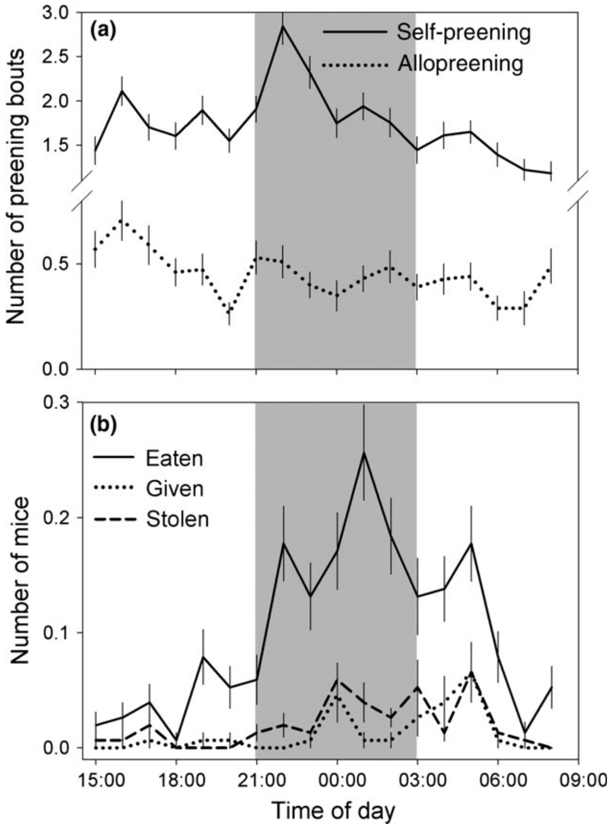

Fig. 3 Timing of self-preening and allopreening activities (a) and feeding, allofeeding and stealing activities (b) in experimental barn owl pairs. Average values per nestling and per hour are quoted ±SE.

The number of allopreening bouts given was positively correlated with the number of self-preening bouts performed by the allopreened individual (Table 2a; 0.08 ± 0.03). The number of allopreening bouts was not related to the allopreener sex, social treatment, coloration or the number of self-preening bouts (Table 2a).

Self-preening in experimental pairs

Nestlings preened themselves with longer bouts in the presence of a sibling than when alone ($17.5 \pm 0.$ 6 vs. 14.9 ± 0.5 s; Table 2b). The mean duration of self-preening bouts was not related to sex or nestling coloration (Table 2b). The number of self-preening bouts did not covary with social treatment, sex or coloration (Table 2b).

Nestlings performed more bouts of self-preening (1.87 ± 0.04 per hour) than allopreening (0.48 ± 0.02; $P < 0.0001$ in a Wilcoxon signed rank test on average values per individual), and self-preening bouts were also longer (17.53 ± 5.57 s vs. 8.30 ± 5.59 s; $P < 0.0001$ in a Wilcoxon signed rank test on average values per individual).

Food sharing, rank and melanism

Three-chick broods

In a previous paper, using the same sample of birds, Roulin *et al.* (2012) showed that seniors were more likely to feed siblings and that darker reddish seniors and middle-born nestlings gave more preys than did lighter individuals. Females and males did not significantly vary in the number of given items, but female nestlings started to feed nest mates earlier than did male nestlings.

Experimental pairs

We observed 33 events of allofeeding between siblings, in 21 of 62 nestlings, with a maximum of 4 items being shared per night (1, 2, 3 and 4 food items were given by 13, 2, 4 and 1 nestlings, respectively). In 45% of the experimental nights, owlets fed nest mates (71% in three-chick broods, Roulin *et al.*, 2012). In only one case, a nestling walked towards its sibling to give a food item from bill to bill, and in the 32 other cases, the allofeeder walked towards its sibling to release a prey item on the floor in front of its sibling.

The probability of giving at least one prey item (Table 3) was not significantly related to the sex of the receiver, the receiver coloration or the interactions between sex and coloration (P-values >0.05). The interaction between social treatment and coloration (Table 3) is explained by the fact that among seniors, the darker reddish individuals fed their sibling more often than did the paler individuals ([model 3 for seniors]: $F_{1,41} = 4.21$, $P = 0.046$, coloration of feeders: −4.06 ± 0.39; coloration of nonfeeders: −4.76 ± 0.14). In juniors, the probability of feeding a sibling was not significantly related to coloration ([model 3 for juniors]: $F_{1,41} = 0.72$, $P = 0.40$).

Reciprocity between food sharing and allopreening

Three-chick broods

Nestlings gave more prey items to a given sibling when they received at least one item from it (Table 4; $P = 0.026$; 1.2 ± 0.5; Fig. 4a). Receiving a prey item from an individual did not affect the number of prey items given to the other sibling (Table 4; −0.2 ± 0.5; Fig. 4b).

The number of items given was related to the interaction between the number of allopreening bouts received and the rank of the allopreener (Table 4; $P = 0.022$; Fig. 5). The significance of this interaction revealed that high-ranking nestlings receiving more prey items performed more allopreening bouts in return (Fig. 5; effect of number of allopreening bouts received, model 4 for seniors: $F_{1,20} = 4.38$, $P = 0.049$, 1.4 ± 0.6; middle-born: $F_{1,20} = 7.76$, $P = 0.011$, 2.0 ± 0.7; juniors: $F_{1,20} = 0.04$, $P = 0.85$, 0.0 ± 0.2). The number of prey items an individual gave to a sibling B was not correlated with the number and mean duration of allopreening events this individual received from the other nest mate C (Table 4; duration: 0.03 ± 0.03; number: −0.56 ± 0.42). The duration of allopreening received was not related to the number of prey items given (Table 4; −0.04 ± 0.04).

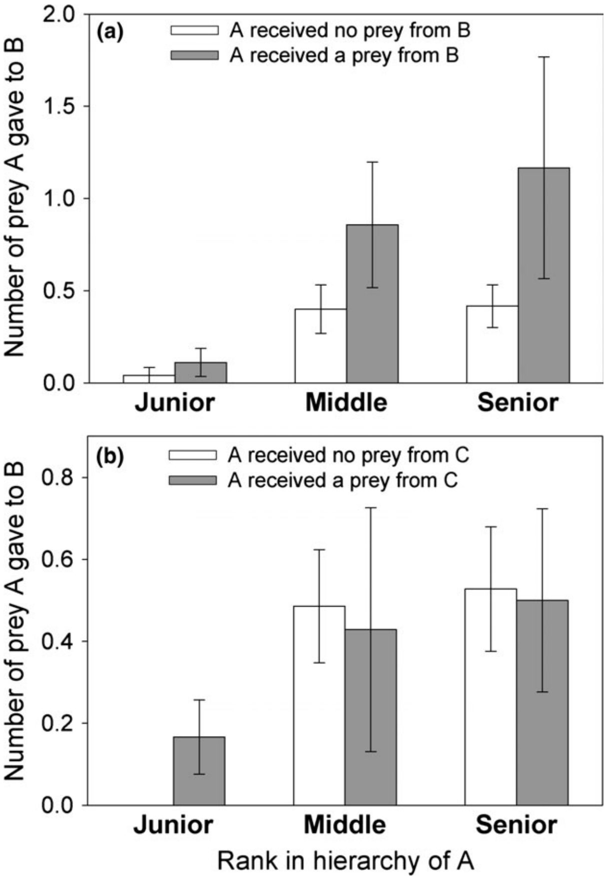

Fig. 4 Reciprocity of food gift in barn owls in three-chick broods. Number of preys given by nestling A (±SE) to B, (a) according to whether A received a prey from B or not and (b) according to whether A received a prey from C or not, for the three hierarchical ranks (junior, middle-born and senior).

Experimental pairs

The probability of sharing at least one food item during the night with a sibling was related to the duration of allopreening bouts received from this sibling in interaction with the period of night considered (Table 5; $P = 0.02$; Fig. 6), but not to the number of

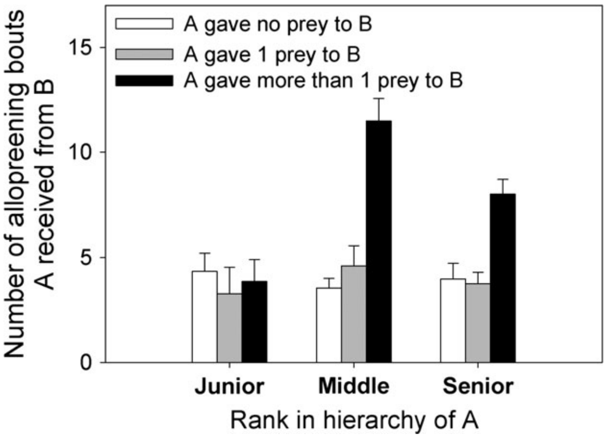

Fig. 5 Reciprocity between food gift and allopreening in barn owls in three-chick broods. Mean number of allopreening bouts received (±SE) in relation to the number of preys given.

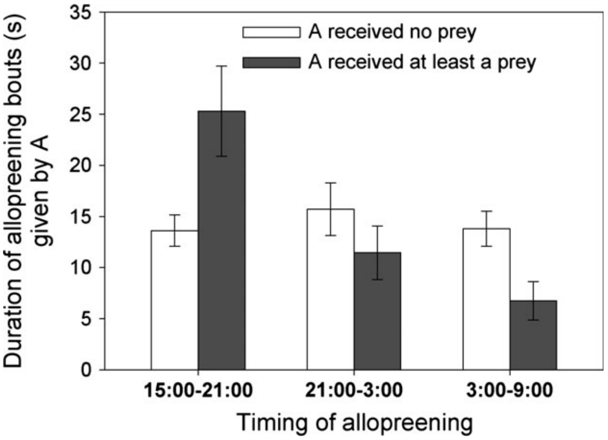

Fig. 6 Reciprocity between food gift and early allopreening in barn owls in experimental pairs. Duration of allopreening bouts (±SE) given by nestling barn owls at different periods of the night in relationship to whether the focal individual received a prey that night.

prey items received from this sibling (Table 5; −0.4 ± 0.3). The probability of sharing at least one prey item was associated with allopreening performed between 15 : 00 and 21 : 00 ($F_{2,55} = 6.74$, $P = 0.012$, 0.3 ± 0.1), but not with allopreening performed between 21 : 00 and 03:00 ($F_{2,55} = 0.45$, $P = 0.50$, 0.1 ± 0.1) or between 03:00 and 09:00 ($F_{2,55} = 2.26$, $P = 0.13$, −0.2 ± 0.2).

Food stealing

Three-chick broods

Senior nestlings stole more prey items when paler, whereas juniors and middle-born nestlings stole more prey items when darker reddish (Roulin *et al.*, 2012), but sex was not significantly related to the number of stolen items.

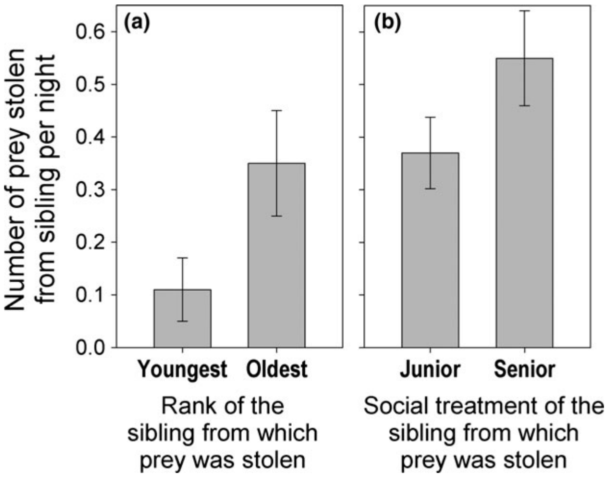

Fig. 7 Prey thefts and rank in age hierarchy. Number of thefts (±SE) per experimental night according to (a) the rank of the sibling being stolen in three-chick broods and (b) the social treatment of the sibling being stolen in the experimental pairs.

Furthermore, nestlings stole more prey items from the oldest sibling (Table 6; oldest vs. youngest: 1.1 ± 0.3; Fig. 7a) if the prey item came from the food stock (interaction between the origin of the prey and the rank of the sibling stolen from: $P = 0.029$), but not if it was directly received from a parent ($F_{1,69} = 1.11$, $P = 0.30$). Nestling rank did not influence stealing (Table 6, senior vs. junior: −0.0 ± 0.4, middle-born vs. junior: −0.2 ± 0.5). Stealing was not correlated with prosocial behaviours such as allopreening and food sharing (Table 6).

Experimental pairs

In 49% of the experimental nights, owlets stole from nest mates (81% in three-chick broods, Roulin *et al.*, 2012). Nestlings stole more often a prey item from a sibling when in junior rather than in senior position (Table 7a; junior vs. senior: 0.5 ± 0.2; Fig. 7b). Hence, nestlings increased the number of prey thefts when they changed from senior to junior position (Wilcoxon signed rank test for individuals that were alternatively junior and senior: $Z = -3.13$, $P = 0.0017$). As a corollary, 34 ± 6% of the prey eaten by juniors were taken out of the corridor by their senior siblings, whereas this value was 17 ± 5% for seniors.

Darker reddish nestlings stole more prey items than paler individuals (Table 7a; 0.3 ± 0.1; Fig. 8). The number of stolen items was related neither to the number and duration of allopreening bouts received nor to the number of items received (Table 7b).

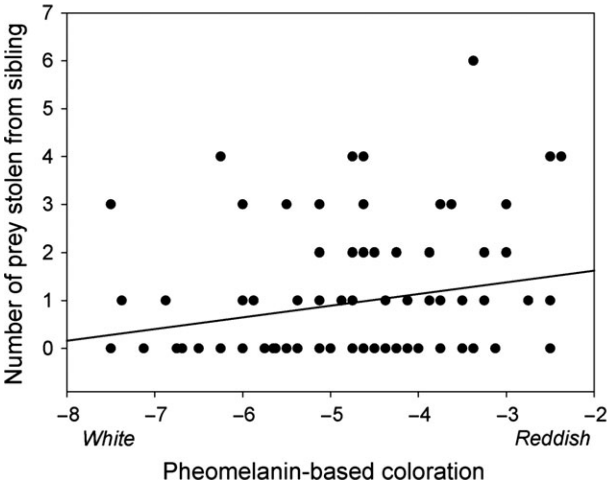

Fig. 8 Prey thefts and coloration in barn owls in experimental pairs. Total number of prey items stolen from sibling over two experimental nights according to the pheomelanin-based coloration of the nestling doing the stealing.

Discussion

Detailed observations in the barn owl showed that nestlings frequently feed and preen each other. Our results support the direct reciprocity hypothesis, as an individual was more likely to be fed or preened by a given sibling when it preened or fed this individual. We found no support for indirect reciprocity, as giving allopreening or food to a sibling did not affect the propensity to be fed or preened by another sibling. This suggests an ability to identify and potentially remember the actions of each sibling and in turn reciprocally direct the behaviour to the correct sibling. This is not surprising because owlets are confined in the same nest cavity for 2 months and hence have ample time to develop complex, repeated social relationships. Furthermore, nestlings are able to recognize their nest mates individually based on vocal cues (Dreiss *et al.*, 2013, 2014, 2015).

Reciprocation was performed with the same commodity (food for food or preening for preening) as well as with a different commodity (preening for food or food for preening). Two observations suggest that allopreening is likely to be a prosocial behaviour beneficial to the owlet recipient. First, received allopreening was positively correlated to self-preening, indicating that allopreened individuals are in need of preening. Second, the most allopreened body regions were those that individuals cannot preen by themselves.

Food sharing and allopreening were apparently not performed to prevent food stealing, as the behaviours were not correlated. A previous study also suggests that food sharing did not follow harassment in the form of begging by the receiver (Marti, 1989). Food stealing was solely related to rank in the within-brood age hierarchy, and experimentally manipulating rank induced a modification of nestlings' propensity to steal. Nestlings stole more often from their older sibling, whereas in experimental pairs, seniors collected prey items from the food stock rather than stealing them from their junior sibling. This suggests that in this context, seniors are producers and juniors scroungers (Barnard & Sibly, 1981).

The propensity to feed or allopreen a sibling was also linked to pheomelanin-based coloration and sex. However, we found mixed support for a 'green-beard' effect (Dawkins, 1976) because the effect of melanin coloration depended upon rank and sex and because the most prosocial phenotypes (darker reddish) were also the ones stealing more food. Darker seniors gave more prey items than paler ones (this study and Roulin *et al.*, 2012), and darker females received longer allopreening bouts than paler females (present study). Darker females may receive longer allopreening bouts in return for the food they gave. In experimental pairs, darker reddish females performed longer bouts of allopreening than paler ones at the end of the night. Darker reddish individuals were therefore more cooperative, but they also generally stole more prey items from their siblings. In three-chick broods, juniors and middle-born nestlings followed the same pattern, but in seniors, paler individuals stole more prey items than darker reddish ones (Roulin *et al.*, 2012).

In this nocturnal species, the proportion of nestlings that are not sired by the male that feeds them is low, with only six extra-pair young of 455 nestlings (1.3%; Henry *et al.*, 2013). This figure is an overestimation because for these extra-pair paternity analyses, we selected double-brooded pairs that have a higher likelihood of extra-pair paternity than single-brooded individuals. The high degree of relatedness between barn owl nest mates may have facilitated the evolution of frequent allopreening and allofeeding behaviours found in this species (Bühler & Epple, 1980; Masurat, 1980; Bunn *et al.*, 1982; Epple, 1985; Marti, 1989; Csermely & Agostini, 1993; Del Guasta, 1998). However, reciprocation may explain part of the variation in cooperative behaviour between barn owl siblings. In primates and some vampire bats, reciprocation has been suggested to play a major role even in unrelated individuals (Schino & Aureli, 2010; Carter & Wilkinson, 2013). Indeed, animals often match the time invested in preening/grooming each other (Stopka & Graciasova, 2001; Manson *et al.*, 2004; Lewis *et al.*, 2007; Adiseshan *et al.*, 2011; Gill, 2012), including young siblings (Mooring & Hart, 1997), and they can even exchange food for grooming (De Waal, 1997; Emery *et al.*, 2007).

The concept of 'biological markets' is useful for understanding the dynamics of reciprocity in cooperative behaviour. Animals face numerous constraints, and investment in one commodity is usually made at the expense of another commodity. The need for reciprocation most likely fluctuates over time and space because such a process is sensitive to the status and behaviour of each participant and to the availability of each commodity in the market, which itself depends on the supply and demand for a particular commodity (Nöe & Hammerstein, 1995). Given that the costs and benefits of investing in different activities differ between individuals (Schneeberger *et al.*, 2012), two scenarios can be proposed to explain the conditions under which conspecifics are expected to exchange the same or different commodities. Under the first scenario, obtaining a given commodity could be relatively cheap for one individual, making that individual potentially willing to exchange the first commodity for another one that is more costly to obtain. In the barn owl, seniors and darker reddish nestlings gave food items to their siblings more often than did juniors and pale-coloured nestlings (present study and Roulin *et al.*, 2012), whereas in experimental pairs, juniors allopreened their siblings for longer bouts than did seniors. Seniors are physically superior to their junior siblings and thus have priority access to food resources. Darker reddish nestlings may require less food to grow (Dreiss *et al.*, 2010), implying that for them, the cost of transferring food to siblings may be relatively low, potentially providing them with more opportunities to exchange food for allopreening than other individuals. Although barn owls reciprocate using different commodities, nestlings also matched the time invested in preening each other, and higher-ranked owlets invested in food sharing according to the number of prey they received. This is compatible with the hypothesis that obtaining a given commodity is relatively costly, making an individual willing to exchange that commodity for the same one. Two arguments support this hypothesis. First, allogrooming is likely to be costly because it can reduce resting time (Dunbar, 1992) and vigilance (Maestripieri, 1993). Second, nestlings preen siblings' body regions that are difficult or impossible to self-preen, such as the head, the back and the neck. Because allopreening may have a hygienic function by removing ectoparasites located on body regions that are difficult to self-preen (Akinyi *et al.*, 2013; Onishi *et al.*, 2013), individuals who are allopreened may have to reciprocally allopreen their sibling to ensure that cooperative allopreening continues. Under our observational conditions, the cost of food sharing was relatively low because food was *ad libitum* in experimental pairs and provided regularly by parents in three-chick broods (Roulin *et al.*, 2012). It would be interesting to investigate the pattern of food sharing and allopreening where their costs and benefits are increased (Schneeberger *et al.*, 2012), that is when food is scarce and nestlings are highly parasitized.

In the present discussion, we have so far envisioned two different sorts of indirect fitness benefits from allofeeding and allopreening siblings: inclusive fitness benefits from helping full siblings and reciprocation. From another perspective, an individual may derive immediate direct benefits rather than indirect benefits from an action directed towards a conspecific (Leimar & Hammerstein, 2010). In mammals and birds, allogrooming and allopreening have been shown to reduce social conflicts and to maintain social bonds. For instance, the levels of circulating corticosterone decreased with the intensity of allopreening received in ravens (*Corvus corax*) (Stoewe *et al.*, 2008) and given in Barbary macaques (Shutt *et al.*, 2007). In wood hoopoes (*Phoeniculus purpureus*), cooperative breeders increase allopreening frequency when they enter the territory of neighbours, where conflicts are more likely (Radford, 2011). By analogy, barn owl nestlings may allopreen and allofeed siblings to reduce the level of sibling competition. It is interesting to mention here that barn owl nestlings are also known to vocally negotiate among each other for priority access to food resources, a behaviour that reduces the level of sibling competition (Roulin, 2002). Reduction in sibling competition by allopreening may be particularly important in darker pheomelanic females, which allopreen their siblings during longer bouts than paler females. This finding suggests that darker and paler females are not equally sensitive to environmental and social factors, a finding that requires further study. If so, allopreening may act as a massage to decrease stress levels in both the groomee and the groomer.

In three-chick broods, owlets more often stole food from their older siblings. Moreover, an individual was more likely to steal food when in a junior rather than in a senior position in experimental pairs, showing that nestling barn owls altered their behaviour according to their rank in the within-brood age hierarchy. This emphasizes the importance of the hierarchical position in shaping nestling behaviour. Although senior siblings in a variety of species have been found to feed their younger siblings or to have food stolen from them (Steele & Hockey, 1995; Yip & Rayor, 2013), this finding is surprising in our laboratory conditions, as food was available *ad libitum* and easily attainable. Food was thus not stolen to save foraging costs. Alternatively, the elder individuals can represent a model for the younger individuals, as they may have access to more rewarding prey items under natural conditions. In some animal species, social learning is indeed an important way of acquiring information about food (Galef & Whiskin, 2001; Rapaport & Brown, 2008; Schwab *et al.*, 2008). Alternatively, giving food and letting siblings steal their meals might be a status signal by which older nestlings can assert their competitive level (Massen *et al.*, 2010).

The results of the two experiments were globally consistent but presented slight differences. Seniors more often fed their younger siblings in three-chick broods but not in experimental pairs. Reciprocation of food sharing was found in three-chick broods but not in experimental pairs. Food sharing was related to the number of allopreening bouts in three-chick broods and to the duration of allopreening in experimental pairs. In three-chick broods, juniors and middle-born nestlings stole more prey items when darker reddish than when paler, whereas the opposite was true for seniors. In experimental pairs, darker reddish nestlings stole more often, independently of their rank in the hierarchy. These differences may be explained by the fact that in experimental pairs, nestlings had no choice of partner or by the fact that three-chick broods interacted for both food stock (prey left in the nest) and food regularly brought by the parents. Several other factors, such as the amount of food and the nestling age, can explain these disparities. In any case, these discrepancies suggest that prosocial and stealing behaviours are context dependent.

Conclusion

The present study is of general interest for several reasons. First, extra-pair paternity is rare in the barn owl and nestlings sharing the same nest are thus full siblings. Therefore, allopreening and allofeeding may have both indirect benefits (kin selection) and direct benefits. These two pathways, which are usually considered separately to explain the evolution of cooperation, can actually work synergistically (Lehmann & Keller, 2006; McGlothlin *et al.*, 2014; Van Cleve & Akcay, 2014), with kin selection being necessary for initial selection for reciprocity (Axelrod & Hamilton, 1981). Second, studying two social behaviours revealed that we have to consider the full range of social behaviours to understand the evolution and stability of cooperation. Indeed, the expression of a given social behaviour was conditional on the expression of the other social behaviour, an effect that may depend on the degree of relatedness between socially interacting individuals (Lehmann & Keller, 2006; Van Cleve & Akcay, 2014). This is fundamental, as reported by Van Cleve & Akcay (2014): ‘natural selection rarely operates through a single pathway, and therefore the various causal components of social evolution need to be integrated and their commonalities and interactions explored’.

Links between social behaviours and coloration were detected in nestlings, and given that adult barn owls do not express complex social behaviours beyond reproductive activities, we must discuss the relevance of our results. First, data are required regarding whether nestlings can assess the coloration of their nest mates and, if so, whether they behave differently in front of a darker or paler sibling. Because it is difficult to manipulate the coloration of the entire body, experiments should be performed in which a focal nestling is placed with either a darker or a paler conspecific. Although coloration may not be clearly visible at night, during daylight hours, there is enough light for humans (and hence probably owls) to perceive slight variations in coloration. Second, the finding that darker females spend more time preening their offspring (Almasi *et al.*, 2013) suggests that coloration is associated not only with the tendency of nestlings to adopt cooperative behaviour but also with parental care. This raises the possibility that reddish coloration may be related to a number of behaviours inside the family. If so, coloration may be related to sibling competition at the nestling stage, and later on, the same trait may be used as a signal of the quality of parental care. Given that the genetic regulation of melanin-based coloration is conserved across vertebrates, including the pleiotropic effects of melanogenic genes (Ducrest *et al.*, 2008), our study indicates that melanin-based coloration may signal not only the extent to which animals are aggressive (Ducrest *et al.*, 2008) but also the extent to which they are cooperative. Because interindividual variation in melanin coloration is mainly explained by genetic factors (Roulin & Ducrest, 2013), this trait may be an honest signal of how individuals behave during social interactions, which could provide information about partner quality (Leimar, 1997).

Acknowledgments

We thank the Swiss National Science Foundation for funding to A.R (31003A-120517), and Robin Abbey-Lee, Laurent Lehmann and two anonymous reviewers for useful comments. The observations were performed under legal authorization of the ‘Service vétérinaire du canton de Vaud’.

References

Adiseshan, A., Adiseshan, T. & Isbell, L.A. 2011. Affiliative relationships and reciprocity among adult male bonnet macaques (*Macaca radiate*) at Arunachala Hill, India. *Am. J. Primatol.* **73**: 1107–1113.

Akinyi, M.Y., Tung, J., Jeneby, M., Patel, N.B., Altmann, J. & Alberts, S.C. 2013. Role of grooming in reducing tick load in wild baboons (*Papio cynocephalus*). *Anim. Behav.* **85**: 559–568.

Almasi, B., Roulin, A. & Jenni, L. 2013. Corticosterone shifts reproductive behaviour towards self-maintenance in the barn owl and is linked to melanin-based coloration in females. *Horm. Behav.* **64**: 161–171.

Axelrod, R. & Hamilton, W.D. 1981. The evolution of cooperation. *Science* **211**: 1390–1396.

Barnard, C.J. & Sibly, R.M. 1981. Producers and scroungers: a general model and its application to captive flocks of house sparrows. *Anim. Behav.* **29**: 543–550.

Blurton Jones, N. 1987. Tolerated theft, suggestions about the ecology and evolution of sharing, hoarding and scrounging. *Soc. Sci. Inform.* **26**: 31–55.

Breed, M.D. 2014. Kin and nestmate recognition: the influence of WD Hamilton on 50 years of research. *Anim. Behav.* **92**: 271–279.

Bshary, R. & Bergmueller, R. 2008. Distinguishing four fundamental approaches to the evolution of helping. *J. Evol. Biol.* **21**: 405–420.

Bühler, P. & Epple, W. 1980. Die Lautäußerungen der Schleiereule (*Tyto alba*). *J. Ornithol.* **121**: 36–70.

Bunn, D.S., Warburton, A.B. & Wilson, R.D.S. 1982. *The Barn owl*. Poyser, London.

Carter, G.G. & Wilkinson, G.S. 2013. Food sharing in vampire bats: reciprocal help predicts donations more than relatedness or harassment. *Proc. R. Soc. B Biol. Sci.* **280**: 20122573.

Clayton, D.H. & Cotgreave, P. 1994. Relationship of bill morphology to grooming behaviour in birds. *Anim. Behav.* **47**: 195–201.

Csermely, D. & Agostini, N. 1993. A note on the social behaviour of rehabilitating wild barn owls (*Tyto alba*). *Ornis Hungarica* **3**: 13–22.

Dawkins, R. 1976. *The Selfish Gene*. Oxford University Press, Oxford.

De Waal, F.B. 1997. The chimpanzee's service economy: food for grooming. *Evol. Hum. Behav.* **18**: 375–386.

Del Guasta, M. 1998. Videoregistrazione dell'attivita ad un nid di bargagianni *Tyto alba* nel Mugello (FI). *Picus* **24**: 85–91.

Dolivo, V. & Taborsky, M. 2015. Norway rats reciprocate help according to the quality of help they received. *Biol. Lett.* **11**: 20140959.

van Doorn, G.S. & Taborsky, M. 2012. The evolution of generalized reciprocity on social interaction networks. *Evolution* **66**: 651–664.

Dreiss, A.N. & Roulin, A. 2010. Age-related change in melanin-based coloration of Barn owls (*Tyto alba*): females that become more female-like and males that become more male-like perform better. *Biol. J. Linn. Soc.* **101**: 689–704.

Dreiss, A.N., Henry, I., Ruppli, C.A., Almasi, B. & Roulin, A. 2010. Darker eumelanic barn owls better withstand food depletion through resistance to food deprivation and lower appetite. *Oecologia* **164**: 65–71.

Dreiss, A.N., Ruppli, C.A., Faller, C. & Roulin, A. 2013. Big brother is watching you: eavesdropping to resolve family conflicts. *Behav. Ecol.* **24**: 717–722.

Dreiss, A.N., Ruppli, C.A. & Roulin, A. 2014. Individual vocal signatures in barn owl nestlings: does individual recognition have an adaptive role in sibling vocal competition? *J. Evol. Biol.* **27**: 63–75.

Dreiss, A.N., Ruppli, C.A., Antille, S. & Roulin, A. 2015. Information retention during competitive interactions: siblings need to constantly repeat vocal displays. *Evol. Biol.* **42**: 63–74.

Ducrest, A.L., Keller, L. & Roulin, A. 2008. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends Ecol. Evol.* **23**: 502–510.

Dunbar, R.I.M. 1992. Time: a hidden constraint on the behavioral ecology of baboons. *Behav. Ecol. Sociobiol.* **31**: 35–49.

Durant, J.M. & Handrich, Y. 1998. Growth and food requirement flexibility in captive chicks of the European barn owl (*Tyto alba*). *J. Zool.* **245**: 137–145.

El Mouden, C., West, S.A. & Gardner, A. 2010. The enforcement of cooperation by policing. *Evolution* **64**: 2139–2152.

Emery, N.J., Seed, A.M., Von Bayern, A.M.P. & Clayton, N.S. 2007. Cognitive adaptations of social bonding in birds. *Philos. Trans. R. Soc. B Biol. Sci.* **362**: 489–505.

Epple, W. 1985. Ethologische Anpassungen im Fortpflanzungssystem der Schleiereule (*Tyto alba* Scop., 1769). *Ökol. Vögel* **7**: 1–95.

Galef, B.G. & Whiskin, E.E. 2001. Interaction of social and individual learning in food preferences of Norway rats. *Anim. Behav.* **62**: 41–46.

Gill, S.A. 2012. Strategic use of allopreening in family-living wrens. *Behav. Ecol. Sociobiol.* **66**: 757–763.

Hamilton, W.D. 1964. The genetical evolution of social behaviour. *J. Theor. Biol.* **7**: 1–16.

Henry, I., Antoniazza, S., Dubey, S., Simon, C., Waldvogel, C., Burri, R. *et al.* 2013. Multiple paternity in polyandrous barn owls (*Tyto alba*). *PLoS ONE* **8**: e80112.

Kober, K. & Gaston, A.J. 2003. Social interactions among breeding Brunnich's guillemots *Uria lomvia* suggest constraints in relation to offspring vulnerability. *The Ibis* **145**: 413–418.

Lehmann, L. & Keller, L. 2006. The evolution of cooperation and altruism - a general framework and a classification of models. *J. Evol. Biol.* **19**: 1365–1376.

Leimar, O. 1997. Reciprocity and communication of partner quality. *Proc. R. Soc. B Biol. Sci.* **B264**: 1209–1215.

Leimar, O. & Hammerstein, P. 2010. Cooperation for direct fitness benefits. *Philos. Trans. R. Soc. B Biol. Sci.* **365**: 2619–2626.

Lewis, S., Roberts, G., Harris, M.P., Prigmore, C. & Wanless, S. 2007. Fitness increases with partner and neighbor allopreening. *Biol. Lett.* **3**: 386–389.

Maestripieri, D. 1993. Vigilance costs of allogrooming in macaque mothers. *Am. Nat.* **141**: 744–753.

Manson, J.H., Navarrete, D., Silk, J.B. & Perry, S. 2004. Time-matched grooming in female primates? New analyses from two species. *Anim. Behav.* **67**: 493–500.

Marti, C.D. 1989. Food sharing by sibling common barn owls. *Wilson Bull.* **101**: 132–134.

Massen, J.J.M., van den Berg, L.M., Spruijt, B.M. & Sterck, E.H.M. 2010. Generous leaders and selfish underdogs: prosociality in despotic macaques. *PLoS ONE* **5**: e9734.

Masurat, H. 1980. Beobachtungen zur Brutbiologie der Schleiereule (*Tyto alba*). *Vogelwelt* **101**: 175–182.

McGlothlin, J.W., Wolf, J.B., Brodie, E.D. III & Moore, A.J. 2014. Quantitative genetic versions of Hamilton's rule with empirical applications. *Philos. Trans. R. Soc. B Biol. Sci.* **369**: 20130358.

Mooring, M.S. & Hart, B.L. 1997. Reciprocal allogrooming in wild impala lambs. *Ethology* **103**: 665–680.

Nöe, R. & Hammerstein, P. 1995. Biological markets. *Trends Ecol. Evol.* **10**: 336–339.

Nowak, M.A. & Sigmund, K. 1998. Evolution of indirect reciprocity by image scoring. *Nature* **393**: 573–577.

Onishi, K., Yamada, J. & Nakamichi, M. 2013. Grooming-related feeding motivates macaques to groom and affects grooming reciprocity and episode duration in Japanese Macaques (*Macaca fuscata*). *Behav. Process.* **92**: 125–130.

Queller, D.C. 2011. Expanded social fitness and Hamilton's rule for kin, kith, and kind. *Proc. Natl Acad. Sci. USA* **108**: 10792–10799.

Py, I., Ducrest, A.L., Duvoisin, N., Fumagalli, L. & Roulin, A. 2006. Ultraviolet reflectance in a melanin-based plumage trait is heritable. *Evol. Ecol. Res.* **8**: 483–491.

Radford, A.N. 2011. Preparing for battle? Potential intergroup conflict promotes current intragroup affiliation. *Biol. Lett.* **7**: 26–29.

Raihani, N.J., Thornton, A. & Bshary, R. 2012. Punishment and cooperation in nature. *Trends Ecol. Evol.* **27**: 288–295.

Rapaport, L.G. & Brown, G.R. 2008. Social influences on foraging behavior in young nonhuman primates: learning what, where, and how to eat. *Evol. Anthropol.* **17**: 189–201.

Roulin, A. 2002. The sibling negotiation hypothesis. In: *The Evolution of Begging: Competition, Cooperation and Communication* (J. Wright & M.L. Leonard, eds), pp. 107–127. Kluwer Academic Press, Dordrecht.

Roulin, A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biol. Rev.* **79**: 815–848.

Roulin, A. & Dreiss, A.N. 2012. Sibling competition and cooperation over parental care. In: *The Evolution of Parental Care* (N.J. Royle, P.T. Smiseth & M. Kölliker, eds), pp. 133–149. Oxford University Press, Oxford.

Roulin, A. & Ducrest, A.L. 2013. Genetics of colouration in birds. *Semin. Cell Dev. Biol.* **24**: 594–608.

Roulin, A., Richner, H. & Ducrest, A.L. 1998. Genetic, environmental, and condition-dependent effects on female and male ornamentation in the barn owl *Tyto alba*. *Evolution* **52**: 1451–1460.

Roulin, A., Ducrest, A.L. & Dijkstra, C. 1999. Effect of brood size manipulations on parents and offspring in the barn owl *Tyto alba*. *Ardea* **87**: 91–100.

Roulin, A., Da Silva, A. & Ruppli, C.A. 2012. Dominant nestlings displaying female-like melanin coloration behave altruistically in the barn owl. *Anim. Behav.* **84**: 1229–1236.

Schino, G. & Aureli, F. 2010. The relative roles of kinship and reciprocity in explaining primate altruism. *Ecol. Lett.* **13**: 45–50.

Schneeberger, K., Dietz, M. & Taborsky, M. 2012. Reciprocal cooperation between unrelated rats depends on cost to donor and benefit to recipient. *BMC Evol. Biol.* **12**: 41.

Schwab, C., Bugnyar, T., Schloegl, C. & Kotrschal, K. 2008. Enhanced social learning between siblings in common ravens, *Corvus corax*. *Anim. Behav.* **75**: 501–508.

Shutt, K., MacLarnon, A., Heistermann, M. & Semple, S. 2007. Grooming in Barbary macaques: better to give than to receive? *Biol. Lett.* **3**: 231–233.

Sigmund, K. 2007. Punish or perish? Retaliation and collaboration among humans. *Trends Ecol. Evol.* **22**: 593–600.

Soares, M.C., Oliveira, R.F., Ros, A.F.H., Grutter, A.S. & Bshary, R. 2011. Tactile stimulation lowers stress in fish. *Nat. Commun.* **2**: 534.

Steele, W.K. & Hockey, P.A.R. 1995. Factors influencing rate and success of intraspecific kleptoparasitism among Kelp Gulls (*Larus dominicanus*). *Auk* **112**: 847–859.

Stoewe, M., Bugnyar, T., Schloegl, C., Heinrich, B., Kotrschal, K. & Moestl, E. 2008. Corticosterone excretion patterns and affiliative behavior over development in ravens (*Corvus corax*). *Horm. Behav.* **53**: 208–216.

Stopka, P. & Graciasova, R. 2001. Conditional allogrooming in the herb-field mouse. *Behav. Ecol.* **12**: 584–589.

Trivers, R.L. 1971. The evolution of reciprocal altruism. *Q. Rev. Biol.* **46**: 35–57.

Van Cleve, J. & Akcay, E. 2014. Pathways to social evolution: reciprocity, relatedness, and synergy. *Evolution* **68**: 2245–2258.

Yip, E.C. & Rayor, L.S. 2013. The influence of siblings on body condition in a social spider: is prey sharing cooperation or competition? *Anim. Behav.* **85**: 1161–1168.

Zahavi, A. 1990. Arabian babblers: the quest for social status in a cooperative breeder. In: *Cooperative Breeding in Birds: Longterm Studies of Ecology and Behavior* (P.B. Stacey & W.D. Koenig, eds), pp. 103–130. Cambridge University Press, Cambridge.

Received 9 November 2014; accepted 5 November 2015