

Begging and Parental Care in Relation to Offspring Need and Condition in the Barn Swallow (*Hirundo rustica*)

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ABSTRACT: Parents are selected to maximize their fitness by allocating care among their progeny in relation to the differential reproductive value of offspring. Nestlings have been hypothesized to signal need for parental care reliably through their begging behavior, but offspring condition as reflected by their reproductive value may likewise affect begging and hence provisioning. We assessed the relative importance of need and condition in determining begging behavior and feeding rate of nestling barn swallows (*Hirundo rustica*) through short-term starvation, a challenge to their immune system with a foreign antigen negatively affecting condition, and brood size manipulation. Food deprivation but not condition or brood size manipulation increased nestling begging rate. Parents fed offspring depending on both need and condition but only when feeding broods that were reduced or of normal size. In enlarged broods, offspring received less food per capita than in reduced broods, and parents did not discriminate among nestlings relative to their need or condition. Thus, nestlings signal their need by increased solicitation. Parents allocate food to offspring dependent on both need and condition, with these effects depending on parental workload as determined by experimental brood size.

Keywords: begging, health status, *Hirundo rustica*, parental care, signaling, starvation.

Reproduction and parental care entail individuals with costs in terms of survival or future reproduction (Lindén and Møller 1989; Partridge 1989; Clutton-Brock 1991;

Lemon 1991; Roff 1992; Stearns 1992; Saino et al. 1999a). A fundamental consequence of sexual reproduction is that parents share only half of their nuclear genetic material with their offspring, as does, on average, each offspring with any of its full siblings, paving the way for the occurrence of a conflict of interests between parents and their progeny, as well as among the offspring (Trivers 1974; Dawkins and Krebs 1979; MacNair and Parker 1979; Godfray 1991, 1995a). Individual offspring are selected to obtain larger parental investment than parents are selected to provide, and individual offspring are selected to obtain a larger share of parental investment than their siblings do. Evolutionary theory of parental investment therefore predicts that natural selection should have favored the evolution of the ability of parents to assess offspring quality, since different fitness rewards may result from allocation of the same amount of resources to offspring differing in quality. Offspring may reveal their needs because features of the phenotype (e.g., body mass) directly reflect their condition. Alternatively, parents may have to infer indirectly the condition through signals produced by the offspring. Parents are predicted to base their decision on signals reliably reflecting offspring reproductive value, more valuable nestlings presumably being those in better condition. In addition, parents may vary their decision about allocation of food according to its availability and offspring need of care (Haig 1990).

Models of offspring signals directed toward parents have attempted to resolve the question whether such signals should reliably reflect the need of offspring (Godfray 1995a, 1995b; reviews in Kilner and Johnstone 1997; Mock and Parker 1997). These models have analyzed this problem by assuming that signalers such as offspring produce a behavior to which another individual may respond. Thus, there is no temporal element in such models. Recently, McNamara et al. (1999) have shown that incorporation of responses into models of evolutionary games can drastically affect the evolutionarily stable strategy, that is, the evolutionarily stable negotiation rules. The reason for such changes in behavior is that negotiations between the chick and the parent and the outcome may differ from

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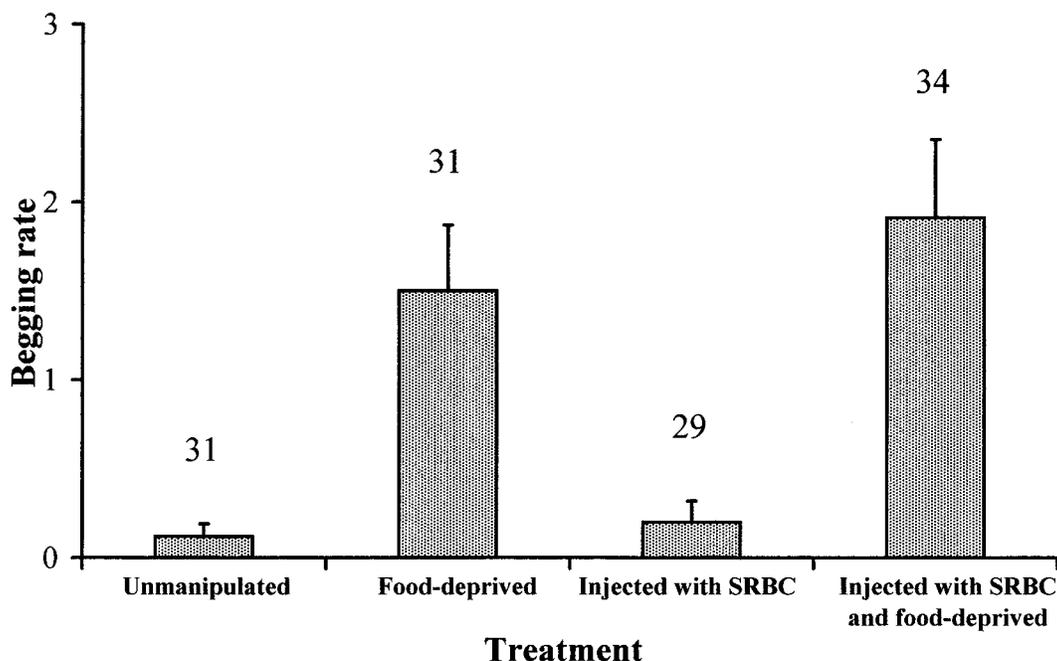


Figure 1: Begging rate (events per minute + SE bar) by unmanipulated nestlings, food-deprived nestlings, nestlings injected with sheep red blood cells (SRBC), and food-deprived nestlings injected with SRBC, in experiment 1. Food deprivation, but not SRBC treatment, significantly affected begging behavior. Sample sizes are the total number of nestlings within each group.

game theory models in which there is no possibility for response (McNamara et al. 1999).

Essential to any argument about parental investment and parent-offspring conflict over critical food resources is the distinction between offspring “need” and “condition.” Here, we define need as the amount of resources requested by an offspring to achieve satiation. This definition is consistent with previous studies where food-deprived nestlings were considered to be more “needy” than their siblings (e.g., Mondloch 1995; Price and Ydenberg 1995; Kilner 1997). However, need does not necessarily reflect “condition.” Individuals of a particular genotype living under given ecological conditions differ in physiological or immunological status, and only a certain status maximizes fitness. We define high “condition” as the status that maximizes fitness and deviations from this state as low “condition.” Thus, condition as used here reflects the inherent, long-term aspect of quality of an offspring such as general health. The difference between need and condition, as we just defined them, is exemplified by the fact that two fully satiated nestlings showing no need of food can still markedly differ in condition because, for example, they have different parasite loads because of differences in their genetic resistance to parasite infestation. Reciprocally, two individuals in the same condition may still have different needs owing, for example, to differences in metabolic efficiency, size, age, or

sexual dimorphism. In addition, satiation and condition vary on different timescales, since hungry nestlings may be well fed after a few feeding bouts, while their condition is unlikely to vary accordingly. This distinction between need and condition also has consequences for the effects on fitness of additional food provisioning in response to a signal of need and a signal of inherent condition. Signals of condition may reflect the quality of an offspring in terms of reproductive value, and parental responses to such signals may, therefore, have a dramatic effect on their own inclusive fitness. However, parental responses to signals of need are likely to have much less strong consequences for fitness. The negotiation between offspring signaler and the parent receiving the signal will depend on the foraging ability of the parent, and parents may work at rates beyond which they do not benefit from responding to offspring signals. The reason for this is that parental lifetime reproductive success to a large extent depends on parental longevity (Clutton-Brock 1988).

Previous studies of offspring signals have shown that offspring of several avian species display signals that have been interpreted as indicators of need of parental care (review by Kilner and Johnstone 1997). Thus, expression of “begging behavior” in birds (Kilner and Johnstone 1997) is enhanced when offspring are deprived of food (e.g., Redondo and Castro 1992; Mondloch 1995; Price

Table 1: Nested ANOVA of per capita feeding rates (number of feeds per nestling and hour) in experiment 1

	Mean square	F	df	P
Brood	28.21	15.17	40	<.001
Injection with SRBC(brood)	5.54	2.98	39	.02
Food deprivation(brood)	10.03	5.39	40	.002
[Injection with SRBC × food deprivation](brood)	7.39	3.97	27	.008

Note: In experiment 1, brood was entered as the main classification factor, and the effects of injection with sheep red blood cells (SRBC; yes or no), food deprivation (yes or no), and their interaction were nested within broods. Effect of factors preceding parentheses is nested within brood; 159 nestlings were considered in this analysis.

and Ydenberg 1995; Kilner and Johnstone 1997), and offspring who beg more usually receive more food than those begging less (Redondo and Castro 1992; Kilner 1995; Mondloch 1995). Passerine nestlings have bright yellow to red mouth colors, and mouth coloration directly affects allocation of food (Götmark and Ahlström 1997; Kilner 1997; Saino et al. 2000). Begging behavior has been considered a manipulative signal that offspring produce to increase their share of care or, alternatively, a signal reliably reflecting offspring need because of differential benefits of a given level of solicitation in relation to need (Godfray 1991, 1995a, 1995b; Kilner and Johnstone 1997). Since parental decisions on allocation of food may depend on offspring need and condition, the relative importance of these factors must be assessed.

Lotem (1998) analyzed begging behavior of barn swallow (*Hirundo rustica*) nestlings by altering both “short-term” and “long-term” determinants of need, that is, hunger as influenced by sib-sib competition and as indicated by body mass and size. Soon after hatching, nestlings in experimentally enlarged broods begged more intensely than nestlings from reduced broods, irrespective of their body condition, as did nestlings in poor condition from reduced broods. Nestlings from enlarged broods begged more than those in reduced broods later in the nestling period, particularly so if in good body condition (Lotem 1998). However, the distinction between short-term and long-term determinants of need in Lotem’s study differs from our definition of need and condition. In particular, we emphasized the distinction between current need and signals of long-term quality such a reproductive value of an offspring.

Here we present the results of three experiments designed to study the effect of condition and need of barn swallow nestlings on begging behavior and parental feeding effort. In the first experiment, the condition of nestlings was experimentally manipulated by injecting them with sheep red blood cells (SRBC), thereby mimicking temporary sickness, reflecting a reduction in reproductive value. While injection with SRBC will cause an increase in production of antibodies, this injection will not resem-

ble other aspects of parasitic infections, such as multiplication by the parasite and direct extraction of host resources by the parasite. Thus, this treatment only represents the reduction in offspring quality caused by the mounting of an immune response. Nestlings injected with SRBC were considered to be in poor condition because they have mounted a response to an experimental challenge to their immune system mimicking a parasitic infection (Roitt et al. 1996; Saino and Møller 1996; Saino et al. 1997a; N. Saino, unpublished data). Sheep red blood cell injection is known to affect other aspects of general condition negatively (Besedovsky et al. 1975; Saino et al. 2000; see also “Methods”). Thus, the SRBC treatment was intended to affect the rate of gain of fitness with increasing food dramatically but also to increase the marginal cost of signaling. Some nestlings of both SRBC-injected and noninjected groups from each brood were either starved for a short while or fed continuously by their parents, thus generating four groups of nestlings from the factorial design. Nestlings subject to food deprivation were considered to be more needy than their siblings that could continuously receive food. Needy offspring were considered to have a low marginal rate of gain of fitness with increasing food but also a low marginal cost of signaling.

The second experiment tested whether injection per se affected nestling begging and food provisioning. The third experiment tested whether differences in need and condition of nestlings depended on ability of parents to provision broods of different size. We therefore manipulated the inclusive fitness cost of offspring signaling by altering brood size, which was either reduced or enlarged by one nestling, thus simulating conditions of relatively large (reduced broods) or small (enlarged broods) availability of food to individual nestlings (Saino et al. 1997b).

More intense solicitation behavior by food-deprived nestlings was predicted for experiments 1 and 3 (not tested in experiment 2), and more intense solicitation behavior by nestlings in enlarged broods was predicted in experiment 3, since they received fewer feedings than those in reduced broods (Saino et al. 1997b). A larger per capita feeding rate to food-deprived nestlings was predicted in

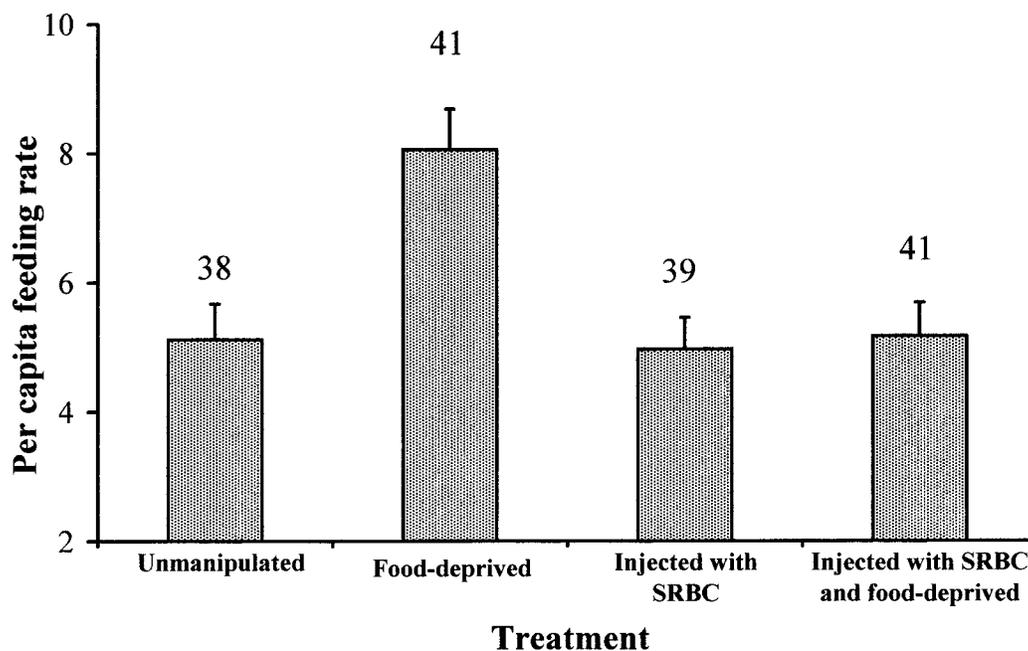


Figure 2: Feeding rates (feedings per hour + SE bar) to unmanipulated nestlings, food-deprived nestlings, nestlings injected with sheep red blood cells (SRBC), and food-deprived nestlings injected with SRBC, in experiment 1. Parents fed more frequently food-deprived nestlings only if they had not been injected with SRBC (see table 1). Sample sizes are the number of nestlings in each group.

all instances, except in the enlarged broods of experiment 3. If injection with SRBC reduces the apparent reproductive value of offspring and if food resources are limiting in experiment 1 and in the enlarged broods of experiment 3, we predicted that injected nestlings would be fed at a lower rate than noninjected ones and differentially so when food-deprived. SRBC-injected nestlings from reduced broods (experiment 3) were predicted to be fed at a relatively higher rate, particularly so when food deprived because of relatively large amount of food available per offspring. In enlarged broods, food resources available per capita were predicted to be so small that parents would allocate fewer feedings to food-deprived nestlings and particularly so when injected with SRBC because of the relatively low reproductive value of these nestlings.

Methods

Study Organism and General Field Procedures

The barn swallow is a small, semicolonial, socially monogamous, insectivorous passerine. Females lay two to seven eggs per clutch and incubate the eggs. Both parents provision offspring with food, males feeding the progeny at slightly lower rates than females (e.g., Møller 1994). Offspring beg for food from their parents while gaping,

jostling, giving typical calls, and opening their yellow to red gape.

The experiments were done in six colonies in cow stables near Milano (northern Italy) during spring 1997–1999. Adult barn swallows were captured in the breeding colonies and individually marked with color bands and markings on breast and belly feathers. The composition of each breeding pair was determined by observation of markings at the nest. Nests were inspected every second or third day to record breeding events and every day around the presumed hatching date.

Experiment 1

This experiment was designed to test the effects of experimental manipulation of nestling condition and satiation on begging behavior and parental feeding allocation in unmanipulated broods. Nestling condition was manipulated by injecting some with a suspension of SRBC, whereas level of satiation was manipulated by preventing some nestlings from receiving food from their parents for two 3-h periods. In 52 first broods, 6 d after hatching nestlings were individually marked. Half (or half the number of nestlings + 0.5 in the case of an odd brood size) were injected intraperitoneally with 3.5×10^7 cells in 30- μ L phosphate-buffered saline (PBS), whereas the others

Table 2: Nested ANOVA of per capita feeding rate (per hour) in experiment 2

	Mean square	F	df	P
Brood	22.52	10.47	23	<.001
Sham inoculation(brood)	2.06	.96	23	.56
Food deprivation(brood)	10.74	4.99	24	.008
[Sham inoculation × food deprivation](brood)	2.11	.98	18	.54

Note: In experiment 2, brood was entered as the main classification factor, and the effects of sham-inoculation with phosphate buffered saline (yes or no), food deprivation (yes or no), and their interaction were nested within brood. Effect of factors preceding parentheses is nested within brood; 98 nestlings were considered in this analysis.

were just handled and put back in the nest. Sheep red blood cell is a multigenic antigen commonly used to test the ability to raise a humoral immune response (e.g., Hudson and Hay 1980; Soulsby 1987; Lochmiller et al. 1991; Roitt et al. 1996), and it has also been shown to elicit an antibody response in the barn swallow (Saino and Møller 1996; Saino et al. 1997a; N. Saino, unpublished data). Inoculation of SRBC was assumed to mimic an infection by a foreign antigen because it elicits an immune response similar to that elicited by real pathogens (e.g., Hudson and Hay 1980; Soulsby 1987; Lochmiller et al. 1991; Roitt et al. 1996). The response to SRBC has been shown to include a stress response with dramatically elevated levels of plasma corticosteroids and catecholamines (Besedovsky et al. 1975). Previous studies on the barn swallow have shown that an injection with SRBC eliciting a humoral response (Saino et al. 1997b) can result in reduced ability of the nestlings to mount a T-cell-mediated cellular immune response (N. Saino, P. Ninni, M. Incagli, S. Calza, R. Sacchi, and A. P. Møller, unpublished data), and reduces the brightness of gape coloration (Saino et al. 2000). Hence, SRBC elicit an immune response by the nestlings and impairs other components of their general condition.

Nestlings who were due to be injected with SRBC were chosen randomly. During the morning of the sixth day after that of injection, one or two nestlings from groups of injected and noninjected individuals were prevented from receiving food from their parents by placing them for 3 h in part of the nest and covered with a thin metal net. This food-deprivation procedure was also repeated the following morning. Three hours after the start of food deprivation, the metal net was removed. Obviously, in broods with two or three nestlings, one or two experimental groups were not represented. It should be emphasized that many nestlings in our study population experience short periods of food deprivation when heavy showers or continuous rain prevent parents from foraging.

At the end of the first food deprivation, all nestlings in each brood were individually marked with small white, blue, or green patches of correction fluid on the forehead to allow individual recognition using binoculars from our

observation position a few meters away, and they were put back in a random position in the nest. Color was assigned randomly. Approximately 20 min after the end of the first food deprivation, we started observations of feeding rates to individual nestlings. We observed each nestling in 41 broods containing 159 nestlings for an average of 3.78 h (0.22 SE) and recorded on average 20.6 feedings per nestling (0.97 SE). In the broods for which we have collected data on feeding rates, there were 41 nestlings that had been food deprived, 41 nestlings that had been deprived of food and had been previously injected with SRBC, 39 nestlings injected with SRBC and not food deprived, and 38 nestlings in a control group of individuals that were neither injected or food deprived.

Immediately after the end of the second food deprivation, 31 broods were put in a swallow nest in a vantage position to record begging behavior. All nestlings were individually marked, and they were stimulated to beg by gently and intermittently touching all parts of the nest rim with a Y-shaped stiff metal wire while recording nestling behavior using a Sony Hi8 video camera. Mean duration of videotaping was 2.42 min (0.08 SE). Twenty minutes after nestlings were videotaped and put back in a random position in their original nest, we started a second session of observations. We videotaped begging behavior of 125 nestlings ($N = 31$ food-deprived nestlings, 34 food-deprived and injected with SRBC, 29 injected, and 31 controls).

Experiment 2

This experiment tested the effect of inoculation per se on feeding rates to individual nestlings. In 24 broods, we repeated all experimental procedures described for experiment 1, the only difference being that part of the nestlings were sham inoculated with 30 μ L of PBS instead of being injected with SRBC. Thus, we had four groups of nestlings: nestlings that had been food deprived for two periods of 3 h ($N = 24$); nestlings that had been food deprived and sham inoculated with PBS ($N = 29$); nestlings inoculated with PBS and not food deprived ($N = 21$); and a control group that was neither inoculated with PBS nor food de-

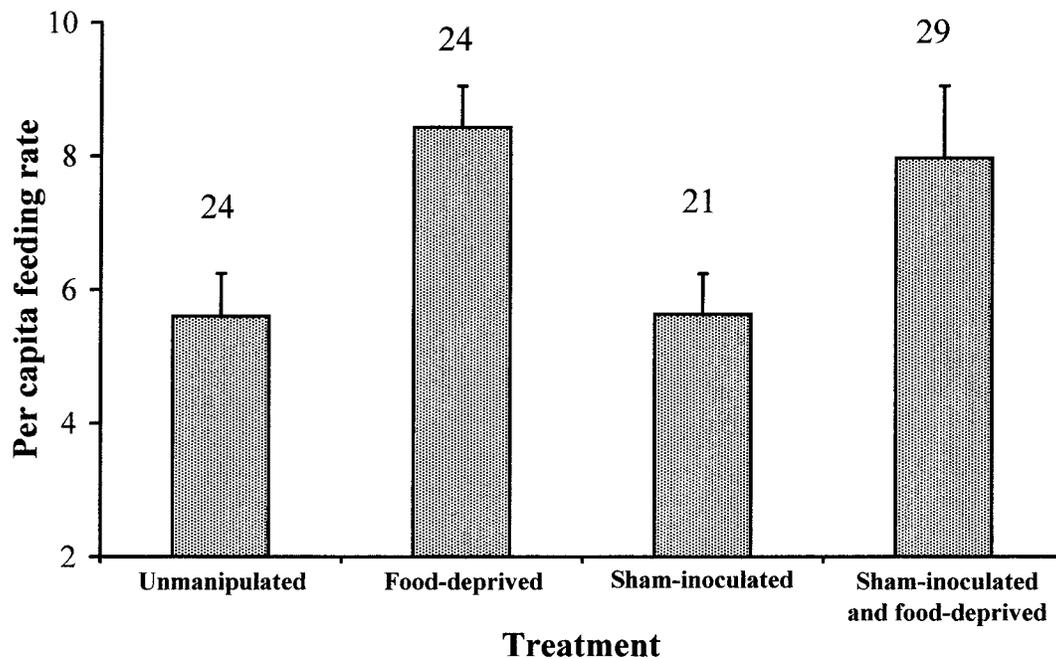


Figure 3: Feeding rates (feedings per hour + SE bar) to unmanipulated nestlings, food-deprived nestlings, nestlings sham-inoculated with phosphate buffered saline, and food-deprived sham-inoculated nestlings prevented from receiving food for two 3-h periods in consecutive days, in experiment 2. Food deprivation had a positive effect on per capita feeding rates (see table 2). Sample sizes are the number of nestlings in each group.

prived ($N = 24$). Hence, this experiment served to control for the effect of inoculation of PBS in which SRBC were diluted in experiments 1 and 3 on parental feeding allocation. An average of 23.4 (1.45 SE) feedings per nestling were recorded during 3.47 h (0.15 SE) of observation.

Experiment 3

We tested the effect of nestling need and condition on begging and feeding under different levels of food availability by means of brood size manipulation. Previous observations and brood size manipulation experiments have shown that the number of feedings per unit time per nestling declines with increasing brood size (Saino et al. 1997a). Brood size manipulation was therefore performed to simulate conditions in which food that could be allocated to individual nestlings was relatively abundant (reduced broods) or scarce (enlarged broods).

This experiment involved a total of 36 pairs of synchronous broods (“dyads” of broods). Within each dyad, we performed an unbalanced cross-fostering within 24 h of hatching so that the size of one randomly chosen brood of the dyad was reduced by one nestling while that of the other was increased by one. Before randomly chosen nestlings were cross-fostered, they were individually marked with color markings on hind limbs. The difference in pre-

manipulation brood size in a dyad was always less than or equal to three. The number of cross-fostered nestlings depended on premanipulation size of both broods in the dyad, according to a predetermined scheme designed to minimize the within-brood difference in the number of cross-fostered and resident nestlings.

Six days after hatching, randomly selected nestlings were injected with SRBC as in experiment 1, whereas all the other nestlings were injected with 30- μ L phosphate-buffered saline (PBS) as a control. Six days after injection, one or two randomly chosen nestlings from groups of SRBC-injected and sham-inoculated nestlings were subjected to a 3-h food-deprivation period as described for experiment 1. After the end of food deprivation, begging behavior was videotaped for 3 min as in experiment 1, and nestlings were then put back in a random position in their original nest. Twenty minutes later, we started observing parental feeding to nestlings that had been individually marked as in experiment 1. Since nestlings were assigned randomly to experimental groups, resident and cross-fostered nestlings were randomly distributed across the four groups. In the remainder of this article, we refer to “complete dyads” of nests as to those for which we have information on feeding rates or begging behavior for both broods belonging to that dyad.

Feeding rates to a total of 214 nestlings ($N = 57$ food-deprived and sham-inoculated nestlings, 73 food deprived

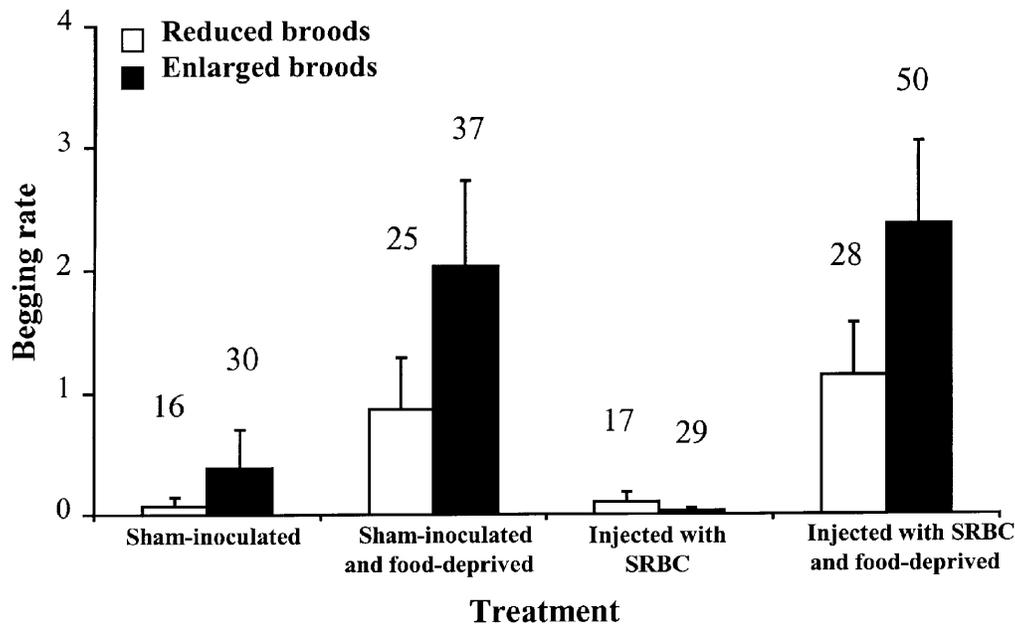


Figure 4: Begging rate (events per minute + SE bars) by sham-inoculated nestlings, sham-inoculated nestlings that were prevented from receiving food from their parents, nestlings injected with sheep red blood cells (SRBC), and injected nestlings prevented from receiving food, in broods whose size was altered (either reduced or enlarged) by one nestling immediately after hatching (experiment 3). Food deprivation, but not SRBC treatment, significantly affected begging behavior. Sample sizes are the total number of nestlings within each group.

and SRBC injected, 41 SRBC injected, and 43 sham inoculated) were recorded in 24 reduced and 27 enlarged broods. Begging behavior was recorded for 232 nestlings ($N = 62$ food-deprived and sham-inoculated nestlings, 78 food deprived and SRBC injected, 46 SRBC injected, and 46 sham inoculated) in 56 broods.

Feeding rate to each nestling (per capita feeding rate) was expressed as the total number of feedings received from either parents per hour. We could clearly observe the assignment of feeds to nestlings because in swallows only a single nestling is fed with a single, large food bolus during each visit. Since the large bolus requires extensive swallowing movements by the nestlings, its identity could readily be ascertained. In a previous study (N. Saino et al., unpublished data), we made observations of nestlings being fed using two observers on a number of occasions. More than 96% of observed cases ($N = 31$) of food delivery were recorded similarly by the two observers. These observations suggest that the assignment of food delivery to individual nestlings was reliable. Frequency of begging was expressed as the number of begging events by individual nestlings per minute (i.e., the number of times that a nestling responded to our stimulation by gaping). When more than one nestling in an experimental group was present in a brood, we used mean begging rate calculated across siblings belonging to that group.

In 13 randomly chosen broods, we recorded begging rate of one randomly chosen nestling immediately after food deprivation and 2 h later by the same procedure described above. Begging rate was significantly repeatable for individual nestlings at different delays after food deprivation (ANOVA: $F = 8.69$, $df = 12, 13$, $P < .001$, repeatability according to Falconer [1989]: $R = 0.63$), indicating that begging rate measured immediately after the food-deprivation period reliably reflected begging rate during recordings of per capita feeding rates.

Statistical Procedures

We used nonparametric Wilcoxon matched-pairs signed-ranks test to compare begging rates between groups within broods in experiments 1 and 3 because of a skewed distribution of begging rates within treatments.

We used a nested ANOVA to test for effects of treatments in experiments 1–3. In the analyses, brood was entered as the main classification factor, while the effects of injection with SRBC, food deprivation, and their interaction were nested within brood, to test for the effect of need and condition on feeding rates. The effect of food deprivation and injection with SRBC on feeding rates in experiment 3 was analyzed separately for reduced and enlarged broods in a nested ANOVA. This allowed us also to include broods

Table 3: Nested ANOVA of per capita feeding rate (per hour) in experiment 3

	Mean square	F	df	P
Reduced broods:				
Brood	31.71	25.03	23	.01
Injection with SRBC(brood)	19.36	15.29	24	.02
Food deprivation(brood)	12.58	9.93	18	.04
[Injection with SRBC × food deprivation](brood)	18.67	16.73	12	.02
Enlarged broods:				
Brood	32.07	6.97	26	<.001
Injection with SRBC(brood)	5.91	1.28	27	.25
Food deprivation(brood)	6.58	1.43	26	.17
[Injection with SRBC × food deprivation](brood)	3.46	.75	23	.76

Note: In experiment 3, brood was entered as the main classification factor, and the effects of injection with SRBC (yes or no), food deprivation (yes or no), and their interaction were nested within brood. Separate analyses were made for enlarged and reduced broods. The effect of factors preceding parentheses is nested within brood. The number of nestlings was 81 for reduced and 133 for enlarged broods. SRBC = sheep red blood cells.

in “incomplete” dyads. Finally, we used nested ANOVA to test for an effect of food deprivation, injection with SRBC and brood size manipulation on per capita feeding rate for the 20 “complete” dyads, in which the term dyad was entered as the nesting factor, and the effects of SRBC, food deprivation, brood size manipulation were nested within that of dyad.

Results

Experiment 1

Food deprivation had a significant effect on begging (fig. 1). Both groups of food-deprived or food-deprived and SRBC-injected nestlings begged at a much larger frequency than their nondeprived siblings (fig. 1; food-deprived vs. nondeprived siblings: $z = 3.99$, $N = 31$, $P < .001$; food-deprived and SRBC-injected vs. nondeprived and SRBC-injected siblings: $z = 3.48$, $N = 24$, $P < .005$). However, SRBC injection did not significantly affect begging rate of food-deprived or nondeprived nestlings (fig. 1; all $P > .10$).

Food-deprived nestlings were fed significantly more frequently than non-food-deprived ones (table 1; fig. 2). Parents fed SRBC-injected offspring significantly less frequently than noninjected siblings (table 1; fig. 2). However, feeding rate to food-deprived nestlings was relatively larger for nestlings that had not been treated with SRBC than for their injected siblings, as indicated by the significant interaction between SRBC treatment and food deprivation (table 1; fig. 2). Indeed, feeding rate of food-deprived nestlings injected with SRBC was very similar to feeding rates of non-food-deprived nestlings, independent of antigen inoculation, whereas feeding rate of food-deprived nestlings not injected with SRBC was approximately 1.6 times larger than that of the other three groups (fig. 2). Hence, parents fed food-deprived nestlings more frequently than

nondeprived ones but only if they had not been injected with SRBC.

Experiment 2

Food-deprived nestlings were fed at a higher rate than their non-food-deprived nest mates (table 2; fig. 3). However, the effects of sham inoculation and its interaction with food deprivation were not statistically significant (table 2; fig. 3), indicating that injection per se did not have an effect on feeding allocation among nestlings.

Experiment 3

Food deprivation markedly increased begging, as in experiment 1. Begging rate of food-deprived nestlings was larger than that of nestlings that could continuously receive food in both reduced and enlarged broods (fig. 4; reduced broods, food-deprived nestlings: $z = 2.52$, $N = 14$, $P < .05$; reduced broods, food-deprived and SRBC-injected nestlings: $z = 2.93$, $N = 17$, $P < .05$; enlarged broods, food-deprived nestlings: $z = 3.18$, $N = 27$, $P < .01$; enlarged broods, food-deprived and SRBC-injected nestlings: $z = 3.40$, $N = 26$, $P < .01$). Begging rate of SRBC-injected nestlings did not differ from that of sham-inoculated ones within food-deprivation treatment, and this result consistently emerged from analyses of both reduced and enlarged broods (fig. 4; reduced broods—non-food-deprived nestlings: $z = 0.45$, $N = 13$; food-deprived nestlings: $z = 1.11$, $N = 25$; enlarged broods—non-food-deprived nestlings: $z = 1.34$, $N = 26$; food-deprived nestlings: $z = 1.38$, $N = 29$; all tests, not significant). Nestlings in enlarged broods did not beg at a higher rate than those in reduced broods. This was indicated by paired comparison of begging rates between corresponding food-deprivation and SRBC treatment groups in reduced and enlarged

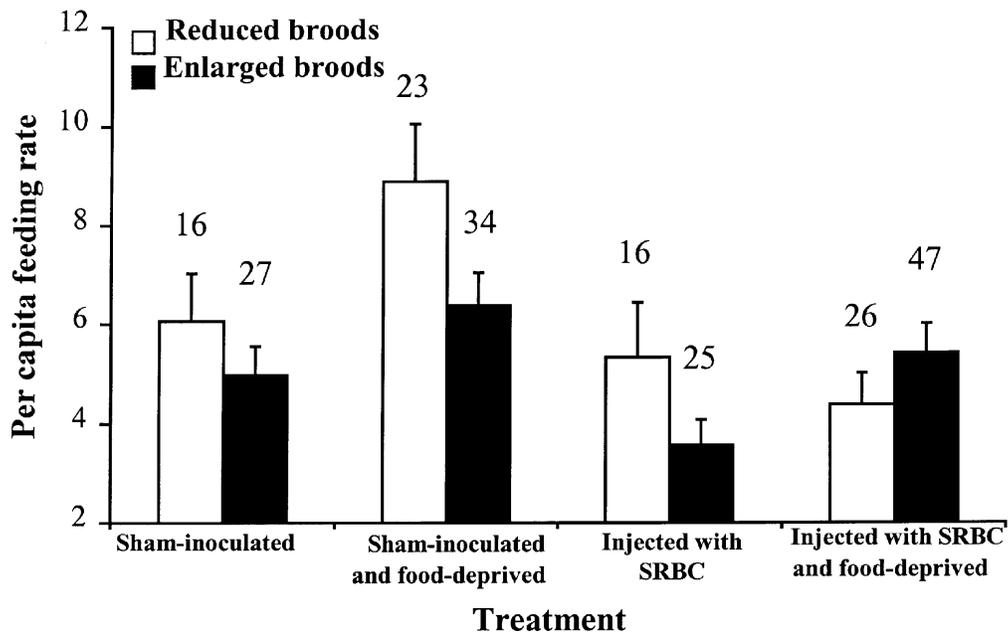


Figure 5: Feeding rates (feedings per hour) to sham-inoculated nestlings, sham-inoculated nestlings that were prevented from receiving food for their parents, nestlings injected with sheep red blood cells (SRBC), and food-deprived and SRBC-injected nestlings, in broods whose size was altered (either reduced or enlarged) by one nestling after hatching (experiment 3). In reduced broods, parents fed more frequently food-deprived nestlings only if they had not been injected with SRBC (see table 3). Sample sizes are the number of nestlings within each group.

broods within “complete dyads” (see “Methods”; Wilcoxon tests; z values always associated with $P > .05$ after Bonferroni correction for four simultaneous tests). In conclusion, food deprivation but not SRBC treatment or brood size manipulation significantly affected begging rates, with food-deprived nestlings begging more than continuously fed ones.

In reduced broods, food-deprived nestlings were fed more than nondeprived ones and SRBC-injected nestlings were fed less than sham-inoculated nestlings (table 3; fig. 5). The effect of food deprivation depended on SRBC treatment, as indicated by the significant interaction (table 3; fig. 5). The positive effect of food deprivation on per capita feeding rate existed only for sham-inoculated nestlings. Indeed, sham-inoculated, food-deprived nestlings received approximately 50% more than sham-inoculated, non-food-deprived ones, while SRBC-injected, food-deprived nestlings received 19% less than SRBC-injected, non-food-deprived ones. These results are therefore consistent with those of experiment 1.

In enlarged broods, effects of food deprivation and injection with SRBC were less marked than in reduced broods, and none of the main effects or the interaction was statistically significant (table 3; fig. 5). In the 20 “complete dyads” for which feeding rates of both broods were recorded, brood

size enlargement significantly reduced per capita feeding rate (effect of dyad: $F = 5.64$, $df = 19, 127$, $P < .001$; effect of brood size manipulation nested within dyad: $F = 1.84$, $df = 20, 127$, $P < .05$).

Brood size manipulation and SRBC treatment had a highly significant effect on per capita feeding rate whereas that of food deprivation did not reach significance ($P = .055$; table 4). The effect of SRBC treatment depended on brood size manipulation, as indicated by the significant interaction (table 4). The difference in per capita feeding rates between sham-inoculated and SRBC-injected nestlings was larger in reduced than in enlarged broods (reduced broods: 3.31 feedings per hour; enlarged broods: 0.92).

Discussion

Food-deprived barn swallow nestlings begged at a higher rate than non-food-deprived ones, as we had predicted. This is consistent with previous studies on birds showing that food-deprived offspring solicit more intensely than better fed ones by increased begging (reviewed in Kilner and Johnstone 1997). In addition, we showed that condition, as affected by inoculation of an antigen, did not affect begging rate. Begging display by barn swallow nest-

Table 4: Nested ANOVA of per capita feeding rate (per hour) in experiment 3

	Mean square	F	df	P
Dyad	46.95	10.28	19	<.001
Brood size manipulation(dyad)	13.94	3.05	20	.004
Injection with SRBC(dyad)	16.11	3.53	20	.001
Food deprivation(dyad)	8.81	1.93	20	.055
[Brood size manipulation × injection with SRBC](dyad)	13.54	2.96	20	.005
[Brood size manipulation × food deprivation](dyad)	5.72	1.25	14	.29
[Injection with SRBC × food deprivation](dyad)	6.85	1.50	18	.17
[Brood size manipulation × injection with SRBC × food deprivation](dyad)	5.17	1.13	8	.37

Note: In experiment 3, dyad was entered as the main classification factor, and the effects of injection with SRBC (yes or no), food deprivation (yes or no), brood size manipulation (reduction or enlargement), and their interaction terms were nested within dyad. Effect of factors preceding parentheses is nested within dyad. This analysis was run on the 20 dyads of broods for which feeding rates per capita were recorded in both broods in each dyad. The number of nestlings was 167. SRBC = sheep red blood cells.

lings should thus be interpreted as a signal of need rather than condition (see the introductory paragraphs), although the possibility exists that the effect of injection with SRBC on begging behavior changed, for example, at a later time. We observed no statistically significant difference in begging rate between nestlings of enlarged versus reduced broods within the antigen injection by food-deprivation treatments. Begging rate of food-deprived nestlings, independent of SRBC treatment, was approximately two times larger in enlarged than in reduced broods (fig. 4), and the difference between food deprived and SRBC-injected nestlings between the two groups of broods did not reach significance. These findings are therefore only partly consistent with those of Lotem (1998), who showed that nestlings in enlarged broods begged at higher intensity than those in reduced broods, presumably because they had received less food (see Saino et al. 1997b). Begging intensity was influenced by nestling "long-term" condition (Lotem 1998), while begging rate was not influenced by SRBC treatment in this study. However, nestling condition was manipulated differently, and the methods used to measure begging behavior differed between this present and Lotem's (1998) study, suggesting that results should be compared with caution.

Parents differentially allocated feeding effort in relation to the condition and need of their offspring in experiment 1. Feeding rate of food-deprived nestlings in relatively good condition was larger than that of control nestlings. However, feeding rate of food-deprived nestlings was larger than that of nestlings that were allowed to receive food continuously only if the former had not been injected with SRBC. This indicates that feeding rate of nestlings that had experienced the same level of food deprivation depended on their condition. That was the case despite the fact that food deprived nestlings in poor condition

begged at the same rate as their food deprived siblings in good condition (fig. 1).

In the second experiment, we showed that food-deprived nestlings were fed at higher rates than nondeprived ones. Moreover, this experiment provided evidence that the innocuous substance (PBS) used to dissolve SRBC did not influence feeding rates. This suggests that injection with SRBC and not the PBS was the determinant of differential feeding rate to SRBC-injected versus noninjected nestlings in experiment 1 and in reduced broods of experiment 3. Taken together, these results indicate that parent barn swallows base their decisions about food allocation on both offspring need and condition (see Christe et al. 1996) and that they assess offspring condition independently of begging rate.

Results from experiment 3 partly contradicted our expectations. The pattern of parental feeding in reduced broods was similar to that observed in broods with unmanipulated size, with food-deprived nestlings being fed more than non-food-deprived ones but particularly so when not treated with SRBC. As predicted, per capita feeding rates were larger in reduced compared to enlarged broods, but feeding allocation in enlarged broods was not significantly affected by need or condition. Hence, under conditions of severe food limitation, parents were less discriminating among their progeny, contrary to our expectations. The effect of injection with SRBC on parental decisions was dependent on brood size manipulation while controlling for the effect of other factors, indicating that nestlings in good condition were fed more when in a reduced compared to an enlarged brood. The results of experiment 3 suggest that when food availability is more limiting, as in enlarged broods, parents allocate feedings more evenly among their offspring than in conditions where food per offspring is relatively more abundant. A

possible a posteriori interpretation of this result is that parental decisions on food allocation are a two-step process. Parents first allocate a certain minimum level of feeding effort to all their offspring, independent of need and condition, to ensure survival, and differentially allocate only resources exceeding that level in relation to offspring need and condition. In our study population, this interpretation is corroborated by the fact that mortality of nestlings is very low (N. Saino, unpublished data). Second, since adult survival is the main determinant of lifetime reproductive success (Clutton-Brock 1988), barn swallows attending enlarged broods may not satiate such broods at the expense of their own survival. Third, parents may be limited by time available to assess offspring need and condition when faced with an enlarged brood and thus be unable to adaptively allocate limiting food resources. A general finding from experiments 1 and 3 is that parents base their parental decisions on signals of need but also assess offspring condition using clues different from the frequency of solicitation behavior. Indeed, begging rate was not affected by SRBC treatment, but parents allocated relatively more feedings to offspring that had not been injected with SRBC.

Signals of offspring need and condition are expected to have evolved under the selective pressure on parents for parental strategies that maximize fitness. The nature of the signals that allow parent barn swallows to allocate parental care based on offspring condition is only partly known (Saino et al. 2000). We can speculate that the quality of begging behavior in terms of loudness or fundamental frequency of calls associated with begging display may reflect offspring condition. Furthermore, barn swallow nestlings, like nestlings of many passerine species (e.g., Harrison 1985), have a brightly yellow to red mouth color directed toward the parents when nestlings beg for food. We have recently shown that injection of nestlings with SRBC results in gape coloration becoming dull (Saino et al. 2000) and that lutein, the predominant carotenoid pigment in barn swallow plasma (Saino et al. 1999b), partly determines gape coloration (Saino et al. 2000). Barn swallow nestlings with a gape experimentally painted red have been shown to receive more feedings than controls (Saino et al. 2000). Saturation or hue of mouth color is therefore affected by condition thus providing parents with a signal of offspring condition. Birds infested by a variety of parasites generally display less brightly colored skin and feathers than uninfested individuals (e.g., Bletner et al. 1966; Kowalski and Reid 1970; Marusich et al. 1972; see also Zuk et al. 1990; Lozano 1994; Shykoff and Widmer 1996). However, the mechanisms mediating this effect of parasitism on coloration are not clear. A likely explanation is that parasitism affects the accumulation of carotenoids responsible for red and yellow integument and mouth col-

oration (e.g., Fox 1976, 1979; Brush 1978; Goodwin 1984; Hill et al. 1994; Stradi et al. 1995; Møller et al. 2000). Carotenoids may be available to hosts in limiting amounts because parasites sequester these substances, which are effective in quenching singlet oxygen and other oxidative compounds released by phagocytic cells and heterophils to destroy the parasite during the respiratory burst (Tizard 1991; Pastoret et al. 1998). Carotenoids are important factors regulating and stimulating host immune functions (Bendich 1989; Chew 1993; Jyonouchi et al. 1994; review in Møller et al. 2000), and they act as efficient free oxygen radicals scavengers and anticarcinogens (Ames 1983; Krinsky 1989; Canfield et al. 1992; Olson 1993; review in Møller et al. 2000). Hence, parasitism may influence pigmentation of mouth tissues since it provokes the host to allocate carotenoids to immunological function, reducing the quantity of pigments stored in peripheral tissues. However, parents are expected to rely on signals of need and condition only if a mechanism prevents offspring from unreliably signaling their quality (Grafen 1990; Godfray 1991, 1995a, 1995b). Honesty of signals might be enforced if a given level of signaling entails nestlings in relatively good condition with smaller costs than nestlings in poor condition. Alternatively, nestlings in poor condition may benefit differentially more than those in good condition from a given level of signaling, as assumed by some models of honest signaling of need by offspring (e.g., Godfray 1991).

The main general conclusion we draw from the results of this study is that need and condition may have different effects on solicitation behavior of offspring. Decisions of adult birds on optimal parental strategies may thus depend on independent and combined assessment of offspring need and condition, and it may vary in relation to the amount of food available to individual offspring as demonstrated by brood size manipulations.

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