

Nest size predicts the effect of food supplementation to magpie nestlings on their immunocompetence: an experimental test of nest size indicating parental ability

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Post-mating sexually selected signals are expected to indicate parental quality. The good parent model assumes that expression of the sexual character positively reflects parental ability, resulting in a potential link between the exaggeration of the character and nestling-fitness traits. We tested this prediction in a population of a monogamous passerine, the magpie (*Pica pica*), for which nest size is known to act as a post-mating sexually selected signal. We provided a food supplement to half of the magpie nestlings in each nest, keeping the other half as control nestlings. We found that food-supplemented nestlings experienced a significantly higher T-cell-mediated immune response and a tendency to an increased condition index. In accordance with the good parent model, we found that nest size was positively related to T-cell mediated immune response for control magpie, whereas this relationship was nonexistent in food-supplemented nestlings. In addition, the difference in T-cell mediated immune response between food-supplemented and control nestlings of the same nest was principally explained by nest size. Based on our results, we discuss that magpie pairs with large nests provided their nestlings with higher quality food as compared to pairs with smaller nests, nest size thereby being an indicator of parental ability. To our knowledge, this is the first study showing a link between a post-mating sexually selected signal and nestling immunocompetence, a trait closely related to fitness in birds. *Key words*: immune response, magpie, parental care, sexual selection. [*Behav Ecol* 15:1031–1036 (2004)]

A wide diversity of cues in the animal kingdom has evolved to signal parental and territory quality, of which many became subject to sexual selection and are used by females as reliable signals of male quality for mate choice. Females choosing high quality males often obtain resources (e.g., parental care, 'good' genes) that are translated into increased offspring quality (e.g., survival), directly or indirectly by differential maternal investment in reproduction (Cunningham and Russell, 2000; Gil et al., 1999; Linville et al., 1998; Møller, 2000; Mousseau and Fox, 1998; Sheldon, 1997). In several bird species, one of these sexual displays evolved in males to attract females is nest size and/or nest-building behavior (Evans, 1997b; Friedl and Klump, 2000; Hoi et al., 1994, 1996; Lens et al., 1994; Soler et al., 1998b). Nest building may indicate genetic quality and/or experience but is also believed to be costly (in terms of energy expenditure and predation risk). Therefore, females could benefit from mating with good nest-building males because only individuals in good condition would be able to build exaggerated nests (Evans, 1997a; Hansell, 2000; Zahavi, 1987). Nest size or nest-building behavior could also indicate parental quality, providing direct benefits for offspring through good parental care (Soler et al., 1998b).

Fledgling condition and the ability to withstand pathogens play a main role for survival during the critical post-fledgling

period in birds (Christe et al., 2001; Hōrak et al., 1999; Stark and Ricklefs, 1998) and essentially depend on the amount and quality of food brought by the parents to the nest (Palomino et al., 1998; Saino et al., 1997; Soler et al., 1996, 1998a, 2001; Stark and Ricklefs, 1998).

Two hypotheses address the relationship between the degree of sexual characters and parental care. The good parent model assumes that expression of the sexual character positively reflects parental ability (e.g., Kirkpatrick, 1985), whereas the differential allocation model assumes that females choose mates for indirect benefits ('good' genes for offspring) and predicts a negative relationship between sexual characters and parental care (Burley, 1986). Empirical support has been found for both hypotheses in several non-monogamous species (e.g., de Lope and Møller, 1993; Linville et al., 1998; Palokangas et al., 1994; Sundberg and Larsson, 1994). However, a number of studies have suggested that in some monogamous species sexually selected traits, like nest building, may act as post-mating sexually selected signals, allowing females to assess males' willingness to invest in parental care and then adjust their reproductive investment accordingly (Soler et al., 1998b). In these cases, sexually selected traits would reliably signal parental quality and should be favored by the good parent process. Thus, because of the fundamental importance of parental care for nestling development, a direct relationship between nestling-fitness traits and males' sexually selected signals related to parental quality can be expected.

We tested this relationship in a population of a monogamous passerine, the magpie (*Pica pica*), from which we demonstrated in previous studies that nest size is a post-mating sexually selected signal used by the female to adjust

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her reproductive investment (i.e., clutch size) (De Neve and Soler, 2002; Soler et al., 2001). In addition, adult great spotted cuckoos (*Clamator glandarius*) use magpie nest size to select nests for brood parasitism (Soler et al., 1995).

Here, we use a food supplementation experiment, providing to half of the magpie nestlings in each nest a high-calorie paste, keeping the other half as control nestlings. Our food supplementation experiment should affect nestling development, and experimental nestlings should experience better condition and/or immunocompetence as compared to control nestlings. Because magpie pairs building large nests would be of better parental quality and provide their nestlings with good quality food (good parent model), we should find a positive relationship between nest size and nestling-fitness traits for control nestlings (Prediction 1), whereas this relationship would not exist for experimental nestlings because food supplements would reduce the influence of parental care on nestling-fitness traits (Prediction 2). In addition, differences in nestling condition and immunocompetence between control and experimental nestlings of the same nest can be used as an indicator of parental feeding ability. If nest size were related to parental feeding ability, it should explain the difference in nestling-fitness traits between control and experimental nestlings of the same nest (Prediction 3a). Moreover, if parents with larger nests provided nestlings with good quality and quantity of food, differences between experimental and control nestlings should be small in those nests, whereas the opposite would be true for nestlings of smaller nests (Prediction 3b).

METHODS

Studied species and study area

Magpies occur throughout large parts of the Holarctic region. The magpie is monogamous, territorial, sedentary, and relatively long-lived for passerine birds, with a well-described biology (extensively reviewed in Birkhead, 1991). Magpies normally build a domed, almost spherical, nest with a stick framework. After the framework is finished, a bowl of mud is built inside and lined with fibrous roots, hair, and grass (nest cup) (Birkhead TR; personal observation.). Both members of the pair participate in nest building, but the male makes significantly more trips to collect mud and large twigs, generally collecting more sticks than does the female (Birkhead, 1991). The size of the nest structure has been suggested to be a reliable signal of territory quality and/or pair quality (Soler et al., 1995). Nest size in magpies acts as a post-mating sexually selected signal, indicating males' quality and willingness to invest in reproduction, and females adjust their reproductive investment according to nest size (Soler et al., 2001).

The experiment was carried out in the spring of 2001 in La Hoya de Guadix (37°18' N, 3°11' W, southern Spain), a high-altitude plateau, approximately 1000 m above sea level. The vegetation is sparse, including cultivated cereals (especially barley) and many groves of almond trees (*Prunus dulcis*) in which magpies prefer to build their nests (see a more detailed description in Soler, 1990). In our study area magpies frequently suffer from brood parasitism by the great spotted cuckoo (e.g., Soler et al., 1998c), but we did not use parasitized nests in the present study.

Experimental procedure

We visited completed magpie nests at least twice a week to record laying date and clutch size. When brood parasitism

occurs, magpies sometimes eject great spotted cuckoo eggs as well as their own damaged eggs (Soler et al., 1997, 1999). However, to detect possible brood parasitism and to ensure that no eggs were missing or damaged during egg laying, nests were also visited every two days during the laying period. After the 18th day of incubation, nests were visited daily to record hatching date.

After clutch completion, we measured the largest and shortest radius of the eggs with a digital caliper (Mitutoyo, 0.01 cm accuracy), as well as nest size with a ruler (0.5 cm accuracy; see De Neve and Soler [2002] for a further description of magpie nest measurement). Egg size and nest size were calculated as the volume of an ellipsoid: $4/3\pi ab^2/1000$, where a is the largest radius and b the shortest.

We provided food supplements to half of the nestlings in each nest during the nestling period. This supplemental food consisted of a high-calorie paste enriched with essential micronutrients (minerals, vitamins, and amino acids; 5 calories per g; Nutri-Calorías, Shering-Plough Animal Health, used as a strong calorie and nutritional supplement for dogs and cats). Two days after the first nestling hatched, each hatchling was weighed and marked with a color on the tarsus. Subsequently, hatchlings were ranked according to their weight. Starting with the heaviest or second heaviest hatchling (alternating between nests), we assigned the food treatment to half of the hatchlings, intermittently according to their weight. The dose and frequency of the food treatment were calculated based on the product instructions for the mean weight of magpie nestlings at eight days of age (50 g) and consisted of 0.1 ml of the liquid food.

We revisited nests subsequently every two days, re-colored the tarsus of all nestlings, and fed the nestlings that were assigned to the treatment during the first visit (seven times during the nestling period). About four days before fledging, when nestlings were about 16–17 days old, we ringed and measured tarsus (digital caliper to the nearest 0.01 cm), wing, and tail length (ruler to the nearest 0.1 cm) and we weighed all nestlings in the nest with a Pesola spring balance (accuracy 0.5 g).

A phytohemagglutinin (PHA-P, Reference number: L8754, Sigma Chemical Co.) injection was used to evaluate the in vivo T-cell-mediated immune response of nestlings (Cheng and Lamont, 1988). We injected fledglings subcutaneously in the right wing web with 0.5 mg of PHA dissolved in 0.1 ml of physiological saline solution (Bausch & Lomb). The left wing web was injected with 0.1 ml of physiological saline solution. We measured the thickness of each wing web at the injection site with a digital pressure-sensitive micrometer (Mitutoyo, model ID-CI012 BS; to the nearest 0.01 mm) before and 24 h after the injection. The T-cell-mediated immune response or wing web index was then estimated as the change in thickness of the right wing web (PHA injection) minus the change in thickness of the left wing web (Lochmiller et al., 1993). We repeated measurements of each wing web three times, and the mean was used in subsequent analyses.

To reduce the effects of possible parental traits not related to parental care but to nest size, we performed a clutch removal experiment in some nests. We removed the eggs of one magpie pair two or three days after clutch completion and brought the clutch to another magpie nest, matched by laying date, that incubated and reared the offspring (foster parents). This would also control for any confounding effects of possible maternal investment (egg size, clutch size) related to parental quality. The clutch from the foster parents was removed and subsequently used in other experiments. Nest size, clutch size, and egg size did not differ significantly between original and foster parents. However, nest size was correlated between original and foster parents, whereas clutch size and egg size were not (Table 1).

Table 1

Paired *t* tests between original and foster parents in nest size, clutch size, and egg size for clutch removal experiments

	Original parents	Foster parents	<i>t</i>	<i>p</i>	<i>r</i>	<i>P</i>
Nest size	0.46 ± 0.09	0.39 ± 0.09	1.07	.29	.70	<.001
Clutch size	7.06 ± 0.17	6.68 ± 0.20	1.65	.11	.21	.26
Egg size	9.62 ± 0.15	9.62 ± 0.11	0.16	.87	.09	.61

In addition, the correlation for these traits between original and foster parents is given. *N* = 31 for all traits.

Statistical analyses

In the analyses we used first, non-parasitized clutches in which at least one control and one food-supplemented nestling survived until fledgling. We obtained a final sample size of 46 nests (31 clutch removals, 15 natural first clutches).

Frequency distributions of nest size after log-transformation, as well as that of all the other variables used, did not significantly differ from a normal distribution (Kolmogorov-Smirnov test for continuous variables, *p* > .15), and we used parametric statistics following Sokal and Rohlf (1995).

Body mass and tarsus length were strongly correlated (*R* = .84, $F_{1,176} = 426$, *p* < .001). However, residuals from this regression were significantly related to two other body size indicators, wing length (*R* = .28, $F_{1,176} = 14.6$, *p* = .0002) and tail length (*R* = .28, $F_{1,176} = 14.7$, *p* = .0002). Therefore, we controlled body mass for both tarsus length and wing length ($R^2 = .77$, $F_{2,175} = 296$, *p* < .0001; partial regression coefficient tarsus length: .50, *p* < .001; partial regression coefficient wing length: .43, *p* < .001). Residuals from this regression were not correlated with tail length (*R* = .057, $F_{1,176} = 0.57$, *p* = .45); therefore, these residuals are likely to present an appropriate index of body condition (Green, 2001).

To test for differences between food-supplemented and control nestlings in T-cell-mediated immune response and condition index, we used a two-factor ANCOVA model where nest (random effect) and treatment (i.e., food-supplemented and control nestlings, fixed effect) were main factors, and the number of nestlings in the nest and laying date were covariates. In this way we estimated the influence of the food supplements on nestlings while taking into account variation among nests.

To test for a relationship between nest size and nestling condition index/T-cell-mediated immune response we performed multiple regression analyses. We used all 46 nests. For nestlings from clutch removal experiments, nest size of the

foster parents was used. We also performed separate analyses for natural first clutches (15 nests) and clutch removal experiments (31 nests) to test for a possible bias.

Analyses were performed using StatSoft (1998), modules “Variance Components” and “Multiple Regression.”

RESULTS

Effect of the food supplements

Food supplements had a significant effect on nestling T-cell-mediated immune response but not on condition index (Table 2). Nestlings receiving the food supplements presented a significantly higher T-cell-mediated immune response and a tendency for higher condition index than control nestlings (Figure 1). Therefore, differences in T-cell mediated immune response between experimental and control nestlings can be used as a variable related to parental quality (see Introduction).

Nest size and nestling-fitness traits

In accordance with Prediction 1, nest size explained significant variation in T-cell-mediated immune response of control nestlings (linear regression: *B* = 0.24 ± 0.11, $t_{1,44} = 2.24$, *p* = .03) but not of food-supplemented nestlings (linear regression: *B* = -0.02 ± 0.09, $t_{1,44} = -0.22$, *p* = .83), suggesting that the food supplement reduced the influence of parental care on T-cell-mediated immune response (Prediction 2). The same results were obtained when performing this analyses for clutch removal experiments and for natural nests separately, though not reaching statistical significance, probably due to lower sample sizes (linear regression clutch removal experiments: *B* = 0.24 ± 0.14, $t_{1,29} = 1.65$, *p* = .11; linear regression

Table 2

Results of two-factor ANCOVAs with T-cell-mediated immune response and condition index as dependent variables and experimental treatment (i.e., food-supplemented and control nestlings) and experimental nest as main factors

	Effect	df effect	MS effect	df error	MS error	<i>F</i>	<i>P</i>
Immune response							
Laying date	F	1	0.53	42.9	0.17	3.10	.08
Number of nestlings	F	1	0.001	43.6	0.17	0.001	.94
Treatment	F	1	1.22	37.9	0.05	23.21	<.001
Nest	R	43	0.17	40.3	0.05	3.28	<.001
Condition index							
Laying date	F	1	187.7	42.8	130.5	1.44	.24
Number of nestlings	F	1	1514.1	43.3	130.5	11.60	.001
Treatment	F	1	71.2	38.9	55.4	1.28	.26
Nest	R	43	130.5	40.2	55.4	2.36	.003

The number of nestlings and laying date are covariates. F = fixed effect, R = random effect, MS = mean squares.

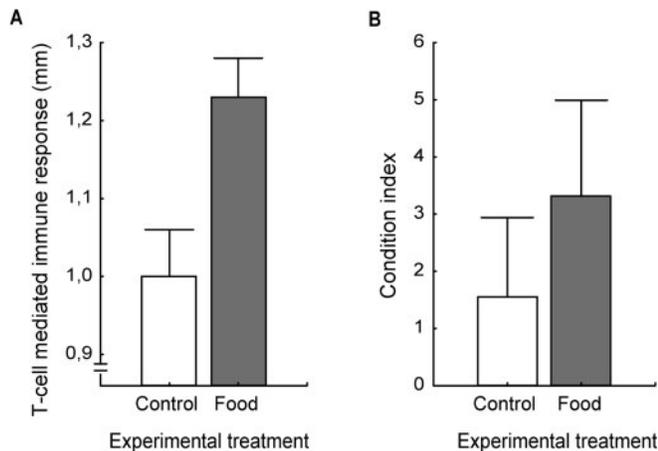


Figure 1
Population marginal means of T-cell-mediated immune response (A) and condition index (B) for food-supplemented and control nestlings. Sample sizes are nests with experimental or control nestlings ($N = 46$). Whiskers are SE.

natural first clutches: $B = 0.25 \pm 0.16$, $t_{1,13} = 1.54$, $p = .15$). Moreover, the slopes did not differ between the two groups (ANCOVA, homogeneity of slopes model: interaction covariable [nest size] and factor [clutch removal and natural first clutches]: $F_{1,42} = 0.31$, $p = .58$).

Because laying date marginally influenced T-cell-mediated immune response (Table 2), we also performed forward stepwise multiple regressions, introducing both nest size and laying date as independent variables. For control nestlings a similar result appeared, with nest size explaining variation in nestling immune response much better than laying date (forward multiple regression: $R^2 = .13$, $F_{2,43} = 3.23$, $p = .049$; B-coefficient nest size: 0.29 ± 0.14 , $p = .05$; B-coefficient laying date: -0.17 ± 0.14 , $p = .24$). For food-supplemented nestlings, again no relationship with nest size appeared and only laying date entered into the regression, not reaching significance (forward multiple regression: $B = -0.24 \pm 0.15$, $F_{1,44} = 2.66$, $p = .11$).

Nest size, however, did not significantly explain variation in condition index, neither in control (linear regression: $B = 0.19 \pm 0.15$, $F_{1,44} = 1.59$, $p = .21$) nor in food-supplemented nestlings (linear regression: $B = 0.06 \pm 0.15$, $F_{1,44} = 0.16$, $p = .69$). After introducing the number of nestlings in the nest as a second independent variable (Table 2), this variable significantly explained variation in nestling condition index (forward multiple regression: $R^2 = .17$, $F_{2,89} = 9.04$, $p = .003$; B-coefficient nest size: 0.15 ± 0.10 , $p = .12$; B-coefficient number of nestlings: -0.39 ± 0.10 , $p < .001$).

Another way to estimate the possible influence of parental care on nestling-fitness traits is to calculate the difference within each nest between food-supplemented and control nestlings in T-cell-mediated immune response and condition index (food-supplemented minus control), and explore a relationship between this difference and nest size. In accordance with Prediction 3a, a forward stepwise multiple regression introducing laying date, the number of nestlings, and nest size as explanatory variables showed that nest size significantly explained variation in the difference in immune response between food-supplemented nestlings and control nestlings, with the number of nestlings in the nest explaining additional variation (forward multiple regression: $R^2 = .21$, $F_{2,43} = 5.91$, $p = .0054$; partial regression coefficient nest size: $-.42$, $p = .0035$; partial regression coefficient number of nestlings: $.24$, $p = .084$; Figure 2).

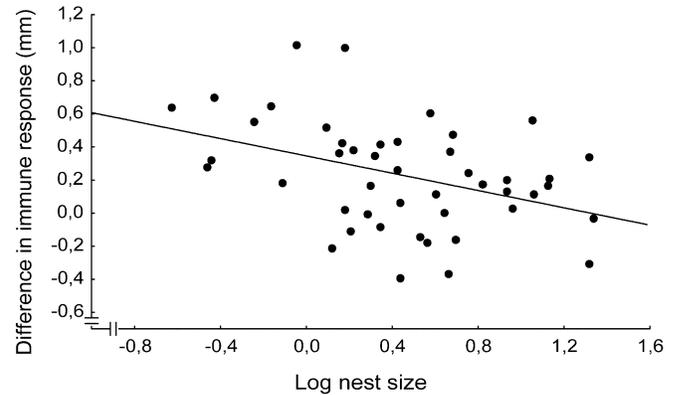


Figure 2
Relation between nest size (log-transformed) and the difference in T-cell-mediated immune response of food-supplemented and control nestlings of the same nest ($r = 2.88$, $N = 46$, $p = .006$).

The negative relationship between the differences in T-cell-mediated immune response and nest size (Figure 2) indicates that differences between food-supplemented and control nestlings were smaller when nest size was larger. This result suggests that pairs with a large nest were able to provide nestlings with good quality food, resulting in similar immune responses for control and experimental nestlings reared in large nests (Prediction 3b).

On the other hand, brood size was the only variable entering into the regression that explained differences in condition index (forward multiple regression, number of nestlings: $-.29$, $F_{1,44} = 4.21$, $p = .046$). However, since we did not find a significant effect of the experimental feeding on nestling condition index, this result cannot be considered as opposing our hypothesis of nest size being an indicator of magpie parental quality.

DISCUSSION

Nestling fitness at the age of fledging depends largely on parental effort in terms of food delivery to their offspring (Clutton-Brock, 1991). Specifically, a large amount of proteins are important for the fast growth that young birds experience during the first days of their life, and they are also important for the development of an adequate immune system (Glick et al., 1983; Lochmiller et al., 1993). Previous studies have demonstrated a reduced T-cell-mediated immune response in cases of protein malnutrition (Gershwin et al., 1985; Lochmiller et al., 1993). However, dietary deficiencies of other kinds of nutrients, such as amino acids (methionine) and vitamins (e.g., antioxidants), have also been shown to affect the optimal development of the immune system and reduce immune function (Chew, 1996; Friedman and Sklan, 1997; Haq et al., 1996; McWhinney et al., 1989; Tsiagbe et al., 1987). We found that a food supplement of high-calorie paste, enriched with essential micronutrients such as vitamins and minerals, resulted in significantly higher T-cell-mediated immune responses and a tendency to an increased condition index for food-supplemented nestlings (Figure 1). Given that the development of the immune system requires a high quality diet, the effects of food supplements were probably particularly noticeable in nestling T-cell-mediated immune response and not in nestling condition index. Nevertheless, because fledging immunocompetence is an important fitness trait affecting survival (Christe et al., 2001; Hórák et al., 1999), parents providing nestlings with a protein-rich diet also

containing vitamins and micronutrients, necessary for the optimal development of the immune system, would experience larger fitness than other parents.

Sexually selected traits, thought to signal parental quality, as is likely the case for nest size in magpies, might predict parental feeding ability. Thus, according to the good parent model, a link between the exaggeration of the character and nestling-fitness traits can be expected. In accordance with this prediction, we found that nest size was positively related to T-cell-mediated immune response for control magpies, probably because nestlings that grew up in larger nests experienced better nutritional conditions due to enhanced parental feeding effort, and consequently they presented improved immunocompetence. On the other hand, no relationship between nest size and immune response appeared for food-supplemented nestlings, indicating that the food supplements compensated for the influence on the development of the immune system of lower quality food received in smaller nests. In addition, the difference in T-cell-mediated immune response between food-supplemented and control nestlings of the same nest was principally explained by nest size. Hence, in small nests, food-supplemented nestlings showed to a larger extent higher immune responses than control nestlings, whereas in large nests control nestlings experienced similar immune responsiveness as compared to food-supplemented nestlings. Because the development of the immune system requires a diet rich in both proteins and essential micronutrients (see above), these results suggest that pairs with large nests provided their nestlings with higher quality food as compared to pairs with smaller nests.

However, we did not measure parental feeding effort per se, and thus we could not distinguish between relative feeding efforts of male and female magpies. Nest size could serve as a predictor for males' provisioning rate, but it is likewise possible that absolute feeding efforts by mates were correlated (Linville et al., 1998). It can be argued that other important environmental factors, such as brood size and laying date, might have confounded our results. This was the case for nestling condition index, which was mainly affected by brood size, with nest size only explaining additional variation in this trait. However, introducing these covariates into the analyses did not change the results with respect to nestling T-cell-mediated immune response. Still, brood size cannot be discarded as an important factor affecting nestling-fitness traits. Differences in T-cell-mediated immune response between food-supplemented and control nestlings were additionally explained by brood size, and differences in condition index were only explained by brood size. In nests with a large brood size, food-supplemented nestlings tended to have higher T-cell mediated immune responses and lower condition indices compared to control nestlings, whereas the opposite tended to occur in nests with few nestlings.

Female birds are also able to differentially allocate substances (carotenoids, particular antibodies, yolk hormones, yolk amount) into their eggs that can significantly influence the development of the immune system (Cunningham and Russell, 2000; Gil et al., 1999; Haq et al., 1996; Royle et al., 1999; Sockman and Schwabl, 2000). Thus, it is possible that female reproductive investment (related to maternal effects on the eggs) was related to sexually selected traits of her mate other than nest size (Gil et al., 1999), thereby influencing the hypothetical relationships between nest size and nestling immunocompetence. However, 67% (31 out of 46) of the clutches in the analyses were clutch removal experiments (the clutch of one magpie pair was put in the nest of another magpie pair), and clutch size and egg size were not significantly correlated between original and foster parents (Table 1). Therefore, maternal effects related to clutch size and egg size

probably did not confound our results, mainly because regression slopes of the relation between nest size and nestling T-cell-mediated immune response did not differ between the two kinds of nests (see Results).

In conclusion, we found, in accordance with the good parent model, that nest size was positively related to T-cell mediated immune response for control magpie, whereas this relationship was nonexistent in food-supplemented nestlings. In addition, the difference in T-cell mediated immune response between food-supplemented and control nestlings of the same nest was principally explained by nest size. Our results support the hypothesis that nest size is an indicator of parental ability in magpies. To our knowledge this is the first study showing a link between a post-mating sexually selected signal and nestling immunocompetence, a trait closely related to fitness in birds.

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