Effects of a food supplementation experiment on reproductive investment and a post-mating sexually selected trait in magpies *Pica pica*

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Food availability is an important factor affecting breeding success in birds. Food supplementation experiments in birds have in general focused on the effects on reproductive success in terms of female investment (laying date, clutch size, egg size), however, it is also known that the estimation of mate quality based on sexually selected signals influences female reproductive investment. In the particular case of magpies, females use nest size, a post-mating sexually selected signal, to assess male's likelihood to invest in reproduction, and accordingly adjust reproductive investment (clutch size). Then, the possible effects of food supplementation on female reproductive investment could be mediated by other variables related to parental quality, such as nest size in magpies. In the present study, we explore if higher food availability in a magpie territory affected both male sexually selected traits (i.e. nest size) and female reproductive investment (laying date, egg size, clutch size). We performed a food supplementation experiment in which we experimentally increased food availability in several magpie territories, keeping others as controls. In food-supplemented territories, males built significantly larger nests and females significantly increased egg size by 4.1% compared to control females. Results suggest that the continuous provisioning of protein rich food allowed magpie females to increase egg size. However, laying date and clutch size did not differ between control and food-supplemented magpie pairs. Food availability also affected the relationship between female reproductive investment and nest size. In control territories, females decreased their egg size in response to a larger nest, whereas a tendency for the opposite relationship was revealed in food-supplemented territories. We discuss the possibility that magpie females adopt different strategies for reproductive investment according to food availability.

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Food availability is an important factor that affects breeding success in birds (Lack 1954). Apart from the high energetic cost of rearing a brood (e.g. Moreno et al. 1997, Verhulst and Tinbergen 1997), it is known that avian egg production and incubation are energetically expensive processes (e.g. Monaghan and Nager 1997, Meijer and Drent 1999). Passerine species depend almost exclusively on dietary nutrients, especially proteins, for egg production. In fact, in many studies that have explored the effects of food supplementation

on female reproductive investment (reviewed by Meijer and Drent 1999), high-protein food supplements generally had a greater effect on egg production than lipid supplements (Williams 1996). In the case of the magpie *Pica pica*, the provisioning of food supplements (fish or dog food) at a continuous rate during the breeding season resulted in an increase in egg size in populations in Sweden (Högstedt 1981) and Canada (Hochachka and Boag 1987). In addition, food supplementation may also result in improved egg quality, as it has been demonstrated in a food supplementation experiment in lesser black-backed gulls *Larus fuscus* (Blount et al. 2002). In this study, carotenoid fed females had increased integument pigmentation, higher plasma concentration of carotenoids and antioxidant activity. In turn, the females produced eggs with high carotenoid concentrations, which may enhance antioxidant activity and chick immune development. Furthermore, like in most passerine species, food supplementation in magpies resulted in an advance in laying date, but no significant effect was found on clutch size (Högstedt 1981, Hochachka and Boag 1987, Knight 1988, Dhindsa and Boag 1989).

On the other hand, food supplementation experiments in birds have in general focused on the effects on reproductive success in terms of female investment (Meijer and Drent 1999). However, the estimation of mate quality based on sexually selected signals could influence female reproductive investment (de Lope and Møller 1993, Wolf et al. 1997). For instance, in zebra finches *Taeniopygia guttata*, females deposited more testosterone in the eggs when mated with more attractive males, which influenced the development of the chick (Gil et al. 1999). In the mallard *Anas platyrhynchos*, females produced larger eggs when mated with preferred males, which resulted in offspring of better condition (Cunningham and Russell 2000). In the particular case of magpies, it is known that females use nest size, a post-mating sexually selected signal, to assess male's likelihood to invest in reproduction. Magpie females invest in reproduction (clutch size) according to their estimation of male parental quality (Soler et al. 2001, De Neve and Soler 2002). So, the effects of food supplementation on female reproductive investment could be mediated by other variables related to parental quality, such as nest size in magpies.

In the present study, by performing a food supplementation experiment, we explore to what extent higher food availability in the magpie territory affects both the male sexually selected trait (i.e. nest size) and female reproductive investment (laying date, egg size, clutch size). In addition, we consider whether the hypothetical relationship between a sexually selected trait (e.g. nest size) and female reproductive investment is mediated by food availability.

Materials and methods

Study area and species

The experiment was carried out during spring 2000 on a magpie population at 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 breed (for a more detailed description, see Soler 1990).

Magpies occur throughout large parts of the Holartic region. They are territorial, sedentary and relatively long-lived for passerine birds, with a well described biology (reviewed in Birkhead 1991). Magpies normally build a domed, almost spherical, nest with a stick framework. 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). Nest size in magpies has been suggested to be a reliable signal of territory quality and/or pair quality (Soler et al. 1995), and final nest size acts as a post-mating sexually selected trait, used by females to adjust reproductive investment (Soler et al. 2001).

Magpies in our study area usually lay 5 to 7 eggs (clutch size ranges from 3 to 10 eggs; Birkhead 1991, Soler et al. 1996). The eggs are incubated exclusively by the female several days after the first egg is laid, but usually before clutch completion, resulting in asynchronous broods (Birkhead 1991). The male frequently feeds the female during incubation (Birkhead 1991). Some nestlings usually die from starvation, especially during the first week after hatching, and the species is considered to adopt the brood-reduction strategy (Husby 1986, Slagsvold et al. 1992, Reynolds 1996).

Experimental design

We considered 6 study plots within the study area because the distribution of trees is patchy. Each plot contains between 20 and 50 magpie territories. We selected randomly within each plot a small area and provided all territories within this area with the food supplement. We began the food-supplementation experiment when territory establishment had occurred in about half of the magpie territories in the whole study area, but before nest-building had started. Magpies usually breed in the same territory for several years and start to defend their territory even before the start of nest-building (Birkhead 1991; pers. obs. of some colour ringed individuals within our study area). Magpies do not change territory within the same year. When, for example, a pair suffers predation during or shortly after egg laying, they build a replacement nest in the same

territory (Birkhead 1991, De Neve and Soler 2002). Moreover, we did not observe any magpie pair change their territory after the start of food-supplementation. Therefore, our experiment probably did not affect the settlement pattern of the magpie pairs, and we most likely obtained a random selection of territories in which magpie pair quality was also randomly distributed. Territories nearby to the food-supplemented territories were kept as a buffer between food-supplemented and control nests, and were not included in the analyses. Some magpie pairs in our study area are colour-ringed (10%), and by observations we ensured that magpies from control plots did not eat from the extra food resources.

One portion of the food supplement consisted of about 150 g of dog food (Selection Junior Royal Canin), mixed with bread and water. The food supplement contained a high percentage of proteins (31.5%), but also contained vitamins and antioxidants (vitamin A, D_3 and E). We provisioned a portion of extra food every two days in old nests within the experimental areas, starting from the beginning of March (before initiation of nest-building) until the end of the breeding season. Old nests within the control areas were visited with the same frequency.

When nest-building started, we searched the whole area for new magpie nests, and visited completed nests every two days to detect laying date. When the clutch was completed, we measured the eggs with a digital calliper (Mitutoyo, accuracy 0.01 cm), and nest size with a ruler (precision 0.5 cm). Nest size and egg size were calculated as the volume of an ellipsoid: $4/3 \ (\pi ab^2)/1000$ (m^3 and cm^3 respectively), a being the largest radius of the ellipsoid and b the smallest.

Data and statistical analyses

We found 208 nests where laying date could be recorded (46 food-supplemented nests and 162 control nests). However, sample sizes were reduced and unequal for the following reasons: out of the 208 magpie pairs, 11 abandoned their clutch during laying and 22 clutches were depredated during laying. Replacement clutches were not included in the present study. Moreover, in our study area magpies suffer frequently from brood parasitism by the great spotted cuckoo *Clamator glandarius* (e.g. Soler and Soler 2000), and in the breeding season of 2000, 56 (26.9%) first clutches were parasitized by the great spotted cuckoo. When brood parasitism occurs, magpies sometimes abandon their clutch in the middle of laying (6 nests), or eject great spotted cuckoo eggs and sometimes eject their own damaged eggs (Soler et al. 1997, 1999). Therefore, although nests were visited frequently, for some parasitized nests (3), clutch size could not be determined and in 12 of the parasitized

nests not all the eggs remained for egg size measurements. Nest size was measured after clutch completion, though we failed to measure nest size in some of the abandoned and depredated nests (12). Since some magpies build their nest on top of nests from previous years (34 nests (16%) in the present study), nest size of these pairs was not included in the analyses. Thus, data were available for 30 food-supplemented nests and 89 control nests, in order to explore links between all variables.

Frequency distribution 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, $P > 0.15$). So, we used parametric statistics following Sokal and Rohlf (1995).

To test for the effects of the food supplementation on nest size (sexually selected signal) and female reproductive investment (clutch size, egg size), we performed two-way ANCOVA, with study plot and experimental treatment (food supply) as factors. In this way we tested the effect of the food supplementation by taking into account possible differences between the 6 study plots in our study area. All analyses were performed with the computer program STATISTICA (StatSoft, Inc. 1998).

Results

Male and female investment in response to food supplementation

After taking into account the possible differences between study plots and controlling for the effect of laying date, the food supplementation experiment had a significant effect on nest size and egg size (Table 1). Nests built in food-supplemented territories were significantly larger than nests in control territories (Table 2). Females laid significantly larger eggs in food-supplemented territories compared to control females (Table 2). Furthermore, females in food-supplemented territories tended to advance laying date as compared to control females, but they did not lay larger clutches after controlling for laying date (Table 1 and 2). Moreover, the interaction term between study plot and experimental treatment was not significant, indicating that the effect of the food supplement was similar in the different study plots (Table 1).

Female investment in response to nest size

Since nest size differed significantly between the 6 study plots (Table 1), we used standardized values for the remaining analyses (to a mean of zero per study plot). Furthermore, laying date influenced clutch size (Table 1), and residuals from the regression between clutch size

Table 1. Results of two factor ANCOVA for the dependent variables: laying date, nest size, clutch size and egg size. Study plot and experimental treatment (i.e. food supplemented and control territories) are the main factors. Laying date is introduced as a covariate in the analyses of nest size, clutch size and egg size.

	df effect	MS effect	df error	MS error	F	P
Laying date						
Study plot	5	438	196	71.98	6.08	< 0.001
Food supplement	1	160	196	71.98	2.22	0.14
Interaction	5	89	196	71.98	1.23	0.29
Nest size						
Laying date	1	0.65	149	0.41	1.56	0.21
Study plot	5	5.74	149	0.41	13.85	< 0.001
Food supplement	1	3.30	149	0.41	7.98	0.005
Interaction	5	0.34	149	0.41	0.83	0.53
Clutch size						
Laying date	1	17.44	153	1.01	17.25	< 0.001
Study plot	5	1.14	153	1.01	1.13	0.35
Food supplement	1	0.11	153	1.01	0.11	0.74
Interaction	5	0.76	153	1.01	0.76	0.58
Egg size						
Laying date	1	0.10	142	0.61	0.16	0.68
Study plot	5	0.73	142	0.61	1.20	0.31
Food supplement	1	3.40	142	0.61	5.59	0.019
Interaction	5	0.98	142	0.61	1.60	0.16

Table 2. Weighted means ($\pm$SE) for reproductive values in food-supplemented and control nests. Results are from two-factor ANCOVAs (see Table 1). Values for nest size are log transformed. Laying date 1 = 1 April.

	Food supplemented		Control	
	Mean $\pm$ SE	N	Mean $\pm$ SE	N
Nest size	5.06 $\pm$ 0.15	39	4.63 $\pm$ 0.06	123
Laying date	25.4 $\pm$ 1.09	46	28.3 $\pm$ 0.74	162
Clutch size	6.92 $\pm$ 0.16	38	6.84 $\pm$ 0.09	128
Egg size (cm^3)	9.85 $\pm$ 0.15	36	9.46 $\pm$ 0.07	119

and laying date (Linear Regression: R = −0.31, $F_{1,164}$ = 17.4, P < 0.0001) were used, hereafter residual clutch size.

Neither egg size nor residual clutch size were related to standardized nest size (Multiple R = 0.08, $F_{2,112}$ = 0.42, P = 0.67; partial regression coefficient egg size: −0.05, P = 0.59; partial regression coefficient residual clutch size: R = 0.07, P = 0.48). However, to test whether the correlation between nest size (covariable) and egg/clutch size (dependent variables) was different in food supplemented and control territories (factor: experimental treatment), we used an ANCOVA (homogeneity of slopes model). The homogeneity of slopes model tests for an interaction between the covariable (nest size) and the factor. The interaction between the experimental treatment and nest size was significant for the dependent variable egg size ($F_{1,111}$ = 12.38, P = 0.0006), but not for clutch size ($F_{1,116}$ = 0.01, P = 0.91). This result indicates that the relationship between nest size and egg size was significantly different in food supplemented and control territories. Egg size was negatively related to nest size in control territories (Multiple R = 0.31, $F_{2,86}$ = 4.45, P = 0.01; partial regression coefficient egg size: −0.30, P =

0.005; partial regression coefficient residual clutch size: R = 0.072, P = 0.48; Fig. 1).

On the other hand, egg size was positively related to nest size in food supplemented territories (Multiple R = 0.40, $F_{2,23}$ = 2.21, P = 0.13; partial regression coefficient egg size: 0.39, P = 0.054; partial regression coefficient residual clutch size: R = 0.14, P = 0.46; Fig. 1). These results suggest that female reproductive investment in response to nest size depended on food availability.

Discussion

In food supplemented territories, females tended to advance laying date with a mean difference of 3 days, laid similar clutch sizes and significantly increased egg size by 4.1% compared to control females (Table 2). It is often assumed that food availability directly influences laying date. This hypothesis has been supported in many species, including the magpie, where food supplementation significantly advanced the onset of laying (Meijer and Drent 1999). However, it is probably a whole complex of proximate factors that influences the timing of breeding. For example, in several passerine species a clear increase in spring temperature triggers egg formation. Breeding too early may also have negative effects on the fitness of parents (e.g. Nilsson 1994). We only observed a slight and non-significant advance in laying date in the present study, suggesting that other factors than food abundance most likely influenced the onset of laying in our study population.

Clutch size and egg size are believed to be largely genetically determined, however, environmental factors can also influence these reproductive parameters. The effects of food supplementation on clutch size are more or less trivial in the literature, and the hypothesis that
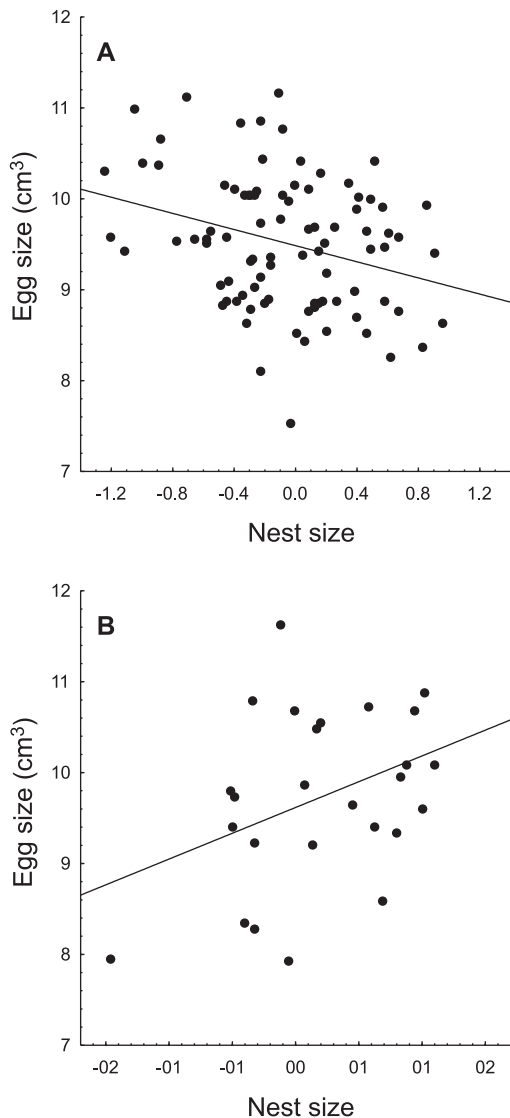
Fig. 1. Linear regressions between nest size (standardized for differences between study plots) and egg size. A. Control territories and B. Food-supplemented territories.

laying date itself controls clutch size, independently of food intake, has been supported in many experimental studies (Meijer and Drent 1999). In accordance with previous findings (Meijer and Drent 1999), magpies did not increase clutch size in response to the food supplements. Still, we did find a significant increase in egg size in response to food supplements. The effect of abundant food resources on egg size has received less attention, and has been observed in very few species (Meijer and Drent 1999, Christians 2002). Christians (2002) focused a review on the effects of environmental factors and a diversity of female characteristics on avian egg size variation. Only in 36% of the studies did supplemental food or enhanced food quality increase egg size. This is

somehow surprising since passerine females depend almost exclusively on dietary nutrients and energy for egg-laying. Still, the effects of supplemental feeding experiments on egg size are most likely influenced by the amount, frequency and kind of food supplied, as well as by the natural food availability in the territory (Meijer and Drent 1999). For example, high-protein supplements have been found to have a greater effect than lipid supplements (Williams 1996, Ramsay and Houston 1997). Our results, together with those from previous studies, indicate that the continuous provisioning of protein rich food (31.5%, see Materials and methods) allows magpie females to increase egg size (Högstedt 1981, Hochachka and Boag 1987). In addition, in a previous experimental study we demonstrated a trade-off between clutch size and egg size in magpies, and egg size was, on average, increased by 4.25% from first to replacement clutches of the same female (De Neve and Soler 2002), which is a similar increase as in the present study between control and food-supplemented females (4.1%). Also, Högstedt (1981) found a significant increase in egg size within magpie females in response to food supplementation. Thus, although egg size has generally been found to be relatively inflexible within individual female birds (Christians 2002), previous findings and results of the present study suggest that a small, but certain flexibility in egg size within individual magpie females exists.

Interestingly, food availability also significantly affected the relationship between female reproductive investment (i.e. egg size) and nest size (post-mating sexually selected signal indicating male likelihood to invest in reproduction). Females invested less in egg size in relation to larger nest size in control territories, whereas an opposite non-significant tendency occurred in food-supplemented territories. Clutch size was not related to nest size, in either food-supplemented or control territories. In a previous study, where nest size was experimentally manipulated, clutch size was positively related to experimentally manipulated nest size (Soler et al. 2001), providing support for the hypothesis that nest size is a post-mating sexually selected signal, indicating the likelihood that the male will invest in parental care. In another experimental study we found support for the hypothesis that female magpies trade-off clutch size for larger egg size (De Neve and Soler 2002). Hence, the existence of a trade-off between egg size and clutch size in magpies could explain the negative relationship between nest size and egg size observed in control territories in the present study. On the other hand, abundant food availability might obviate the trade-off between clutch size and egg size, which would explain the opposite tendency in the relationship between egg size and nest size in food-supplemented territories. Our results would then suggest that food availability during laying may constrain especially female

investment in egg size. Females mated with high quality males (large nest size) would trade-off clutch size for smaller eggs, since they may expect good parental care from their mate. However, when food is very abundant, females can invest in both clutch size and egg size, obviating the trade-off. Consequently, the detected effect of food supplementation on egg size could be mediated by a larger nest size in food-supplemented territories. Even so, with the present experimental design we could not distinguish the specific effect of food-supplementation, independently of nest size, on female reproductive investment.

In conclusion, magpie females used external environmental conditions (i.e. food availability) as well as nest size, a sexually selected signal indicating parental quality, for reproductive decisions. Higher food availability in the territory affected both male (nest size) and female reproductive investment (egg size). Finally, food availability affected the relationship between nest size and egg size.

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