

Differential maternal investment counteracts for late breeding in magpies *Pica pica*: an experimental study

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Reproductive success in many avian populations declines throughout the breeding season. Two hypotheses have gained attention to explain such a decline: the “timing” hypothesis proposes that deteriorating food availability causes the decline in reproductive success (causal effect of breeding time), whereas the “quality” hypothesis proposes that individuals of lower phenotypic quality reproduce at the end of the breeding season, causing the correlation between breeding time and breeding success. We tested both of these hypotheses in a monogamous single breeder, the magpie *Pica pica*, by experimentally inducing some pairs to lay a replacement clutch, after removal of the first clutch. The first clutch was left in the nest of another magpie pair (matched by laying date and clutch size), and incubated and raised by these foster parents. In this way we obtained two clutches from the same magpie pair with full siblings raised in conditions of the first and second reproductive attempts. High quality pairs (with laying dates in the first half of the breeding season) reached similar breeding success in replacement clutches as compared to first clutches of the same female. In addition, experimental pairs reared significantly more offspring of similar quality in their replacement clutches as compared to late-season first clutches, thereby suggesting that late season first clutches were produced by pairs of lower phenotypic quality. Results indicate that high quality pairs trade-off clutch size for larger eggs in replacement clutches, which could help magpie pairs to partly compensate for poorer environmental conditions associated with a delayed breeding attempt.

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Laying date is a prime factor influencing reproductive success of birds. Late season broods usually reach lower reproductive success than early season broods (e.g. Price et al. 1988, Verhulst et al. 1995, Moreno 1998, Morrison 1998, Verboven and Visser 1998). There exist two hypotheses explaining the general seasonal decline in reproductive success: the “timing” and the “quality” hypothesis. The “timing” hypothesis proposes a causal link between the timing of breeding (decrease in food supply) and reproductive success (Brinkhof et al. 1993, Verboven and Verhulst 1996, Svensson 1997, Verboven and Visser 1998). Alternatively, the “quality hypothesis”

poses that the decline in breeding success during the season is due to a weakening in individual quality (e.g. age or breeding experience, foraging ability, territory quality, etc.; Moreno 1998, Hipfner et al. 1999, Hansson et al. 2000, Nilsson 2000, Christians et al. 2001). A frequently used approach to discriminate between these two hypotheses is to induce a delay in breeding time by forcing females to lay a replacement clutch via experimentally removing the first clutch (Verhulst et al. 1995, Svensson 1997, Hansson et al. 2000, Nilsson 2000, Christians et al. 2001). Following this experimental approach, and in line with the “timing” hypothesis,

one should expect a decrease in breeding variables of individuals with experimentally delayed laying date according to the seasonal trends observed in control first clutches. On the other hand, if individual quality *per se* predicts variation in life history traits, experimentally delayed laying date would not, or to a lesser extent, influence breeding success.

However, both hypotheses are most likely not mutually exclusive. High quality individuals may reach similar breeding success in an experimentally delayed breeding attempt, though this does not necessarily mean that deteriorating environmental conditions do not affect reproductive investment. Individual phenotypic quality (i.e. body mass, territory quality, etc.) changes during the breeding season due to the costs associated with reproduction. However, relative phenotypic quality (with respect to the whole population) should be more or less constant along the breeding season, or even increase in good quality individuals, because of different effects of the costs associated with reproduction in high- and low-quality individuals. Then, according to the "quality" hypothesis, individuals of high quality, faced with a delayed breeding attempt, may be able to compensate for the negative effects of late breeding, while other individuals of lower phenotypic quality may not be able to do so.

In this study, we tested whether the magpie *Pica pica*, a monogamous single breeder passerine, was able to compensate for an experimentally delayed breeding date by means of a complementary egg removal-exchange experiment. The removed first clutch was placed in another nest (matched by laying date and clutch size) and was incubated and reared by foster parents, allowing us to estimate breeding success and nestling quality (i.e. condition and immunocompetence) of first and replacement clutches of the same magpie pairs. Extra-pair paternity is very low in our population, and magpies usually do not change partner within the same breeding season (Parrot 1995). One advantage of this experimental method is that genetic differences between broods reared at different stages of the season are controlled for, thereby reducing variation caused by genetic effects. In addition, we can compare reproductive investment (clutch size, egg size) of the same female in first and replacement clutches. We also use the experimental results and reproductive success of pairs with late season first clutches to evaluate and discuss the importance of both the "timing" and the "quality" hypotheses explaining the general seasonal decline in breeding success described above.

Materials and methods

Species and study area

Magpies occur throughout large parts of the Holarctic region. They are territorial, sedentary and relatively

long-lived for passerine birds, with a well-described biology (extensively reviewed in Birkhead 1991). They lay a single clutch in spring, from March to May, in their western European range (clutch size ranges from 3 to 10 eggs; Birkhead 1991). In the case of predation during egg laying or incubation, magpies are able to lay a replacement clutch close to the original nest (Birkhead 1991, Sorci et al. 1997). Replacement nests as well as replacement clutches are usually smaller than those in first attempts, but egg size of replacement clutches increases, suggesting a trade-off between clutch size and egg size in magpies (Clarkson 1984, cited in Birkhead 1991).

The experiment was carried out in spring 1999 and was repeated in spring 2001 in La Hoya de Guadix (37°18'N, 3°11'W, southern Spain), a high altitude plateau, approximately 1000 m a.s.l., where about 200 magpie pairs breed. The vegetation is sparse, including cultivated cereals (especially barley) and many groves of almond trees *Prunus dulcis* in which magpies prefer to build their nest (see a more detailed description in Soler 1990). In our study area magpies frequently suffer from brood parasitism by the great spotted cuckoo *Clamator glandarius* (e.g. Soler et al. 1998b). Because parasitized nests were not used in the present study, and some of the nests in the duplicate (those with offspring from first and replacement clutches) suffered from a high predation rate (Table 1), sample sizes in 1999 were largely reduced, and therefore, the experiment was repeated in 2001. Since magpie pairs tend to use the same territory in different years (Birkhead 1991, pers. obs.), and since in 2001 we avoided using successful experimental territories of 1999, a pseudo-replication problem is unlikely.

Experimental procedure

At the beginning of the breeding season we started to look for new magpie nests in the study area. When nests were finished, each nest was visited at least twice a week to record laying date. 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 visited every two days during the laying period. Two or three days after clutch completion, we removed one of a couple of clutches matched by laying date and clutch size (hereafter duplicate), inducing magpies to build a new nest and lay a replacement clutch (magpie pair A). We then measured and brought the eggs of clutch A to the other magpie nest (magpie pair B), that incubated and raised the offspring from nest A. The eggs from pair B were removed, measured, and subsequently used in other experiments.

One week after removing the eggs from magpie pairs A, we started to look for the replacement nest. Replace-

Table 1. Factors that influenced the final sample sizes and that lead to unbalanced sample sizes. **N experiments**: number of removal experiments of first clutches and complete replacement clutches. **N egg size**: nests where all eggs could be measured. **Parasitized**: in first clutches: after the experiment was performed (loss of hatchlings); in replacement clutches: during egg laying or after clutch completion (possible loss of egg size and loss of hatchlings). **Depredation eggs**: of non-parasitized (loss of hatchlings). **Depr. Nestlings**: Depredation of nestlings before body condition was measured. **Duplicates**: data of first and replacement clutches of the same female.

	1999		2001	
	First	Replacement	First	Replacement
N Experiments	33	23	46	38
N egg size	32	16	43	34
Parasitized	5	8	2	13
Depredation eggs	3	2	5	6
N hatchlings	24	13	36	19
Depr. Nestlings	10	4	4	8
N fledglings	14	9	32	11
Duplicate clutch size		23		38
Duplicate egg size		16		34
Duplicate hatchlings		8		12
Duplicate fledglings		7		7

ment nests were also visited every two days. With this experimental approach we obtained two complete clutches (first and replacement clutch) from the same magpie pair. Foster parents reared the first clutch, and the real parents reared the second clutch. Laying date and clutch size did not differ between real and foster parents' first clutches (Table 2). Moreover, neither egg size, nor nest size, an indicator of parental quality in magpies (Soler et al. 2001, De Neve and Soler 2002), differed significantly between real and foster parents (Table 2), thereby suggesting that foster parents were probably of similar quality as the real parents.

After clutch completion, we measured the largest and shortest radius of the eggs of first and replacement clutches with a digital calliper (Mitutoyo, 0.01 cm accuracy), as well as the 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: $(4/3 \times \pi \times a \times b^2)/1000$, where a is the largest radius and b the shortest.

Because the probability to build a replacement nest and lay a second clutch declines during the breeding season (Birkhead 1991), experimental removal of

clutches was done until the 12th of May in 1999 and until the 7th of May in 2001 (onset of breeding in about 70% of the magpie pairs).

To detect hatching date and success, we visited magpie nests every day after the 18th day of incubation. Around 4 days before fledging, when nestlings were about 17 days old, we ringed and measured tarsus (digital calliper to the nearest 0.01 cm) and wing length (ruler to the nearest 0.1 cm) and weighed (Pesola spring balance, accuracy 0.5 g) all nestlings in the nest. Further, a phytohemagglutinin-P (PHA-P, 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 a solution of 0.5 mg PHA dissolved in 0.1 ml physiological saline solution (Bausch & Lomb Co.). As a control, the other wing web was injected with 0.1 ml physiological saline solution. We measured the thickness of the 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 hours 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 (control injection; Lochmiller et al. 1993). We repeated measurements of each wing web three times, resulting in a high repeatability (Wilks'-Lambda > 0.85, $P < 0.0001$, for all measures). We used mean values in subsequent analyses.

We also followed up first clutches of magpie pairs with laying dates similar to those of experimental replacement clutches ($N = 12$; 5 in 1999 and 7 in 2001). However, we did not have a control group of first clutches at the beginning of the breeding season, since we used all available nests at that stage for the clutch-removal experiments.

Statistical analyses

We carried out a total of 33 clutch removal experiments in 1999 and 46 experiments in 2001. We found 23 (70%) and 38 (82%) complete replacement clutches in 1999 and 2001, respectively. However, final sample sizes of duplicated nests with first and replacement clutches and fledglings of the same female were largely reduced (7 duplicates in both years), due to brood parasitism

Table 2. Results of paired t-tests of breeding values of the real parents (magpies pairs A) and the foster parents (magpie pairs B), which reared the first clutch of magpie pairs A. Sample sizes are unequal because of brood parasitism in some foster parents nests ($N = 10$), and because nest size was not measured in all foster parents' nests. Laying date: 1 = 1st of April. Values are means \pm SE.

	Real parents	Foster parents	df	t	P
Laying date	19.8 \pm 0.80	19.7 \pm 0.80	78	0.32	0.75
Clutch size	6.62 \pm 0.13	6.54 \pm 0.11	73	0.56	0.57
Egg size (cm ³)	9.60 \pm 0.10	9.50 \pm 0.07	68	0.65	0.52
Nest size (m ³)	0.16 \pm 0.01	0.15 \pm 0.01	55	1.05	0.30

and depredation of eggs and nestlings (see experimental procedure, Table 1). We used mean values per nest of egg size and nestling traits to avoid pseudo-replication. Frequency distributions of all variables did not significantly differ from a normal distribution (Kolmogorov-Smirnov test for continuous variables, $P > 0.15$), and we used parametric tests following Sokal and Rohlf (1995).

We tested all used variables for a possible bias between years. Laying date, clutch size and nestling immune response differed significantly between years. In 2001 magpies started to reproduce earlier, laid larger clutches and nestlings had higher immune responses than in 1999 (Table 3). Therefore, except when comparing breeding values of the same female, we used standardized values to a mean of zero, by subtracting mean values of these traits for each of the study years.

As a body condition index we used residuals of body mass controlled for tarsus length. Body mass and tarsus length were strongly correlated ($R = 0.825$, $F_{1,178} = 378.9$, $P < 0.0001$), and residuals from this regression were not correlated with wing length (another body size indicator; $R = 0.017$, $F_{1,178} = 0.055$, $P = 0.81$). Therefore, the use of these residuals as a body condition index is appropriate (Green 2001).

To compare reproductive values of the same female in first and replacement clutches we used paired t-tests. At first, we compared reproductive values using all available duplicates for the respective variables (Table 1). However, since the final duplicate sample size of nests with fledglings was largely reduced, it is possible that this subset of nests did not represent the complete dataset. Therefore, we tested this possibility by analysing comparisons again only using this subset of nests.

To compare reproductive values of late season first clutches and replacement clutches, t-tests for independent variables were used. To look which of the measured variables explained variation in nestling immune response, we used a forward multiple regression with as explanatory variables clutch size, egg size and laying date, and nestling immune response as dependent variable. We performed this regression with first clutches and replacement clutches separated. All analyses were performed with the computer program STATISTICA (StatSoft, Inc.1998).

Results

Comparisons between first and replacement clutches of the same female

Mean values of different reproductive variables of first and replacement clutches, and results of paired t-tests, using all duplicates available for each variable (see Material and methods) are shown in Table 4. First clutch size was significantly larger than the replacement clutch size (Table 4), whereas mean egg size of the first clutch was significantly smaller than the mean egg size of the replacement clutch (Table 4). No other reproductive values differed significantly between the first and second reproductive attempts of the same female (Table 4). However, hatching success of the first clutch tended to be lower than that of the replacement clutch (Table 4).

This result might be interpreted as a consequence of the manipulation of first clutches (transporting eggs from the original nest A to the rearing nest B). However, differences in egg size between first and replacement clutches explained the significant variation in differences in hatching success between first and replacement clutches (Multiple Regression: $R^2 = 0.33$, $F_{2,17} = 4.11$, $P = 0.035$; partial regression coefficient differences in egg size: 0.57, $P = 0.01$; partial regression coefficient differences in clutch size: 0.04, $P = 0.83$). Moreover, hatching success of late-season broods tended to be lower than that of early season broods (Table 5). Therefore, although we cannot rule out methodological problems, the above regression suggests that the higher hatching success of replacement clutches was probably due to the larger size of those eggs.

For comparison of nestling condition and immune response, we obtained a final "duplicate" sample size of 14 pairs of nests. Then, to test whether this subset represented the whole dataset, we compared reproductive values between the first and the replacement clutches, using only the duplicates where fledging was reached (Table 5). In general, the same trends are observed with this subset of clutches, with the exception of clutch size, that only showed a tendency to be smaller in replacement as compared to first clutches (Table 5). Brood reduction tended to be higher in replacement

Table 3. Mean breeding values (\pm SE) from the breeding season in 1999 and 2001 and results of independent t-tests. Laying date: 1 = 1st of April.

	Mean 1999	Mean 2001	df	t	P
Laying date	33.3 \pm 1.5	26.0 \pm 1.3	134	3.52	0.0006
Clutch size	6.3 \pm 0.1	6.7 \pm 0.1	134	2.02	0.045
Egg size (cm ³)	9.74 \pm 0.13	9.78 \pm 0.10	123	0.26	0.78
Number of hatchlings	4.7 \pm 0.3	5.1 \pm 0.2	64	1.24	0.22
Percent hatchlings	71.6 \pm 4.3	74.2 \pm 3.2	64	0.49	0.62
Number of fledglings	3.6 \pm 0.2	4.1 \pm 0.2	64	1.29	0.20
Percent fledglings	55.0 \pm 3.65	58.4 \pm 3.12	64	0.68	0.49
Condition index	0.29 \pm 2.63	0.27 \pm 1.38	64	0.21	0.83
Immune response	0.82 \pm 0.40	1.03 \pm 0.51	64	2.42	0.018

Table 4. Mean (\pm SE) breeding values of first (1st clutch) and replacement clutches (2nd clutch) of the same female, and the results of paired t-tests. Mean (\pm SE) values of the difference between first and replacement breeding values of the same female are also shown. Laying date: 1 = 1st of April.

	1st clutch	2nd clutch	df	t	P	Mean difference
Laying date	19 \pm 0.9	39 \pm 1	60	34.97	< 0.0001	21 \pm 0.59
Clutch size	6.8 \pm 0.1	6.3 \pm 0.1	60	3.7	0.0005	1.0 \pm 0.1
Egg size (cm ³)	9.65 \pm 0.12	9.88 \pm 0.14	49	3.12	0.003	0.45 \pm 0.05
Number of hatchlings	4.95 \pm 0.29	5.45 \pm 0.23	19	1.46	0.16	1.14 \pm 0.23
Hatchling success (%)	71.6 \pm 4.3	80.4 \pm 2.6	19	1.90	0.07	17.7 \pm 2.7

clutches, though not significantly so (Table 5), and then, the number of fledglings was very similar in the first and replacement clutches of the same female (Table 5). In addition, a light tendency was observed for a lower body condition and immune response in replacement clutches compared to first clutches (Table 5). However, differences were far from significant. Even after controlling condition index for the number of nestlings in the nest (RES condition), and controlling immune response for the body condition index (RES immune), no significant differences appeared (Table 5).

Comparisons with late season first clutches

Results of unpaired t-tests between late season first clutches and experimentally induced replacement clutches, and late season first clutches and early season first clutches are given in Table 5. Results of t-tests were very similar using real data or data standardized for differences between years. Therefore, results are given with real data, to ease comparisons between different kinds of nests (Table 5).

Replacement clutches did not differ in laying date from late season first clutches. However, late season first clutches showed a clear tendency for lower breeding success as compared to experimental replacement

clutches. The first had significantly or marginally significant lower clutch size, number of hatchlings, hatching success, number of fledglings and fledgling success (Table 5). Brood reduction was very similar between both groups.

No significant differences were revealed in nestling condition index and immune response between the three groups of nests. However, a small tendency for a decrease in immune response (controlled for condition index: RES immune response) from early first clutches to replacement clutches to late season clutches could be detected. Condition index, after controlling for the number of nestlings (RES condition), was very similar in early season compared to late season broods, being higher than in replacement broods (Table 5).

Female trade-off in reproductive investment

Clutch size and egg size of first and replacement clutches of the same female were positively related (Linear Regression: clutch size: $R = 0.38$, $N = 61$, $t = 3.18$, $P < 0.001$; egg size: $R = 0.84$, $N = 50$, $t = 10.8$, $P < 0.001$). In general, there is no relationship between clutch size and egg size in magpies (Birkhead 1991). However, the number and size of the eggs may vary among females. Therefore, to demonstrate a trade-off between clutch size

Table 5. Results of paired t-tests comparing breeding values of first (1st clutch) and replacement clutches (2nd clutch) of the same female using only data from nests within the duplicates. Unpaired t-tests tests of differences in breeding values of late season first clutches (Late clutch), and experimentally induced replacement clutches (2nd clutch), and early season first clutches (1st clutch). Values are means (\pm SE). Laying date: 1 = 1st of April; RES condition = condition index controlled for the number of nestlings; RES immune = standardized immune response (for years) and controlled for condition index. P-values ≤ 0.1 are in bold.

	1st clutch	2nd clutch	Late clutch	Paired t-test: 1st–2nd clutch		Unpaired t-test: 2nd–Late clutch		Unpaired t-test: 1st–Late clutch	
				P	df	P	df	P	df
Laying date	18.5 \pm 2.5	38.6 \pm 2.57	35 \pm 2.5	< 0.001	14	0.37	25	< 0.001	25
Clutch size	7.1 \pm 0.3	6.7 \pm 0.2	5.9 \pm 0.4	0.24	14	0.07	25	0.027	25
Egg size	9.41 \pm 0.23	9.64 \pm 0.24		0.048	14				
N Hatchlings	4.6 \pm 0.4	5.4 \pm 0.3	3.7 \pm 0.4	0.11	14	0.004	25	0.11	25
% Hatchlings	66.1 \pm 5.5	80.0 \pm 4.4	61.2 \pm 6.2	0.044	14	0.018	25	0.56	25
% Brood reduction	13.3 \pm 4.4	26.5 \pm 5.3	26.4 \pm 7.9	0.10	14	0.99	25	0.15	25
N Fledglings	3.9 \pm 0.4	3.9 \pm 0.3	2.5 \pm 0.3	0.88	14	0.007	25	0.007	25
% Fledglings	55.9 \pm 4.4	57.4 \pm 4.5	43.1 \pm 5.5	0.82	14	0.054	25	0.078	25
Condition index	1.06 \pm 2.54	−4.01 \pm 3.20	5.63 \pm 3.29	0.25	13	0.051	24	0.28	24
RES condition	0.77 \pm 2.82	−4.51 \pm 3.31	0.68 \pm 3.07	0.24	13	0.25	24	0.98	24
Immune response	1.01 \pm 0.11	0.92 \pm 0.10	0.94 \pm 0.10	0.53	13	0.92	22	0.62	22
RES Immune	0.49 \pm 0.88	0.08 \pm 0.78	0.28 \pm 0.63	0.74	13	0.74	22	0.51	22

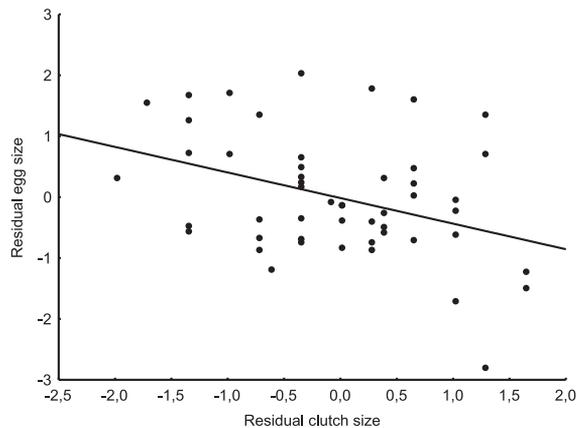


Fig. 1. Relation between variation in clutch size from first to replacement clutches (residual clutch size) and variation in egg size from first to replacement clutches (residual egg size). $Y = -0.01 - 0.42 X$

and egg size, comparisons of clutches of the same females are needed. If there is such a trade-off, variation in clutch size between first and replacement clutches of the same female should explain the variation in egg size. So, we used residuals of the two previous regressions as an estimation of variation in clutch size and egg size from first to second clutches of the same female (De Neve and Soler 2002). In accordance with the previously suggested trade-off between clutch size and egg size, variation in egg size between first and replacement reproductive attempts was explained by variation in clutch size, with a negative relation between both of them (Linear Regression: $R = -0.37$, $F_{1,48} = 7.83$, $P = 0.007$; Fig. 1).

Egg size and clutch size of replacement clutches explained the significant variation in nestling immune response, with laying date explaining additional variation (Forward Multiple Regression: $R^2 = 0.40$, $F_{3,16} = 3.56$, $P = 0.038$; partial regression coefficient for egg size = 0.71, $P = 0.009$; partial regression coefficient for clutch size = 0.69, $P = 0.014$; partial regression coefficient for laying date = 0.45, $P = 0.063$). However, no significant relationship appeared for first clutches, laying date being the only variable entering the analysis (Forward Multiple Regression: $R^2 = 0.06$, $F_{1,44} = 2.91$, $P = 0.09$; regression coefficient for laying date = -0.25). So, the resolution of the trade-off between clutch size and egg size in replacement clutches, favouring large eggs, possibly explained variation in nestling immune response.

Discussion

Food abundance for yellow-billed magpies *Pica nuttalli* in California was most abundant in April and May, and reached its lowest levels during the dry summer months

August and September (Verbeek 1973 cited in Birkhead 1991). Hence, although we do not have direct measures on food availability during the breeding season in our study area, replacement clutches and late season first clutches were most likely to be faced with poorer environmental conditions as first clutches. Consequently, if reproductive investment was unaffected by environmental conditions ("timing" hypothesis), reproductive success of replacement clutches would be lower than that of the first clutches of the same magpie pair. However, we found no support for this prediction. Breeding success (number of fledglings) of the same magpie pair in replacement clutches was similar to that of first clutches (reared by foster parents). In addition, different variables related to breeding success were significantly higher for replacement clutches than for late-season first clutches of other magpie pairs (Table 5). These results indicate that early breeders were of better parental quality than late breeders.

It can be argued that nestlings fledged from replacement broods and late-season first broods may experience a harsher environment during their post-fledging period. They will not only be younger and less experienced than early fledged birds, but they may also experience higher intra-specific and inter-specific competition for food. In fact, recruitment rate of late season fledglings has often been found to be lower than that of early season fledglings (Verhulst et al. 1995, Morrison 1998, Verboven and Visser 1998, Hansson et al. 2000, Naef-Daenzer et al. 2001), also in magpies (Birkhead 1991). Consequently, because offspring quality is an important factor influencing survival possibilities (Stark and Ricklefs 1998, Hórak et al. 1999, Christe et al. 2001, Dubiec and Cichon 2001), parents that want to compensate for the handicap of hatching and fledging late in the season, should provide their offspring with a significant fitness boost (Naef-Daenzer et al. 2001). However, we did not find support for this prediction, since nestling quality, in terms of immunocompetence and condition index, was similar between different kinds of nests at different stages of the season. Thus, although the replacement broods fledged a similar number of good quality nestlings compared to early first broods, nestlings were not provided with a fitness boost. In this context, differential investment in replacement clutches did not fully compensate for the effect of hatching late in the season. Still, another main cause of mortality during the early post-fledging period is predation (Naef-Daenzer et al. 2001), but there is no evidence of a higher predation probability of fledglings late in the breeding season for magpies. Hence, although parents probably did not fully compensate for the experimentally delayed breeding time, the significantly larger number of fledglings of similar quality in replacement clutches compared to late-season first clutches suggests that reproductive success of replacement clutches was larger than that of late-season

first clutches. In this perspective, high quality females did compensate for the negative effects of late breeding by raising a larger number of good quality offspring, whereas pairs of late season first clutches were not able to do so. So, the main advantage of breeding at the beginning of the season, for high quality pairs, apart from keeping the possibility of a replacement breeding attempt, would be better survival probabilities for fledglings (Birkhead 1991). In the case of a failure of the first breeding attempt, these pairs were able to reach a higher reproductive success in the replacement clutches, compared to pairs with a first breeding attempt late in the season, supporting the quality hypothesis in magpies.

One potential problem with our experimental design could be that the lack of differences in breeding success between first and replacement clutches were confounded by differences in parental quality (foster parents – real parents). However, laying date and clutch size of foster parents, that reared the nestlings from the first clutches, were matched to those of the real parents, and no differences in egg size and nest size were found between pairs of nests (Table 2). In addition, variances of reproductive success in the first and the replacement clutches were similar (see Table 4, Levene's test for homogeneity of variance: $F_{1,28} = 0.32$, $P = 0.58$), making a bias in parental quality unlikely.

Still, the lack of statistically significant differences in breeding success (number and quality of fledglings) between the first and the replacement clutches of the same female is somehow surprising. Females rearing a replacement clutch invested twice in egg production and incubated a few days more than other females. Variation in the previous reproductive effort between females was minimized because all magpie nests were experimentally depredated at the same stage, two or three days after clutch completion. In addition, the laying date of replacement clutches was on average delayed by 21 days with respect to the first clutches, which certainly would affect environmental conditions (e.g. lower food availability; Verbeek 1973, cited in Birkhead 1991, Sorci et al. 1997). These extra costs suffered by experimental females (e.g. Rosenheim 1999) could be the reason why nestlings of replacement clutches experienced lower, but not significantly so, condition index and immune response than the full siblings of first clutches (see Results). However, those costs were, at least partially, compensated for, because replacement clutches had larger reproductive success than late season first clutches with similar laying date.

The question then, is how magpies counteracted the costs related to late breeding for reproductive success? One likely answer comes from the result that magpie females changed their reproductive investment in replacement clutches by trading off their clutch size for larger eggs. An alternative explanation for this observed trade-

off between clutch size and egg size could also be that magpies trade-off clutch size with timing of breeding. By laying a small clutch, magpies could speed up their breeding attempt by starting incubation earlier. However, magpies do not start the incubation after clutch completion, but rather after the laying of the fourth egg, independently of clutch size. So, a direct link between clutch size and the start of incubation for speeding up breeding is unlikely, since magpies can start incubation independently of clutch size. In addition, if clutch size was explained by the timing of breeding, the clutch size of replacement clutches would be similar to that of late season first clutches (with similar laying dates). Although the clutch size of replacement clutches was not significantly larger than the clutch size of late season first clutches, our results seem to support that both timing and quality may play a role in determining clutch size, and that the seasonal decline in clutch size in magpies may probably be due to variation in female quality (Christians et al. 2001).

Larger eggs in replacement clutches mainly led to a higher hatching success in replacement clutches. In addition, offspring coming from larger eggs most likely experienced an advantage during the critical first days after hatch compared to offspring coming from smaller eggs. This is because in many species egg size may affect hatchling mass and nestling growth (Williams 1994, Belliure et al. 1999, Royle et al. 1999, Lipar and Ketterson 2000), and sometimes has persistent effects on nestling fitness traits (Smith and Bruun 1998, Styrsky et al. 1999, Hipfner 2000, Styrsky et al. 2000). Moreover, the amount and quality of nutrients (i.e. albumins) in the eggs may indirectly affect nestling immunocompetence mainly because of the well-known relationship between nutrition and immunocompetence (Chandra and Newberne 1977, Gershwin et al. 1985). Future nestling immunocompetence can also be mediated by differential allocation of carotenoids, special antigens or immunoglobulins into the eggs (Haq et al. 1996, Heeb et al. 1998, Gasparini et al. 2001, Blount et al. 2002), which in some cases has been shown to be related to intra-clutch egg size (Royle et al. 2001, Blount et al. 2002). Unfortunately, we do not have data on egg composition or the concentrations of such substances for magpies and consequently, we are not able to distinguish the possible effects of egg composition on the nestling immune response. Anyhow, clutch size and egg size of replacement clutches explained 40% of variation in nestling T-cell mediated immune response (see Results).

On the other hand, the parents could also increase the feeding effort to reach a similar breeding success as in the first clutches, because the amount and quality of food brought by the parents is a main factor influencing nestling condition and immunocompetence (Soler et al. 1996, 1998a, 2001, Saino et al. 1997, Palomino et al. 1998, Stark and Ricklefs 1998). We did not measure the

possible variation in feeding effort, which would be an interesting possibility for further research. Differential parental investment (i.e. feeding effort) may be correlated with a differential maternal investment (i.e. egg size). Since we did not manipulate these variables, we cannot distinguish between the relative importance of maternal and parental investment in reproductive success of replacement clutches. Still, our results suggest that the larger eggs of replacement clutches most likely helped compensating for the costs associated with rearing a brood late in the season. In addition, our findings are in agreement with some recent studies suggesting that female birds may trade-off their clutch size for larger eggs in late season broods or in areas with low food availability in order to improve survival possibilities of their offspring (Smith and Bruun 1998, Styrsky et al. 1999, Nilsson 2000, De Neve and Soler 2002).

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