Evolutionary Ecology Research, 2004, **6**: 415–431

Genetic, environmental and maternal effects on magpie nestling-fitness traits under different nutritional conditions: a new experimental approach

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ABSTRACT

Rearing full siblings under different environmental conditions allows partitioning of the total phenotypic variance of a trait into its genetic and environmental components. This, in natural bird populations, is usually achieved by cross-fostering experimental designs. We estimated genetic and environmental components of nestling-fitness traits using an alternative experimental approach in a magpie (*Pica pica*) population. Two broods of full siblings were reared under contrasting environmental conditions of first and replacement clutches. With this approach, potential maternal effects related to differences in clutch size and egg size could also be partially evaluated. In addition, the nutritional condition of half of the nestlings within each nest was manipulated by providing a calorie-rich paste enriched with micronutrients. Our results are only indicative because of very low sample sizes. In food-supplemented nestlings, the heritability estimates of tarsus length, body mass and T-cell-mediated immune response tended to be higher compared with control nestlings. No causal conclusions could be drawn for changes in heritability estimates of body mass and T-cell-mediated immune response; for tarsus length, the results suggest a lower potential to adapt to poor nutritional conditions. Furthermore, we found some indication that maternal effects related to clutch/egg size inflated causal estimates of phenotypic variance in tarsus length.

Keywords: body mass, food supplementation, immune response, magpie, maternal effects, *Pica pica*, quantitative genetics, tarsus length.

INTRODUCTION

Nestling body mass and the ability to mount an efficient immune response just before fledging provide a relatively good prediction of an individual's survival probabilities and are

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therefore closely related to fitness in birds (Lindén *et al.*, 1992; Dawson and Bortolotti, 1997; Hõrak *et al.*, 1999; Christe *et al.*, 2001; Merilä *et al.*, 2001; Naef-Daenzer *et al.*, 2001). Most phenotypic traits are the outcome of a combination of genetic, environmental and maternal components. However, selection will lead to evolutionary change only if a trait is heritable (Falconer, 1989). In natural bird studies, most phenotypic variation in nestling morphological traits has been found to be environmentally determined, with a weaker additive genetic component (Merilä, 1996; Sheldon, 1997; Potti *et al.*, 1999; Cadée, 2000; Christe *et al.*, 2000; Kunz and Ekman, 2000; Tella *et al.*, 2000b). Some genetic components of nestling immunocompetence have also been detected in poultry (Cheng and Lamont, 1988; Cheng *et al.*, 1991). However, these findings are not necessarily applicable to wild species (Sorci *et al.*, 1997a) and, to our knowledge, only two field studies found evidence for family-related components in T-cell-mediated immune responsiveness in wild bird populations (Saino *et al.*, 1997; Brinkhof *et al.*, 1999; Christe *et al.*, 2000; Tella *et al.*, 2000b).

Furthermore, life-history traits are often phenotypically plastic (Stearns, 1989). Heritability estimates and additive genetic variances for the same trait can vary with environmental conditions, implying that the rate at which traits evolve can vary under different environmental conditions (Hoffmann and Parsons, 1997; Merilä, 1997; Hoffmann and Merilä, 1999). A number of bird studies have found that heritability estimates of morphological traits are consistently lower in poor than in good environmental conditions (Larsson *et al.*, 1997; Merilä, 1997; Merilä and Sheldon, 1999; Kunz and Ekman, 2000), leading to the suggestion that adaptation under poor conditions may be constrained by lack of genetic variation (Merilä, 1997). However, information on possible differences in the expression of genetic variability under different environmental (nutritional) conditions for immunocompetence is lacking in the literature.

The aims of this study were: (1) to use an alternative experimental approach to evaluate causal components of phenotypic variation on nestling-fitness traits (body mass, tarsus length and T-cell-mediated immune response) in a population of magpies (*Pica pica*); and (2) to identify possible differences in the expression of genetic variability under different nutritional conditions.

The relative importance of additive genetic causes (including a quarter of dominance variance and maternal effects if present) as well as environmental causes of resemblance among siblings is usually evaluated in experiments where full siblings are reared in different environments (Emerson *et al.*, 1988; Falconer, 1989). Cross-fostering experiments are in general used to break the correlation between environmental and genetic components of variance in nestling traits (Merilä, 1996; Potti *et al.*, 1999; Christe *et al.*, 2000; Kunz and Ekman, 2000; Sheldon, 2000; Tella *et al.*, 2000a). Here, we experimentally induced some magpie pairs to lay a replacement clutch, through removing their first clutch soon after clutch completion, leaving it in the nest of another magpie pair (matched by laying date and clutch size). The removed clutch was then incubated and reared by foster parents. Extra pair paternity is very rare in our magpie population (Parrot, 1995). Then, in this way we obtained two clutches from the same magpie pair with full-sibling nestlings reared under conditions of first and second reproductive attempts, by parents of similar quality in terms of laying date and clutch size.

Recently, more and more evidence has emerged that maternal effects, through clutch size and differential investment in egg size and egg composition, can significantly influence nestling growth and development (Sanz, 1997; Smith and Bruun, 1998; Styrsky *et al.*, 1999;

Lipar and Ketterson, 2000), as well as the development of the immune system (Heeb *et al.*, 1998; Gil *et al.*, 1999; Cunningham and Russell, 2000; Gasparini *et al.*, 2001). However, because in cross-fostering experiments nestlings are exchanged several days after hatching, possible confounding maternal effects acting before the exchange cannot be taken into account. In the case of magpies, females differentially invest in replacement clutches compared with first clutches by trading-off their clutch size for larger eggs (De Neve and Soler, 2002). By using the current experimental approach, possible maternal effects on nestling traits related to differences in clutch size and/or egg size among females, and for the same female in first and replacement clutches, can be partially taken into account.

In addition, throughout the nestling period, we experimentally manipulated the nutritional status of half of the nestlings in each nest by providing them with a dose of high-calorie paste. In this way, causes of phenotypic variation in different nestling traits can be estimated under different nutritional conditions.

MATERIALS AND METHODS

Study area and species

The experiment was conducted in spring 2001 in 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 nest (for a more detailed description, see Soler, 1990).

Magpies, distributed throughout large parts of the Holarctic region, are territorial, sedentary and relatively long-lived for passerine birds (for an extensive review of their well-described biology, see Birkhead, 1991). In spring from March to May in their Western European range, they lay a single clutch ranging in size from 3 to 10 eggs (Birkhead, 1991). In the case of predation during egg laying or early incubation, magpies can lay a replacement clutch, normally close to the original nest (Birkhead, 1991; Sorci *et al.*, 1997b). Replacement nests as well as 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; De Neve and Soler, 2002).

In our study area, magpies frequently suffer brood parasitism by the great spotted cuckoo (*Clamator glandarius*) (J. Soler *et al.*, 1995, 1999; M. Soler *et al.*, 1998); however, parasitized nests were not used in the present study.

Experimental procedure

The experiment was performed during the breeding season of 2001. When the building of each new magpie nest was finished, we visited the nest at least twice a week to record the laying date. When brood parasitism occurs, magpies sometimes eject great spotted cuckoo eggs and sometimes eject their own damaged eggs (M. Soler *et al.*, 1997, 1999). Therefore, during the laying period, we visited nests every 2 days to detect possible brood parasitism and to ensure that no eggs were missing or damaged during egg laying. Two or three days after clutch completion, we matched nests and transferred one of the clutches (magpie pair A) to the nest of the other magpie pair (magpie pair B). In this way, we simulated

predation in the nest of magpie pair A, inducing the pair to build a new nest and lay a replacement clutch. Magpie pair B incubated and reared the offspring from magpie pair A. We measured the eggs of magpie pairs A and B when carrying out the experiment. The clutch from pair B was removed and used for other experiments. One week after removing the eggs from magpie pair A, we started to look for the replacement nest and also visited the replacement nest every 2 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 real parents reared the second clutch.

After clutch completion, we also measured the eggs of the replacement clutch. The longest and shortest radius of the eggs was measured with a digital calliper (Mitutoyo, 0.01 cm accuracy). Egg size was calculated as the volume of an ellipsoid: $4/3\ \pi ab^2/1000$ (cm^3), where a is the largest radius and b the shortest.

Because the probability of building a replacement nest and laying a second clutch declines over the breeding season (Birkhead, 1991), clutches were experimentally removed only until 7 May. To record the hatching date and hatching success, we visited nests daily after the 18th day of incubation.

Throughout the nestling period, half of the nestlings in each nest received a food supplement, consisting of a high-calorie paste enriched with essential micronutrients (minerals, vitamins and amino acids; 5 calories per gram; 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, we weighed and marked each hatchling with a colour on the tarsus. Subsequently, hatchlings were ranked according to their weight. Starting with the heaviest or second heaviest hatchling (alternating between nests), half of the hatchlings, intermittent according to their weight, were assigned the food supplement (hereafter control and food-supplemented nestlings, respectively), consisting of 0.1 ml of the high-calorie paste. Afterwards, we visited nests every 2 days and re-coloured the tarsus of all nestlings, and gave the food supplement to the nestlings assigned the treatment during the first visit (seven times over the nestling period). Based on the manufacturer's instructions, the dose and frequency of the food supplement were calculated for the mean weight of magpie nestlings at 8 days of age (50 g).

About 4 days before fledging, when nestlings were about 16–17 days old, we ringed, measured tarsus length (digital calliper, accuracy 0.01 cm) and weighed (Pesola spring balance, accuracy 0.5 g) all nestlings in the nest.

A phytohaemaglutinin (PHA-P, Sigma Chemical Co.) injection was used to evaluate the *in vivo* T-cell-mediated immune response of the 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 and Lomb Co.). The left wing web was injected with 0.1 ml of 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). Measurements of each wing web on each occasion were repeated three times, and the mean was used in subsequent analyses.

For a total of 43 clutch removal experiments, we found 35 (81%) complete replacement clutches. However, due to a high rate of brood parasitism of experimental nests (34%) and predation of eggs and nestlings in unparasitized experimental nests (35%), the final sample

sizes of duplicated nests with first and replacement fledglings of the same female were largely reduced (seven duplicates).

Statistical analyses

Full-sib analyses

Three kinds of analyses were performed to test for the effect of the food treatment, as well as genotype–environment interactions and genetic and environmental components on nestling-fitness traits.

To test for differences between food-supplemented and control nestlings in T-cell-mediated immune response, body mass and tarsus length (dependent variables), we used a two-factor analysis of variance (ANOVA) in which nest was considered a random factor and food treatment (i.e. food-supplemented and control nestlings) a fixed factor. Degrees of freedom of the error term were estimated using the Satterthwaite method (Sokal and Rohlf, 1995). We were then able to estimate the influence of the food treatment on nestlings while taking into account variation among nests, using nests as independent data points (see degrees of freedom in Table 1). With this analysis, a significant interaction of nest × food treatment would indicate that the influence of the food treatment on nestling traits differed among nests.

Genetic variation in reaction norms to the treatment, manifested as genotype–environment interactions, was estimated using mixed-model analyses of variance. Nest of rearing (i.e. first and replacement clutches) and nest of origin (i.e. duplicates: two full clutches of the same magpie pair) were considered random factors, and food treatment (i.e. food-supplemented and control nestlings) a fixed factor. The following interactions were estimated using this model: (1) The interaction origin × food represents the interaction between the nest of origin (two full clutches of the same magpie pair) and the food treatment. This interaction determines whether the influence of food treatment on nestling-fitness traits was affected by the genotype, independent of the nest of rearing (i.e. first and replacement clutches). (2) The interaction rearing × food determines if the influence of food treatment on nestling-fitness traits depended on the rearing conditions of first and replacement clutches (nest of rearing). (3) The origin × rearing interaction establishes whether the influence of rearing conditions of first and replacement clutches (nest of rearing) on nestling-fitness traits depends on the genotype (nest of origin). The interactions origin × food and origin × rearing are genotype–environment interactions. Also, the three-way interaction origin × rearing × food was estimated in this model. The three-way interaction indicates that genotype–environment interactions are affected by environmental conditions.

To estimate possible differences in genetic effects under different environmental conditions (i.e. nutritional condition due to the food treatment), we performed two-factor random-nested analyses of variance (main factors: nest of rearing nested within nest of origin). The analyses were performed on food-supplemented nestlings and control nestlings separately. In these analyses, the nest of origin represents the two full broods of the same magpie pair, and estimates half of the additive genetic variation ($\frac{1}{2}V_A$), but also includes one-quarter of the dominance variance ($\frac{1}{4}V_D$) and maternal effects if present (V_{M1}). We use the term 'additive genetic component' hereafter to refer to the nest of origin (including part of the dominance variance and maternal effects). The nest of rearing (nested within nest of

origin) estimates environmental variation (V_E) due to different rearing conditions of the first and replacement clutches; but also maternal effects, if present, due to differential maternal investment in first and replacement clutches (V_{M2}). Error variance equals random environmental variations – that is, individual environmental variance (V_{Ei}) plus $\frac{1}{2}V_A$ and $\frac{3}{4}V_D$. Thus, we calculate the total phenotypic variance by summing all variance components. The broad-sense heritability (including maternal effects and dominance variance in V_A) is defined as $h^2 = V_A/V_P$ (Falconer, 1989). Variance components were calculated by equating the observed mean squares to the expected mean squares, and negative variance components were set to zero. Standard errors of heritability estimates were calculated using the jackknife technique as described by Sokal and Rohlf (1995). All analyses were performed using StatSoft (1998), module 'Variance Components'.

Maternal effects

Egg size and clutch size from first and replacement clutches were used as an estimate of female investment and were subsequently introduced as covariates into the previous two-factor nested ANOVA designs. In this way, we can explore whether maternal effects related to the variation in egg size/clutch size explained part of the effect of rearing environment or part of the family-related effect on different nestling traits. In addition, we examine whether maternal effects related to clutch/egg size differed for food-supplemented and control nestlings.

Egg size and clutch size not only differ among females (nest of origin), but also differ – though to a lesser extent – for the same female (differential investment in first and replacement clutches, nest of rearing) (De Neve and Soler, 2002). Then, although variation within the same clutch cannot be included in the analyses, variation among the nests of rearing from the same female exists. If by introducing the covariates the variation explained by the nest of origin declines, this would indicate maternal effects related to clutch size and/or egg size among females on the nestling trait. On the other hand, if the variation accounted for by the nest of rearing declines, this would suggest that maternal effects related to differential investment in clutch size/egg size by the same female in first and replacement clutches affected the trait. If by introducing covariates the variance components do not change, maternal effects related to clutch size/egg size would not greatly affect the trait.

The frequency distributions of variables did not significantly differ from a normal distribution (Kolmogorov-Smirnov test for continuous variables, $P > 0.15$).

RESULTS

Effect of the food treatment and genotype–environment interactions

When variation among nests was taken into account, the food treatment had a marginally significant influence on the nestling T-cell-mediated immune response, though not on nestling tarsus length or nestling body mass (Table 1). Nestlings receiving the food supplements showed a stronger T-cell-mediated immune response (Table 2).

However, for all three traits, a significant interaction between the nest and the food treatment appeared (Table 1). This interaction suggests that the effect of the food treatment on nestling traits differed among nests and could involve possible genotype × environment interactions.

Table 1. Results of two-factor analyses of variance testing for differences in T-cell-mediated immune response, tarsus length and body mass between food-supplemented and control nestlings

	d.f. effect	MS effect	d.f. error	MS error	*F*	*P*
Immune response						
Food treatment	1	4812	12.85	1406	3.42	0.09
Nest	13	3304	12.14	1268	2.60	0.05
Food × nest	13	1218	28	569	2.14	0.04
Tarsus length						
Food treatment	1	0.73	13.78	9.78	0.07	0.79
Nest	13	31.38	12.22	8.52	3.68	0.01
Food × nest	13	8.16	28	3.47	2.35	0.03
Body mass						
Food treatment	1	308	12.23	682	0.45	0.51
Nest	13	804	12.27	635	1.26	0.34
Food × nest	13	607	28	243	2.50	0.02

Note: Nest is a random factor and food treatment (i.e. food-supplemented and control nestlings) a fixed factor. Degrees of freedom of the error term were estimated using the Satterthwaite method.

Table 2. Population marginal means (± standard error) of T-cell-mediated immune response, tarsus length and body mass of food-supplemented and control nestlings

	Food-supplemented ($n = 30$)	Control ($n = 26$)
Immune response (mm)	1.12 ± 0.05	0.97 ± 0.05
Tarsus length (mm)	47.00 ± 0.37	47.20 ± 0.40
Body mass (g)	142.6 ± 3.1	138.9 ± 3.3

Genotype–environment interactions were explored in a mixed-model ANOVA, in which we considered nest of rearing and nest of origin as random factors and food treatment as a fixed factor (see Materials and methods). Fledgling body mass and tarsus length showed a significant three-way interaction of origin × rearing × food. This indicates that two-way interactions were affected by the third factor and so genotype–environment interactions were affected by rearing conditions for these traits. T-cell-mediated immune response showed a marginally significant origin × food interaction, with a separate significant origin × rearing interaction (Table 3). The origin × food interaction indicates that nestlings invested the food supplement in a different way in their immune system, depending on their origin (Fig. 1).

Heritability under different nutritional conditions

Control nestlings showed a small additive genetic component for tarsus length, though not for body mass or T-cell-mediated immune response. The heritability estimate for tarsus length was low with a large standard error (Table 4). On the other hand, food-supplemented

Table 3. Results of genotype–environment interactions in a mixed-model ANOVA

	d.f. effect	MS effect	d.f. error	MS error	*F*	*P*	VC	VC%
Immune response								
Origin × food	6	2043	6.20	670.2	3.05	0.09	354.5	17.2
Rearing × food	1	0.037	5.49	631.3	0.00	0.99		
Origin × rearing	6	4691	5.66	588.0	7.98	0.01	1116	54.4
Origin × rearing × food	6	587.5	28	569.2	1.03	0.42	10.1	0.5
Body mass								
Origin × food	6	609.6	5.76	719.5	0.85	0.58		
Rearing × food	1	2.431	5.64	719.1	0.00	0.95		
Origin × rearing	6	889.1	5.87	675.4	1.32	0.37	58.1	9.9
Origin × rearing × food	6	662.5	28	242.6	2.73	0.03	231.9	39.4
Tarsus length								
Origin × food	6	8.63	6.00	9.93	0.87	0.56		
Rearing × food	1	2.79	6.33	9.47	0.29	0.60		
Origin × rearing	6	14.6	6.00	9.93	1.47	0.33	1.28	3.9
Origin × rearing × food	6	9.93	18	3.47	2.86	0.03	3.57	26.2

Note: Nest of origin and nest of rearing are considered random factors and food treatment a fixed factor. VC is the absolute value of the variance component of the interaction. VC% is the proportion of total phenotypic variation explained by the interaction. Negative values were set to zero and are not given in the table.

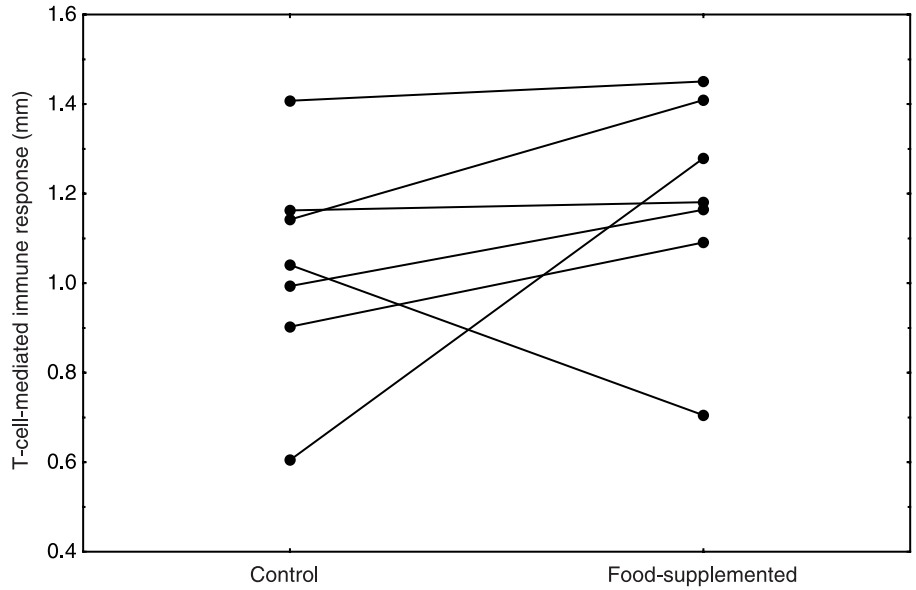

Fig. 1. Genotype–environment interaction (nest of origin × food treatment) for nestling immune response as revealed by a mixed-model ANOVA. Each line represents one nest of origin. Two-way interaction: $F_{6,6} = 3.05$; $P = 0.09$.

nestlings showed additive genetic components for all three traits, though only reaching significance for tarsus length. Heritability estimates were still low for body mass and nestling immune response, though considerably increased for tarsus length (Table 4).

By introducing covariates (egg size and clutch size) into the previous two-factor nested analyses of variance, potential maternal effects related to clutch size/egg size on nestling-fitness traits could be partially considered. Covariates did not explain significant variation in T-cell-mediated immune response (Table 5). For both control and food-supplemented nestlings, variance components of T-cell-mediated immune response were generally maintained after introducing covariates (Table 4). However, heritability estimates were low with large standard errors in both cases.

On the other hand, nestling body mass showed a tendency for opposite effects when introducing covariates (Table 4). In control nestlings, clutch size explained significant variation in body mass (Table 5). Total phenotypic variance largely declined due to a fall in the variation of the nest of rearing. This suggests that maternal effects related to differential investment in clutch size/egg size by the same female in first and replacement clutches affected nestling body mass. For food-supplemented nestlings, covariates did not explain significant variation in body mass. Total phenotypic variance in body mass was similar when introducing covariates; however, variation accounted for by the nest of origin was set to zero. This suggests maternal effects related to differences in clutch/egg size among females.

Furthermore, clutch size and egg size explained significant and marginally significant variation in tarsus length for control and food-supplemented nestlings, respectively (Table 5). The additive genetic component for tarsus length disappeared for control nestlings, and decreased considerably for nestlings receiving the food supplements, resulting in low heritability estimates (Table 4). This result indicates that maternal effects related to clutch size/egg size among females affected tarsus length.

Table 4. Heritability estimates ($h^2 \pm$ standard error) for magpie nestling T-cell-mediated immune response, body mass and tarsus length as estimated from two-factor nested analyses of variance (nest of rearing nested within nest of origin)

	Control nestlings					Food-supplemented nestlings				
	$h^2 \pm$ SE	V_A	V_E	V_{Ei}	V_P	$h^2 \pm$ SE	V_A	V_E	V_{Ei}	V_P
Without covariates										
Immune response	—	—	1.95*	0.36	2.31	0.19 ± 0.15	0.21	0.18	0.72	1.11
Body mass	—	—	540*	209	749	0.22 ± 0.07	98	74	268	440
Tarsus length	0.25 ± 0.23	2.9	5.1*	3.37	11.37	0.61 ± 0.10	8.54*	1.96	3.54	14.04
With covariates										
Immune response	—	—	1.73*	0.36	2.09	0.13 ± 0.21	0.13	0.18	0.72	1.02
Body mass	—	—	83	209	292	—	—	158	268	426
Tarsus length	—	—	1.07*	3.37	4.44	0.28 ± 0.16	2.74	3.63	3.54	9.91

Note: The number of 'nest of origin' is 7. V_A = additive genetic variance component (including ¼ dominance variance and potential maternal effects). V_E = environmental variance due to the nest of rearing (first and replacement clutches). V_{Ei} = individual environmental variance. V_P = total phenotypic variance. Covariates are egg size and clutch size. * $P < 0.05$.

Table 5. Fixed effects of covariates (clutch size and egg size) introduced in two-factor nested analyses of variance (nest of rearing nested within nest of origin) of food-supplemented and non-food-supplemented nestlings

	Non-food-supplemented		Food-supplemented	
	F	*P*	*F*	*P*
Immune response				
Clutch size	0.52	0.48	0.83	0.39
Egg size	0.23	0.64	1.97	0.21
Body mass				
Clutch size	19.81	0.001	0.16	0.69
Egg size	1.69	0.25	1.08	0.34
Tarsus length				
Clutch size	48.67	<0.001	4.02	0.08
Egg size	13.37	0.035	1.19	0.32

Note: Degrees of freedom for nest of origin = 6; degrees of freedom for nest of rearing = 5.

DISCUSSION

Heritability under different nutritional conditions

Rearing full siblings under different environmental conditions allows partitioning of the total phenotypic variance of a trait into its genetic and environmental components. While in natural bird populations this is usually achieved by cross-fostering experimental designs (e.g. Merilä, 1996, 1997; Christe *et al.*, 2000), in the present study we estimated genetic and environmental components of nestling-fitness traits using an alternative experimental approach. In our model, every nest of origin represented two nests of full siblings reared under conditions of first and replacement clutches (nests of rearing). Cross-fostering experiments use variation between sibling and non-sibling nestlings reared in the same environmental conditions. Since in those analyses the nest of origin is nested within the nest of rearing, information about how siblings develop in different environments is lost. Given that the relative estimation of different components explaining variation in a trait relies on knowing how full siblings develop that trait under different environmental conditions (Falconer, 1989), we think that our experimental design has some advantages. With the current approach we were also able to take into account potential confounding maternal effects related to differences in clutch size and/or egg size. Furthermore, we manipulated nutritional condition for half of the nestlings within each nest by providing high-calorie paste enriched with essential micronutrients.

We estimated genetic and environmental components of variation in nestling body mass, tarsus length and T-cell-mediated immune response. In addition, we also explored possible

changes in heritability estimates under different nutritional conditions (food-supplemented and control nestlings). Heritability estimates of morphological traits in vertebrates have been generally found to be lower under poor (or stressful) than favourable conditions (e.g. Merilä and Sheldon, 1999), although in some studies with *Drosophila melanogaster* the opposite pattern was found (Imasheva *et al.*, 1999; de Jong and Imasheva, 2000; Fowler and Whitlock, 2002). Brood-manipulation experiments are usually employed and provide an indirect way of altering food availability for each nestling (Larsson *et al.*, 1997; Kunz and Ekman, 2000). However, in the present study we addressed whether nutritional status is really a factor underlying heritability changes, and whether the pattern of lower heritability estimates under poorer nutritional conditions also applies to immunocompetence (Hoffmann and Parsons, 1997). Our results reveal no additive genetic variation for body mass and T-cell-mediated immune response for control nestlings (unmanipulated nutritional status), and only a low genetic component for tarsus length. Very low additive genetic components for T-cell-mediated immune response have also been found in several recent studies with other species (Christe *et al.*, 2000). Phenotypic traits closely related to fitness, as is the case for body mass and immunocompetence, usually show low heritability estimates (Mousseau and Roff, 1987). However, low heritability of fitness traits does not necessarily mean that there is no additive genetic variation in these traits, but rather that this variation may be masked by higher environmental variances (Merilä and Sheldon, 1999). In our study, environmental variation among different nests of the same origin (first and replacement clutches) was probably substantial (21 days mean difference in laying date), which could have masked any variation explained by additive genetic components in nestling body mass and T-cell-mediated immune response. Nevertheless, for both of these traits, an additive genetic component was revealed in food-supplemented nestlings; however, this was not significant and with low heritability estimates. Still, the additive genetic component and heritability estimate for tarsus length increased considerably in food-supplemented nestlings. Therefore, although heritability estimates should be deemed indicative because of low sample sizes and high standard errors (especially for T-cell-mediated immune response), our results suggest that heritability estimates for both morphological traits and immunocompetence changed under different nutritional conditions.

The proximate cause for lower heritability estimates in control nestlings compared with heritability estimates in food-supplemented nestlings might have been that additive genetic components of variance were smaller, or that additive genetic components were similar accompanied by a larger environmental variance in control nestlings (Hoffmann and Parsons, 1997; Merilä and Fry, 1998). We found some evidence for the first reason, since the genetic variance was nil (body mass and immune response) or lower (tarsus length) in control nestlings compared with nestlings receiving the food supplements. However, environmental variation (differences between first and replacement clutches; V_E) was repeatedly lower in food-supplemented nestlings (see Table 4). A third explanation for heritability changes between two environments could be the presence of genotype–environment interactions, which exist when different genotypes have different phenotypic responses to environmental variation. In those cases, the adaptation is promoted in heterogenous environments, leading to the maintenance of genetic variation (Merilä and Fry, 1998). Genotype–environment interactions may be caused by cross-environment genetic correlations less than one (different genes control the expression of genetic variation under favourable versus poor conditions) and by differences in additive genetic variance.

Cross-environment genetic correlations, however, do not imply a lower genetic potential to evolve under poor nutritional conditions, but suggest that different genotypes may be favoured under different conditions. Hence, only lower additive genetic variance suggests lower potential to evolve (Merilä and Fry, 1998). We found a marginally significant genotype–environment interaction with respect to the food treatment (origin × food) for nestling T-cell-mediated immune response (see Fig. 1). This suggests that the way in which nestlings invested the food supplements for development of the immune system was related to their genetic origin. However, it was not possible to distinguish between the potential causes of this genotype–environment interaction (cross-environment genetic correlations less than one, or differences in additive genetic variation). Nor could we calculate cross-environment genetic correlations, since no additive genetic variation in nestling T-cell-mediated immune response was revealed for control nestlings (see Table 4). This was probably due to low sample sizes. Thus, because of the genotype–environment interaction revealed for nestling T-cell-mediated immune response, differences in heritability estimates for T-cell-mediated immune response could be caused either by a change in additive genetic component or by cross-environment correlations. The first would suggest a lower potential to evolve under poor nutritional conditions. The second would indicate that different genotypes are favoured under different nutritional conditions for this trait. Genetic plasticity for morphological traits has been found in some bird populations (Gebhardt-Henrich and Van Noordwijk, 1991; Merilä, 1997), though not in others (Smith and Wettermark, 1995; Merilä, 1996). To our knowledge, only one study has found a non-significant genotype–environment interaction for nestling house martin (*Delichon urbica*) immunocompetence in response to a manipulation of the intensity of nest infestation with an ectoparasite (Christe *et al.*, 2000).

Maternal effects

One potential complication in estimating additive genetic variance with full-sib analyses may be confounding maternal effects, since they could inflate additive genetic variance and heritability estimates. Maternal effects are usually thought to be small, although it cannot be ruled out that considerable variance among full siblings might occur before the exchange of nestlings in cross-fostering experiments. This variation may be due to common environmental or maternal effects, which are subsequently indistinguishable from additive genetic effects (V_A). In a population of collared flycatchers (*Fidecula albicollis*), Merilä (1997) found that heritability estimates for tarsus length from parent–offspring regressions were consistently lower than those from full-sib analyses, suggesting that early common environmental or maternal effects were present for this trait. Also, differences in initial size, due to hatching asynchrony, might differentially affect nestling fitness, and inflate the estimates of V_A (Saino *et al.*, 2001). In the present experiment, this kind of variation was partially controlled for, since variances were calculated from complete broods of full siblings, containing their natural size hierarchies and reared under different environmental conditions. In addition, brood size and differences in competition among nestlings might affect fitness traits. We did not manipulate brood size, though clutch size was positively related to brood size; this was only marginally significant because of low sample size (Spearman $R = 0.51$; $t_{12} = 2.08$; $P = 0.06$).

Furthermore, a growing number of studies have provided clear evidence that within-clutch variations in egg size and egg composition can affect nestling-fitness traits not only

during the early nestling period, but also at fledgling age (Haq *et al.*, 1996; Lipar and Ketterson, 2000; Sockman and Schwabl, 2000; Royle *et al.*, 2001; Blount *et al.*, 2002). In the present study, we were not only able to partially control for potential maternal effects related to differences in clutch size/egg size among females (variation added to the nest of origin), but also within females, due to differential investment in first and replacement clutches (variation added to the nest of rearing) (De Neve and Soler, 2002). In accordance with the findings of Merilä (1997), our results indicate that maternal effects related to variation in clutch size/egg size among females were confounded with causal estimates of additive genetic variance on tarsus length. Total phenotypic variance, additive genetic variance and heritability estimates were largely reduced when introducing covariates, for both nestlings receiving the food supplements and those that did not. The same tendency was revealed for body mass, though this could be discerned only in food-supplemented nestlings, since control nestlings showed no additive genetic component for body mass. On the other hand, we found no support for the idea that maternal effects related to clutch size/egg size would have affected estimates of additive genetic variance of nestling immune response.

Furthermore, it is also quite possible that the contribution of maternal effects to the resemblance between relatives may vary under different environmental (nutritional) conditions and may generate erroneous indications about the magnitude and difference in heritability estimates (Merilä, 1997). Hence, by introducing covariates (egg size/clutch size), any possible environment-dependent expression of maternal effects could also be revealed if differences were found between food-supplemented and control nestlings. However, we found no direct support for this prediction, since the same tendencies in the additive genetic component and total phenotypic variation were found for food-supplemented and control nestlings when introducing covariates. Still, a tendency for opposite effects was revealed for nestling body mass. Our results suggest that maternal effects related to differences in clutch/egg size within females (first-replacement clutches) affect body mass of control nestlings, whereas there was a tendency for maternal effects related to clutch/egg size among females for food-supplemented nestlings (Table 4). In addition, clutch size explained significant variation in body mass and tarsus length for control nestlings, while this was less the case or not at all for food-supplemented nestlings (Table 5). This effect was especially noticeable in the larger decrease in total phenotypic variance in control nestlings when covariates were introduced in the analyses (Table 5). Still, it is worth mentioning that we have no information on the covariation between egg size and egg composition in magpies. Therefore, since the amount of nutrients and other health-related components may vary in part independent of clutch size and egg size (e.g. Fernie *et al.* 2000), not all maternal effects related to clutch size/egg size were considered.

These results, in any case, underline the idea that maternal effects related to clutch size/egg size and/or differential maternal investment can confound causal estimates of the hypothetical genetic and environmental components of morphological traits in nestling magpies.

In conclusion, our results indicate a change in heritability estimates of magpie-nestling tarsus length and body mass under different nutritional conditions. These changes are probably caused by a higher additive genetic component under better nutritional conditions. Then, magpies might have a lower potential to adapt to poor nutritional environments for nestling tarsus length and body mass. In addition, our results indicate that maternal effects related to clutch size/egg size confounded causal estimates of phenotypic

variance in tarsus length of magpie nestlings. On the other hand, an additive genetic component of the T-cell-mediated immune response was revealed only in food-supplemented nestlings. Because a marginally significant genotype–environment interaction was revealed, the change in heritability of the T-cell-mediated immune response under different nutritional conditions might be caused by a change in the additive genetic component or by cross-environment genetic correlations less than one.

Because the sample sizes in this study were very low and the standard errors of heritability estimates were large, our conclusions must be considered with caution. However, this alternative experimental approach, given also the tendencies we found with respect to maternal effects, provides a new perspective for estimating genetic and environmental components of phenotypic variation of fitness traits in natural bird populations under different nutritional conditions. However, studies with other species are necessary to provide support for our findings.

ACKNOWLEDGEMENTS

This study was supported by the European Community (Postgraduate grant project ERBFM-BICT983079 to L.D.N.), by 'Dirección General de Enseñanza Superior e Investigación Científica' (project PB97-1233-CO2-O2 to J.J.S. and M.S.) and by the research group 'Comportamiento y Ecología Animal' (CV207 to T.P.C.). We thank N.B. Davies for a linguistic check of the manuscript. We are grateful to D. Martín-Galvez for helping with the fieldwork.

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