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Environmental and genetic variation in the haematocrit of fledgling pied flycatchers *Ficedula hypoleuca*

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Abstract We report a field study of the haematocrit of pied flycatcher (*Ficedula hypoleuca*) nestlings when close to fledging. First a descriptive study was conducted of both fledgling and adult haematocrit over 2 years to analyse correlates of variation in this trait. Then a swapping experiment was performed to see whether variation among fledglings had a measurable genetic component. Average fledgling haematocrits were lower than those of their male and female parents. Intraclass correlations among sibships in fledgling haematocrit were high in both years, indicating that the estimates of resemblance were inflated, probably by common environmental effects. Fledgling haematocrits were unrelated to date and number of young in the nest. Fledglings with a high haematocrit were heavy and had thick breast muscles. There were no significant relationships between the average fledgling haematocrit and those of the adults caring for them. Nest mite ectoparasites negatively affected fledgling haematocrit. The haematocrits of adults did not differ between sexes or years and in both sexes were unrelated to breeding date, body mass, age, clutch size or number of young reared. Females, but not males, caring for fledglings in nests infested by mites had a lower haematocrit than those rearing young in mite-free nests. The cross-fostering experiment indicated that almost all measured variation in haematocrit was explained by the nest where the bird was reared (67.2% of the explained variance), not by their nest of origin (7.8%), meaning that there was a very small, non-significant resemblance in the haematocrit of genetically related sibs when reared in different environments while unrelated nestlings reared in the same nestbox had similar haematocrits. The low proportion of variance explained by the familial component may be due to the high connection of haematocrit to fitness.

Key words Birds · Blood · Cross-fostering experiment · Heritability · Repeatability

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Introduction

The haematocrit, or packed cell volume in relation to the volume of whole blood, is increasingly being used as an index of physiological performance in studies of wild bird populations (Brown 1996). Many researchers have assumed that haematocrit levels reflect condition and disease status of an animal (Merilä and Svensson 1995; Svensson and Merilä 1996; Yamato et al. 1996; Dawson and Bortolotti 1997a; Merino and Barbosa 1997; Saino et al. 1997a, 1997b; Wanless et al. 1997). Low haematocrit values may indicate anaemia, and may therefore be related to difficulties in oxygen uptake and transport (Phillips et al. 1985) and thus in anabolic metabolizable energy which may impair flight performance and affect survival. In adult birds, an increase in the haematocrit is considered to be an adaptive response which enhances the uptake and efficiency of transfer of oxygen to the muscular tissues during spells of intense muscular activity (e.g. Saino et al. 1997a). Furthermore, a linear relationship between haematocrit and heart weight has been observed, indicating an adaptation of heart mass to changes in cardiac output and haematocrit to meet the demands of increased basal metabolic rate (Yahav et al. 1997).

There is less information about the causes and consequences of variation in the haematocrit of nestling birds, the main focus of researchers having been the implications of blood haemoglobin content, and thus haematocrit, in the thermoregulatory capacities of chicks (Shilov 1973, cited in O'Connor 1984; Bech and Klaasen 1996). In addition, recent work has shown that, as in adult birds, the haematocrit of nestling birds is negatively affected by blood-sucking ectoparasites due to draining of considerable amounts of blood (Johnson et al. 1991; Johnson and Albrecht 1993; Hurtrez-Boussès et al. 1997; Merino and Potti 1998; Moreno et al., in press). This may have dramatic effects on nestling survival in some well-studied systems (e.g. Richner et al. 1993).

Here we report a field study of the haematocrit of pied flycatcher (*Ficedula hypoleuca*) nestlings when close to fledging. We first made a descriptive study of both fledglings and adults haematocrit over 2 years to analyse correlates of variation in this trait. We then asked whether the variation we observed might have a measurable genetic component. To our knowledge, the genetic component of variation in blood parameters has never been studied in a wild bird population, although the issue has been addressed in poultry because the haematocrit value of broilers may be inherited and can serve as an indicator of partial resistance to the ascites syndrome in cold-stressed broilers (Shlosberg et al. 1996). To address the estimation of heritability in a free-ranging bird population we conducted a swapping experiment in one year to partition the variance in haematocrit into familial and environmental components. We chose this approach because estimates of genetic variation are difficult to obtain under natural conditions, although the resemblance between relatives may be estimated, because higher resemblance between related individuals may arise from a variety of non-genetic causes (Falconer 1989; Boag and van Noordwijk 1987; Cheverud and Moore 1994; Roff 1997; Lynch and Walsh 1998). For example, common rearing environments and maternal or paternal effects may potentially be responsible for a similarity beyond that due to the effect of shared genes (e.g. Schluter and Gustafsson 1993; Potti and Merino 1994; Potti 1999a). Swapping eggs or young between nests, i.e. breaking the correlation between genetic and environmental effects, may reveal these kind of effects (e.g. James 1983; Alatalo and Lundberg 1986; Rhymer 1992; Smith 1993; Thessing and Ekman 1994; Smith and Wettermark 1995; Gustafsson and Merilä 1994; Merilä 1996).

Methods

General methods

A pied flycatcher population breeding in an old oak (*Quercus pyrenaica*) forest in central Spain was studied in 1994, 1995 and 1997 (Potti and Merino 1994; Merino and Potti 1995, 1998). On day 13 of nestling age (hatching date = day 1) all nestlings were measured for tarsus length (distance between bending points) to the nearest 0.05 mm with a dial calliper, and weighed to the nearest 0.1 g, using a spring balance. To obtain a measure of the growth of skeletal muscles in fledglings we scored the combined thickness of the pectoralis major and supracoracoideus muscles (breast muscles hereafter) on the right side of the fledgling's breast with portable ultrasound thickness equipment (Krautkrämer Branson, USK 7B) using a 10-MHz contact transducer (e.g. Newton 1993; Winkler and Allen 1996; Aparicio 1998).

Blood samples were taken from the wing vein of most nestlings by venipuncture. A capillary tube was immediately centrifuged at 11,500 rpm for 8 min in a portable centrifuge (Compur 1101, Bayer diagnostics Ltd., Germany) to obtain the haematocrit value.

In 1994 and 1995, parents were captured while incubating (females) or feeding nestlings (males), and they were measured (tarsus, mass) and bled in the same way as fledglings. They were aged following criteria in Karlsson et al. (1986) and Potti and Montalvo (1991). As an index of breeding phenology the laying date, i.e. the date of appearance of the first egg in the clutch, was used. Only unmanipulated nests that were not used for other experiments were included in computations, as other studies in the same population have shown significant effects of experimental treatments on fledgling haematocrit (Merino and Potti 1998; Moreno et al., in press; J. Potti and O. Frías unpublished work; see Discussion).

To score ectoparasite abundances in nests, we used categorical records of mite (*Dermanyssus gallinoides*) abundance (low or high) recorded on the day the measurements of fledglings were made. These scores were highly predictive indices of intensities of mite infestations as shown by mite counts in Berlese funnels (Merino and Potti 1995, 1996). After the nestlings fledged the nest material was dismantled to score for presence or absence of buried *Protocalliphora azurea* blowfly pupae (Merino and Potti 1995).

Within-nest repeatabilities (R) of haematocrit values were estimated by calculating the intraclass correlation coefficient, i.e. the ratio of the among-nests variance to the sum of both the among-nests and within-nest variances, expressed as a proportion. The intraclass correlation thus estimates the fraction of the total phenotypic variance attributable to factors causing resemblance between sibs of the same family. Variance components were estimated by one-way ANOVA (Sokal and Rohlf 1981; Lessells and Boag 1987). Only nests where more than one fledgling haematocrit were available were entered into these computations. In full-sib analyses, heritability is estimated as twice the intraclass correlation coefficient of chick measurements (Falconer 1989; Arnold 1994). The standard error of the intraclass correlation was calculated following Becker (1984). The repeatability was also used for assessing the measurement error of fledgling haematocrit. To that end, two capillaries were assayed for haematocrit in 94 fledglings in 1997 and the mean value was used in calculations.

To assess the stability or consistency of the haematocrit within adult birds as they age, which may give a cue on its broad-sense heritability (i.e. genetic variation, including non-additive variation) and its upper limit (Falconer 1989; Boag and van Noordwijk 1987) we also used the intraclass correlation coefficient. Repeatability is estimated by making repeated (at yearly intervals in this case) records in a sample of breeding adults and then calculating the ratio of the among-individual variance to the sum of both the among-individuals and within-individual variances (Falconer 1989; Boag and van Noordwijk 1987; Lessells and Boag 1987; Boake 1989). A high repeatability indicates that variation within individuals is much smaller than among different individuals. Repeatability is low if measurements within individuals are very different.

Cross-fostering experiment

In 1997, a cross-fostering experiment was conducted to separate environmental and familial components of variance in fledgling haematocrit values. Broods were created where approximately the same numbers of nestlings from two different families were raised together in the same nest. The experiment was restricted to nests with original clutch sizes of five to seven eggs, those most common

in our population. We established nest triplets with the same hatching date (±1 day) and clutch size. On day 1 of nestling age (day 0 being the hatching day of most of the brood), two or three nestlings were reciprocally transferred in cotton bags between two of the nests in the triplet, the third one being left as a control for manipulation effects. Nest transfers were performed within 15 min. The down of hatchlings, fostered and unfostered, was distinctively clipped and their nails painted to allow individual identification until banding, on day 6 after hatching. As a result of this design, all nests remained with the original number of eggs hatched, so that breeding effort of adults and intra-nest competition were not manipulated (cf. Merilä 1996). Adult birds thus initially cared for two or three of their own chicks plus two or three fostered chicks, although mortality during the nestling stage due to starvation and total or partial predation by weasels (*Mustela nivalis*) and great spotted woodpeckers (*Dendrocopos major*) resulted in unbalanced sample sizes for both fostered and unfostered nestlings. Control nests allowed us to ascertain whether cross-fostering itself had any effect on both fledgling haematocrit and nestling mortality. Fledglings were bled at 13 days of age as described above, while some of their mothers (but not their fathers) were measured, weighed and bled on the same day as fledglings.

Genetic and environmental effects on offspring haematocrit were estimated using an approach similar to that of Smith and Wettermark (1995) and Merilä (1996). We used a two-factor mixed model nested ANOVA where the main effects, which were considered random effects, were duplicate (a pair of nests) and nest of origin (nested within duplicates). The term duplicate accounts for differences in fledgling haematocrit between pairs of nests. Within duplicates, the variation due to nest of origin estimates variation attributable to genetic transmission ($0.5V_A$) but also includes one-quarter of the dominance variance (V_D) and common environmental variance (V_{EC}), including any premanipulation parental effects (V_P) if present. The model was run in SPSS (Norusis 1991). Statistics are two-tailed.

Results

Fledgling and adult haematocrits

We measured the haematocrit of 222 fledglings in 54 nests in 1994 and 118 fledglings in 35 nests in 1995. There were no differences between years in average within-nest fledgling haematocrit ($F_{1,79} = 0.02$, $P = 0.89$). We first checked for the possibility that haematocrit values were influenced by sampling hour (Dawson and Bortolotti 1997a). There was no relationship between sampling hour and haematocrit in either fledglings or their parents (fledglings: $r = -0.05$, $n = 85$, $P = 0.63$; adult males: $r = 0.06$, $n = 121$, $P = 0.53$; adult females: $r = 0.04$, $n = 128$, $P = 0.65$). The measurement error of fledgling haematocrit was low, as shown by the high consistency of measurements in 94 fledglings assayed twice in 1997 ($R = 0.89$, SE = 0.02, $F_{93,187} = 17.13$, $P < 0.00001$). Values of within-nest fledgling haematocrits were significantly lower than those of their parents, either males (*t*-tests, $t = 18.05$, $P < 0.0001$) or females ($t = 16.60$, $P < 0.0001$).

One first approximation to the genetic and common environmental effects on fledgling haematocrit is calculating the intraclass correlation among sibships. The correlations were $r = 0.77$ ($F_{51,219} = 15.51$, $n_0 = 4.22$, SE = 0.04, $P < 0.0001$) in 1994 and $r = 0.72$ ($F_{27,110} = 11.11$, $n_0 = 3.94$, SE = 0.07, $P < 0.0001$) in 1995, which amounts to heritabilities of 1.54 ± 0.08 (SE) and 1.44 ± 0.14, respectively. As heritability ranges from 0 to 1, these high values indicate that the estimates are inflated, probably by common environmental effects. Within-nest averages of fledgling haematocrit were used in the remaining computations to avoid pseudoreplication.

There were no significant relationships between the average fledgling haematocrit and those of the adults caring for them ($r = -0.19$, $n = 69$, $P = 0.11$, and $r = 0.17$, $n = 65$, $P = 0.18$, for male and female parents respectively). Neither were fledgling haematocrits related to laying date ($r = 0.03$, $n = 79$, $P = 0.82$), maternal clutch size ($r = 0.11$, $n = 79$, $P = 0.36$) or number of young in the nest ($r = 0.15$, $n = 78$, $P = 0.20$). Fledglings with a higher haematocrit had thicker breast muscles ($r = 0.27$, $n = 80$, $P = 0.01$) and a higher mass ($r = 0.23$, $n = 80$, $P = 0.04$), but not longer tarsi ($r = 0.13$, $n = 80$, $P = 0.25$).

Nest mite ectoparasites negatively affected the haematocrit of nestling pied flycatchers ($F_{1,77} = 14.86$, $P = 0.0002$; Fig. 1). Although nests infested by blowfly larvae had chicks with lower haematocrits than those free of blowflies, the difference was not significant ($F_{1,74} = 1.84$, $P = 0.18$). There were no interactions (Merino and Potti 1995) between the two types of parasites affecting fledgling haematocrit (interaction term, $F_{1,74} = 0.75$, $P = 0.40$).

As for the haematocrits of adults, they did not differ between sexes or years (two-way ANOVA: for year factor, $F_{1,193} = 1.54$, $P = 0.22$; for sex differences, $F_{1,103} = 1.30$, $P = 0.26$; interaction year × sex, $F_{1,193} = 0.89$, $P = 0.36$). They were unrelated in both sexes to breeding date, body mass, age (Fig. 2), clutch size or number of young reared (in all tests, $P > 0.10$). However, females caring for fledglings in nests infested by mites had a lower haematocrit than those rearing young in mite-free nests ($F_{1,72} = 8.09$, $P = 0.006$, Fig. 2). The haematocrit of male parents did not differ in relation to

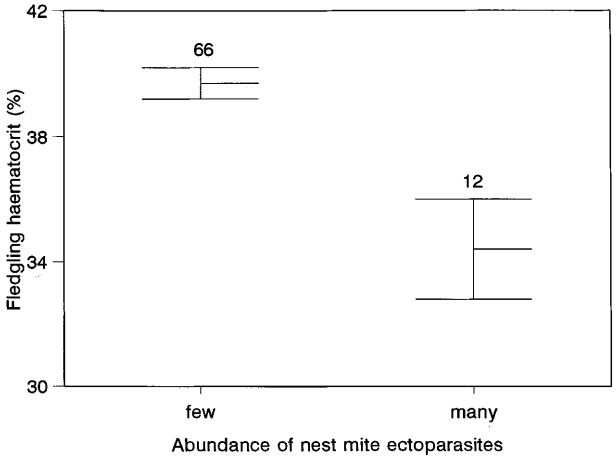

Fig. 1 Nest mite ectoparasites negatively affect the haematocrit of fledgling pied flycatchers: means ± 1 SE (*numbers* are sample sizes)

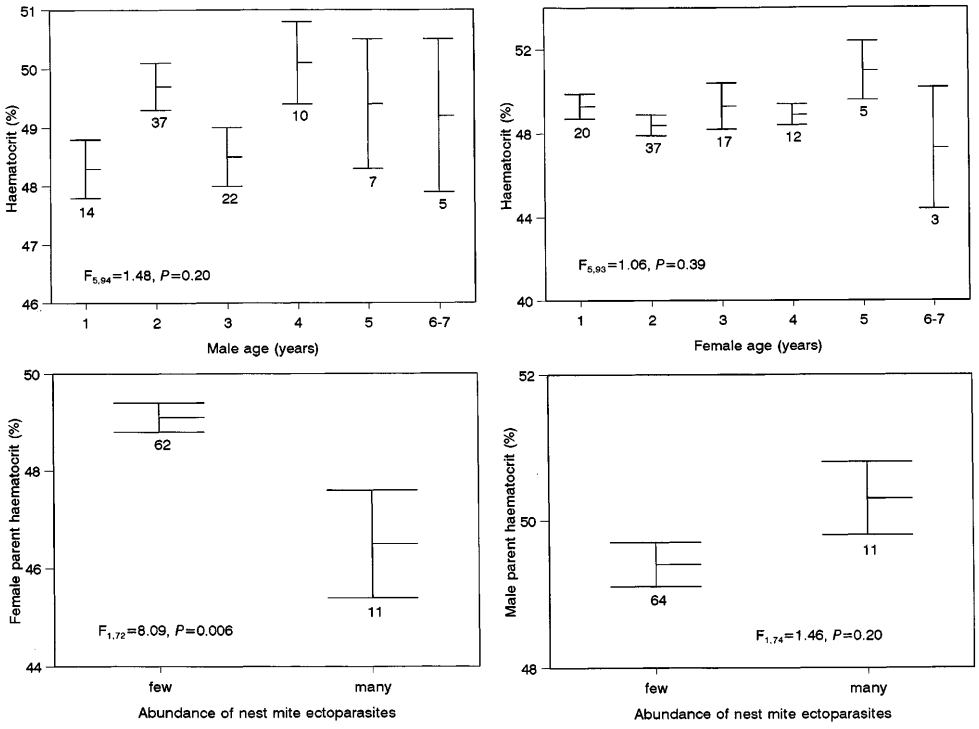

Fig. 2 Variation of the haematocrit of male and female parents in relation to age and their nest's status of parasitization by mites: means ± 1 SE (*numbers* are sample sizes)

their nests' status of parasitization ($F_{1,72}$ = 1.46, P = 0.23, Fig. 2). Neither did the haematocrits of male and female parents differ in relation to the presence or absence of blowfly larvae in their nests ($F_{1,71}$ = 1.99, P = 0.16, and $F_{1,71}$ = 2.48, P = 0.12, for males and females, respectively).

We recorded the haematocrit of 20 parents, 8 females and 12 males, which bred in 2 successive years. Haematocrit values were significantly repeatable across years in both sexes (females: R = 0.80, S.E. = 0.10, $F_{7,15}$ = 8.86, P = 0.003; males: R = 0.69, SE = 0.12, $F_{11,23}$ = 5.48, P = 0.003), indicating within-individual consistency in the haematocrit of adult birds. There was no relationship between the change in haematocrit and the change in mass between succesive years within birds in either sex (Spearman rank correlations, $P > 0.20$).

Cross-fostering experiment

In 1997, the year when the swapping experiment was done, there were no apparent effects of either mites or blowfly parasites on either fledgling tarsus length or mass ($F_{1,31}$ = 0.18, P = 0.68, and $F_{1,31}$ = 1.28, P = 0.27, respectively). This was probably due to their very low levels of infestation due to inclement weather conditions in that year (Merino and Potti 1996; S. Merino and J. Potti, unpublished work).

We first assessed whether moving hatchlings between nests had any effect on their haematocrit at fledging by comparing the mean haematocrits in control broods vs those of moved hatchlings. There was no effect of the experimental manipulation on fledgling haematocrit as these did not differ between non-fostered (mean ± SD 41.15 ± 5.19, n = 12) and fostered fledglings (mean 40.60 ± 5.90, n = 19; t-test, t = 0.26, P = 0.80). There was high chick mortality in 1997, with an average of 1.5 ± 1.5 (SD) chicks in experimental nests dying between hatching and the nestling age of 13 days, apparently due to starvation. However, for those nests where at least one young fledged, there were no differences between control and experimental nests in the number of chicks that died (respective means ± SD: 1.7 ± 1.3, n = 13, and 1.3 ± 1.3, n = 24; $F_{1,36}$ = 1.01, P = 0.32).

The two-factor nested ANOVA with main effects duplicate and nest of origin (nested within duplicates) indicated that all measured variation in haematocrit was explained by the nest where the bird was reared (67.2% of the variance), not by their nest of origin (7.8%), the error variance accounting for the remaining 25% (Table 1). This means that there was a very small, non-significant resemblance in the haematocrit of genetically related sibs when reared in different environments while unrelated nestlings reared in the same nestbox had similar haematocrits.

Discussion

We found that the haematocrits of fledgling pied flycatchers were lower than those of adult birds and were independent of date of the season and number of sibships, while being strongly affected by the presence of blood-sucking ectoparasitic mites in nests. There was an apparent strong component of environmental variation

Table 1 Results from two-way nested ANOVA of fledgling haematocrit in cross-fostered pied flycatcher broods where at least one offspring from each treatment survived until the day of measurement. The data are analysed in relation to rearing environment and family of origin (nested within rearing)

Source of variation	SS	*df*	MS	*F*	*P*	r^2(%)
Rearing	1992.72	13	153.29	6.82	< 0.0001	67.2
Origin	230.42	13	17.72	0.79	0.666	7.8
Residual	741.57	33	22.47			
Model	2223.14	26	85.51	3.80	< 0.0001	75.0

affecting fledglings' haematocrit in a nest, as reflected in the inflated heritability estimates of haematocrit values when using sibships in familial estimates of resemblance. This was experimentally confirmed by switching individuals soon after hatching, thereby breaking the correlation between genotypes and a common environment (Alatalo and Lundberg 1986; Merilä 1996, 1997; Gustafsson and Merilä 1994).

As in other studies in several avian orders (ostriches: Brown and Jones 1996; penguins: Merino and Barbosa 1997; raptors: Dawson and Bortolotti 1997a, 1997b; terns: Bech and Klaasen 1996), we also found that fledglings of pied flycatchers, a passerine species, had haematocrits significantly lower than those of adult breeding birds. This raises the question of whether the haematocrit value may be considered the same trait in fledglings and in adult birds, as occurs with some morphological measures (e.g. tarsus length; Alatalo and Lundberg 1986; Potti and Merino 1994). Even though we had not enough data to test whether the haematocrit of an individual fledgling is related to its haematocrit when adult, we found that the haematocrit was repeatable (i.e. consistent) within individual birds as they age. This accords with findings that individual condition, measured as body mass standardised with respect to tarsus length, is repeatable in pied flycatchers (Potti 1999a, 1999b). Furthermore, this index of condition was consistent between the fledgling and the adult age, supporting accumulating evidence that many components of individual performance may be determined early in life (reviewed in Bernardo 1996). In the case of haematocrit, there must be a "catch-up" of fledgling to adult values, but it is unknown at present whether adult values are dependent to some extent on nestling history (Horak 1994; Potti 1999a, 1999b).

The lower haematocrit in fledglings may partly be due to a constraint, namely the fact that the avian blood system is not yet fully developed in fledglings 13 days old (Jones and Johansen 1972; Hughes 1984). Alternatively, however, the haematocrit may be lower in fledglings because, as in molting adults, plasma volume expands to facilitate the vascularization of growing feather quills and this is not accompanied by an increase in number of erythrocytes (Chilgren and deGraw 1977). Yet another explanation may involve an adaptive component to the higher haematocrits of adults because the highly mobile, energy-demanding adults need to have a high haematocrit to meet increased energy demands during breeding (e.g. Merino and Barbosa 1997) while reduced activity of fledglings should be associated with low haematocrits. In nestling passerines, the blood haemoglobin content almost doubles from hatching until homeothermy is achieved, a change that has been interpreted as related to increased metabolism and capacity for heat production (O'Connor 1984; but see Bech and Klaasen 1996). Nestling pied flycatchers achieve chemical thermoregulation (i.e. by physiological heat production) around 8–10 days after hatching (O'Connor 1984). Haematocrit values have been interpreted as proportional to metabolic activity during periods of days to weeks preceding blood sampling (Carpenter 1975; Ots et al. 1998). Therefore, haematocrit values at fledgling may be integrated measurements of nestling growth and welfare, reflecting past conditions, including physiological constraints related to thermoregulation, and disease. Unfortunately, our knowledge of disease in relation to blood physiology in wild birds is still in its infancy and no studies can be used at present as a guidance to which range, among a wider spectrum of haematocrit values, may reflect health or disease situations in particular species in the wild. Higher than "normal" values may not always be necessarily good indicators of a prime health (e.g. Shlosberg et al. 1996), as they may instead indicate compensatory increases in concentration of red blood cells to cope with disease or even haematological disorder (e.g. human polycithemia; Erslev and Gabuzda 1985). In natural settings, however, we can expect that high haematocrits will more often be associated with health than with disease, as mortality from disease will probably severely bias sample sizes in favour of birds with prime condition-related high haematocrits.

Despite the relative paucity of previous information, there are, in fact, some studies indicating that high haematocrit values in wild birds are relatively good indicators of prime condition and health rather than being positively associated with disease. Svensson and Merilä (1996) found that birds with high levels of subcutaneous fat had high haematocrit values and low sedimentation rate levels, indicating that the haematocrit level of an individual is associated with its nutrition and state of health. Rattner et al. (1987) reported positive correlations between haematocrit and weight change during growth in ducklings. We found that fledglings in good condition as scored by their mass and development of breast muscles also had high haematocrits which were, however, unrelated to another measure of growth performance, tarsus length (Alatalo and Lundberg 1986; Potti and Merino 1994; Moreno et al. 1997). Fledgling mass is usually interpreted as a highly sensitive measure of growth conditions (van Noordwijk et al. 1988; Alatalo et al. 1990; Haywood and Perrins 1992; Potti 1999a, b), while the growth of tarsi is dependent to a higher degree on additive genetic variation (Alatalo and Lundberg 1986; but see Moreno et al. 1997). The within-nest correlation between fledgling mass, haematocrit and

width of pectoral muscle suggests that the haematocrit is also sensitive to conditions experienced during growth. In another study on the same population, Potti (1999b) found that fledgling mass was affected by both the quality of natal nests (presumably reflecting quantity and/or quality of food around its surroundings; Lundberg and Alatalo 1992, pp. 174–175) and the abundance of ectoparasitic mites (Merino and Potti 1995, 1996). Previously, we have found in experimental studies on the same population that supplementary food (mealworms) during the nestling phase raises the haematocrit of fledglings (Merino and Potti 1998; Moreno et al., in press; J. Potti and O. Frías, unpublished work) in relation to that of control birds, which was important in one year when blood loss was caused by blowfly larvae, corroborating the link between haematocrit, health and nutritional condition. With respect to the influence of parasites in the present study, intensities of nest parasitism (sensu Margolis et al. 1982) by both mites and blowflies were very low in the year of the swapping experiment (1997) due to the unusually cold weather in that year (see also Merino and Potti 1996). Hence we did not expect to find effects of parasites (Merino et al., in press), and this expectation was confirmed. However, the effect of mites on fledgling haematocrit was large in the other two study years, confirming the detrimental roles these parasites have on the growth of nestling pied flycatchers (Merino and Potti 1995, 1996). In any case, in the light of parasite, in particular mite, detrimental effects on fledgling haematocrit, the absence of negative effects in the year of the cross-fostering experiment simplified our analyses, by reducing the environmental variance, for the presence of genetic effects on fledgling haematocrit, the major question we were interested in.

There were no consistent significant relationships between the haematocrit of fledglings and those of their parents in both the natural and experimental settings. Relationships of either sign may be predicted: if traits are comparable in both age classes and haematocrit retains genetic variance we could expect to find a certain degree of parent-offspring resemblance. However, if environmental variance in haematocrit is large, as this study demonstrates, it well could be that nests where the fledgling haematocrit is low are those that are being tended by parents with high haematocrits due to, e.g., low breeding effort. Little is known about the relationships of the haematocrit to physiological costs of reproduction. By means of brood manipulation experiments, Horak et al. (1998) showed that parent great tits (*Parus major*) caring for enlarged broods had higher haematocrits than those whose brood size was decreased. They interpreted this finding as a response to the requirement of an elevated blood oxygen-carrying capacity during increased work load (see also Saino et al. 1997a), which would imply an adjustment of parental haematocrit to brood demand.

We found no evidence for the presence of significant additive genetic variation in fledgling haematocrit of pied flycatchers. Leaving aside non-additive genetic variance and epistatic effects as causes of resemblance, the high resemblance in haematocrit values between sibships sharing nests in natural situations that we observed is most likely due to a shared environment, as the experimental results indicated. A theoretical argument to explain low heritability of haematocrit is its high connection to individual condition, hence fitness, which makes likely the depletion of genetic variation due to strong and constant selection, with all remaining variation being environmentally caused (''Fisher's fundamental theorem''; Gustafsson 1986; Mousseau and Roff 1987; Roff 1997; see also Price and Schluter 1991).

The heritability in its narrow sense, i.e. the proportion of phenotypic variation in a trait which is due to the additive effects of genes, is computed as a ratio and thus variation in heritability values may be due to either variation in the numerator (genetic variance) or denominator (total variance, including environmental variance and non-additive genetic variance). Some observational and experimental studies on the heritability of morphological traits in wild birds have found considerable differences in the amount of additive genetic variance expressed under different environmental conditions. In general, it is not yet clear whether additive genetic variance of physiological and life-history traits is more likely to be expressed under stressful or benign conditions, and experimental work on a variety of taxa have pointed to both posibilities being almost as common (Hoffman and Parsons 1991). Overall, however, heritabilities of morphological traits in wild bird populations have been found to be lower in poor than in good environments (Gebhardt and van Noordwijk 1991; Larsson and Forslund 1991; Merilä 1996, 1997; Hoffman and Parsons 1997). When compared with long-term data, the year we made our cross-fostering experiment was poor from the standpoint of reproductive success and average measurements of fledglings, which were lower than long-term averages (J. Potti, unpublished work). The possibility remains, therefore, if there is differential expression of genetic variance in haematocrit under different environmental conditions, that some degree of additive genetic variance may be expressed in ''good'' years. Results of this study suggest, however, that the heritability of fledgling haematocrit should be very low.

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