Ibis (2007), **149**, 128–134

Haematocrit is weakly related to condition in nestling Barn Swallows *Hirundo rustica*

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Currently, there is no agreement about the suitability of haematocrit (the proportion of blood volume occupied by packed red blood cells) as a predictor of condition in birds. In order to clarify this point, genetic and environmental components of phenotypic variation for a number of traits were estimated in nestling Barn Swallows *Hirundo rustica* by means of a partial cross-fostering experiment. The studied traits were haematocrit, two morphological traits used as estimates of condition in birds (body mass and body mass relative to body size) and a morphological trait that presumably is not associated with condition (tarsus length). First, we found that body mass relative to body size was related to haematocrit, supporting the suggestion that haematocrit reflects condition in this species. Secondly, we found that the coefficient of residual (i.e. environmental) variation of haematocrit was larger than that of tarsus length, but smaller than those of body mass or body mass relative to body size. Under the hypothesis that traits closely related to condition (and, ultimately, to fitness) are characterized by large residual variance, these results also suggest that haematocrit is related to condition, but that this relationship must be weak. Therefore, the use of haematocrit as an estimate of condition is not recommended. Finally, heritabilities of the studied traits were calculated, differing significantly from zero only for tarsus length, the trait with the smallest residual variation. However, a consistent pattern in the relationship between heritability and genetic variation was not found.

Haematocrit is the proportion of blood volume occupied by packed red blood cells, and many bird studies have assumed that haematocrit levels reflect condition (e.g. Johnson & Albrecht 1993, Svensson & Merilä 1996, Simon *et al.* 2004). However, some studies have cast doubt on the utility of haematocrit as an estimate of condition, at least in birds. For example, studies on both adult (Dawson & Bortolotti 1997a) and nestling (Dawson & Bortolotti 1997b) American Kestrels *Falco sparverius* showed that haematocrit was a poor estimate of health, weight, nutritional status or food abundance. Although declines in avian haematocrit from malnutrition or disease have been well documented (e.g. Sturkie & Griminger 1986, Whitworth & Bennett 1992, Hurtrez-Boussès *et al.* 1997, Hoi-Leitner *et al.* 2001), the studies on American Kestrels suggest that haematocrit levels do not vary with condition when body condition is within the normal range. Supporting this idea, many studies in birds have not found significant relationships between haematocrit levels and factors presumably affecting condition, such as ectoparasites (e.g. Saino *et al.* 1998, O'Brien *et al.* 2001), or between haematocrit levels and estimates of condition (e.g. Villegas *et al.* 2002) or fitness (Bearhop *et al.* 1999).

Although studies of a direct relationship between haematocrit and estimates of condition in natural populations of birds have found contradictory results, one study using a completely different approach to the problem suggested a strong link between haematocrit and condition. By means of a partial cross-fostering

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experiment in nestling Pied Flycatchers *Ficedula hypoleuca*, Potti *et al.* (1999) found that the variation in haematocrit explained by the nest of origin (genetic variance) was small and non-significant. The authors concluded that this small genetic variance could be explained by a strong connection between haematocrit and condition (and, ultimately, fitness), because characters associated with fitness are subject to strong selection, which, in turn, will deplete genetic variation [Fisher's (1930) fundamental theorem of natural selection].

Potti *et al.*'s (1999) explanation of small genetic variance for haematocrit might, however, be misleading. As suggested by Price and Schluter (1991), and later corroborated by several studies investigating wild populations of birds (Kruuk *et al.* 2000, Merilä & Sheldon 2000, McCleery *et al.* 2004), traits closely related to fitness are characterized by large residual variance (residual variance is all phenotypic variance except additive genetic variance). By contrast, the relationship between fitness and additive genetic variance is far from clear, with some studies showing large additive genetic variance in traits closely related to fitness (Houle 1992, Pomiankowski & Møller 1995, Kruuk *et al.* 2000, Merilä & Sheldon 2000), and another showing no relationship (McCleery *et al.* 2004). In any case, traits closely related to fitness do not all show small additive genetic variances, and it is inappropriate to assume that a trait with small genetic variance is closely related to condition or fitness.

Although the conclusion reached by Potti *et al.* (1999) was not consistent with their results, the approach they used could still be useful to explore the possible relationship between haematocrit and condition. If haematocrit is strongly connected to condition, we would expect large residual (i.e. environmental) variance for this character, whereas small residual variance would be expected if the connection is weak. This prediction is based on the assumption that a trait closely related to condition will be also related to fitness, because traits reflecting condition will have a strong effect on survival and, hence, on fitness. With these ideas in mind, we studied genetic and environmental components of phenotypic variation for haematocrit and a number of morphological traits in nestlings of a passerine bird, the Barn Swallow *Hirundo rustica*. The aim of this study was to investigate the possible link between haematocrit and condition using two different approaches. First, we tested the relationship between haematocrit and an estimate of condition (body mass relative to body size). Secondly, we calculated residual variation for haematocrit, for morphological traits traditionally used as estimates of condition in birds (body mass or body mass relative to body size) and for a morphological trait that presumably is not associated with condition (tarsus length). If the link between haematocrit and condition is strong, we would expect the residual variance of haematocrit to be similar to that of body mass or relative body mass. By contrast, if there is no link between haematocrit and condition, we would expect the residual variance of haematocrit to be similar to that of tarsus length. Finally, estimates of additive genetic variance and heritability were also calculated, allowing comparisons between traits and tests for significant differences from zero, respectively.

METHODS

Field methods

The study was carried out near Badajoz, southwestern Spain, in 1995. For a description of the study area, see de Lope (1983). Barn Swallows are small insectivorous passerines (*c.* 20 g) that feed on the wing. European populations spend the winter in Africa south of the Sahara. For information on the general biology of this species see Cramp and Simmons (1988) and Møller (1994). Adult Barn Swallows were caught in mist-nets early after arrival on the breeding grounds (February–April), and every individual was measured and provided with a numbered metal ring and a unique colour combination of plastic rings. Barn Swallows bred colonially in farmhouses, colony size ranging from 19 to 50 breeding pairs. Nests were visited at least every second day to determine laying and hatching dates, clutch and brood size, and breeding success (number of nestlings surviving to the age of 12 days old).

In order to separate genetic and environmental components of variation in nestling phenotype, a partial cross-fostering experiment was conducted. Two broods hatching on the same day were matched and constituted a dyad. The experiment was restricted to nests with clutch size of 4–6 eggs, the most common in our population. Two randomly chosen chicks in one nest were exchanged with two randomly chosen chicks of the other nest of the dyad when chicks were 1 day old. Chicks of this age cannot be ringed, so they were individually marked with waterproof markers on their toes and claws. When chicks were 12 days old they were weighed (with a Pesola spring balance to the nearest 0.25 g), provided with a numbered metal ring, and the length of right and left tarsus measured (with a digital calliper to the nearest 0.01 mm). Tarsus length was determined as the mean value of right and left measurements. Simultaneously, a blood sample was taken from the brachial vein in a 9-μL capillary tube and centrifuged at 600 *g* for 7 min. The haematocrit was assessed as the proportion of capillary length occupied by packed red blood cells in relation to capillary length occupied by all blood components. Capillary tubes were measured with a 10× magnifying lens with measuring scale to the nearest 0.1 mm. Repeatability of nestling haematocrit was not estimated, but haematocrit of adult Barn Swallows in the same breeding season and obtained with exactly the same methodology was highly repeatable ($R = 0.91$, $P < 0.0001$; Saino *et al.* 1997). Only dyads with both nests having at least one original chick and one foster chick surviving to the age of 12 days were included in the analyses (21 dyads in total).

Statistical analyses

Genetic and environmental components of nestling phenotypic variation were calculated using mixed-model nested ANOVAs with type III sums of squares, following Merilä (1996) and Merilä and Fry (1998). The terms included in the model were dyad, nest of rearing, nest of origin, and the interaction between nest of rearing and nest of origin. All factors were considered as random effects. Nest of rearing, nest of origin and their interaction were nested within dyad. The term dyad reflected variation among pairs of nests (e.g. temporal variation), and nest of rearing reflected variation between nests of the same dyad, thus both representing environmental effects. The term nest of origin estimated genetic effects, specifically half of the additive genetic variance, one-quarter of the dominance variance and maternal effects. Statistica 6.0 (StatSoft 2001), the program used to perform these analyses, provided estimates of variance components using type III decomposition. All negative variance components were set to zero.

Our experimental design did not allow us to separate additive genetic variance from maternal effects. Nevertheless, the variance component of the term nest of origin was used as an estimate of half of the additive genetic variance. This estimate is reasonable for the case in which maternal effects and dominance variance are negligible. This may not be universally appropriate as some studies of Barn Swallows have shown that maternal effects may be important for traits such as immunity (Saino *et al.* 2002a, 2002b). However, immunity has not been investigated in the present study and the importance of maternal effects for haematocrit, weight or tarsus length in this species is unknown. Estimates of additive genetic variance may also be influenced by the presence of extra-pair paternity. We do not know if any of the broods included in the experiment had mixed paternity, but a study carried out in the same population showed that 17.8% of all offspring was due to extra-pair paternity (Møller *et al.* 2003). Maternal effects and extra-pair paternity would have opposite effects, either inflating or reducing, respectively, our estimates of additive genetic variance.

Phenotypic variance was assessed simply as the sum of all variance components, including error. Phenotypic variance minus additive genetic variance gave us an estimate of residual variance ($V_R = V_P - V_A$). In order to permit comparison of different traits, coefficients of variation (CV) were calculated (CV = 100 ($V^{0.5}/X$), where V is the variance and X the mean character value). Heritability (h^2) was calculated by dividing the additive genetic variance by the phenotypic variance ($h^2 = V_A/V_P$). Standard errors (se) of heritabilities were estimated using the jackknife method (computations were repeated with each pair of nests left out in turn; see Sokal & Rohlf 1981, pp. 796–797). Heritabilities were considered significant when $h^2 - 2\text{se} > 0$.

Tarsus length and haematocrit did not differ significantly from a normal distribution (Shapiro–Wilks' W-test, $P > 0.10$), but body mass of nestlings had to be x^3-transformed for normality before further analyses. Residuals from the regression of (transformed) body mass on tarsus length ($F_{1,181} = 15.15$, $P < 0.001$, $R^2 = 0.077$) were used as an index of body condition. The absolute value of the smallest residual was added to all other residuals to avoid negative values. This transformation was necessary because, otherwise, CVs of residual body mass could not have been calculated (mean value of residuals was zero and any number divided by zero is infinite). Residual body mass followed a normal distribution without transformation. Differences between CVs were tested using variance-ratio tests according to Zar (1996, pp. 144–146).

The relationship between haematocrit and condition was tested using the model described above (mixed-model nested ANOVA) with haematocrit as the dependent variable, but including the index of body condition (i.e. residual body mass) as a fixed effect covariate. We then followed a backward stepwise procedure, eliminating all variables and interactions that did not explain a significant amount of the variance from the model.

All statistical tests were two-tailed and the level of significance was set at 0.05.

RESULTS

The nest in which chicks were reared explained a significant proportion of the variance in all studied traits (haematocrit, tarsus length, body mass and residual body mass), suggesting a significant environmental effect on the phenotypic variation of these traits (Table 1). By contrast, the nest of origin (i.e. family) explained a significant proportion of the variance in all traits except haematocrit (Table 1). This result suggests a significant genetic effect on all traits except haematocrit. However, only tarsus length showed significant heritability, whereas body mass and residual body mass heritabilities were marginally non-significant, and haematocrit was far from heritable (Fig. 1). Coefficients of additive genetic variation (CV_A) for body mass and residual body mass were significantly larger than those of haematocrit and tarsus length (Fig. 2a). Coefficients of residual variation (CV_R) for body mass and residual body mass were also significantly larger than those of haematocrit and tarsus length (Fig. 2b), and CV_R for haematocrit was significantly larger than that of tarsus length (Fig. 2b). Finally, the relationship between haematocrit and residual body mass while controlling for environmental effects (i.e. variation among nests and between nests of the same dyad) was statistically significant (Table 2). We are aware that comparisons between tarsus length and residual body mass are not appropriate because the former was used to calculate the latter. This is the reason why we have also shown comparisons between tarsus length and body mass, yielding in all cases qualitatively similar results (Fig. 2).

Table 1. Results from mixed-model nested ANOVAS with haematocrit, tarsus length, body mass and residual body mass (body mass relative to body size) of nestling Barn Swallows as dependent variables, and nest of rearing and nest of origin (both nested within dyad) as factors. All effects are considered as random. Var. (%) is the percentage of variation accounted for by each factor in the model.

	df	*F*	Var. (%)
Haematocrit			
Dyad	20	2.41*	21.3
Nest of rearing (dyad)	21	2.27*	15.5
Nest of origin (dyad)	21	1.21	2.5
Nest of rearing × Nest of origin (dyad)	21	0.81	0.0
Error	99		60.6
Model	83	2.16***	
Tarsus length			
Dyad	20	0.66	0.0
Nest of rearing (dyad)	21	2.40*	12.0
Nest of origin (dyad)	21	4.56***	30.4
Nest of rearing × Nest of origin (dyad)	21	0.60	0.0
Error	99		57.6
Model	83	1.95***	
Body mass			
Dyad	20	2.15*	22.5
Nest of rearing (dyad)	21	4.40***	19.6
Nest of origin (dyad)	21	3.40**	13.8
Nest of rearing × Nest of origin (dyad)	21	0.53	0.0
Error	99		44.1
Model	83	3.15***	
Residual body mass			
Dyad	20	3.56**	38.2
Nest of rearing (dyad)	21	2.64*	13.0
Nest of origin (dyad)	21	2.12*	8.9
Nest of rearing × Nest of origin (dyad)	21	0.81	0.0
Error	99		39.9
Model	83	3.87***	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

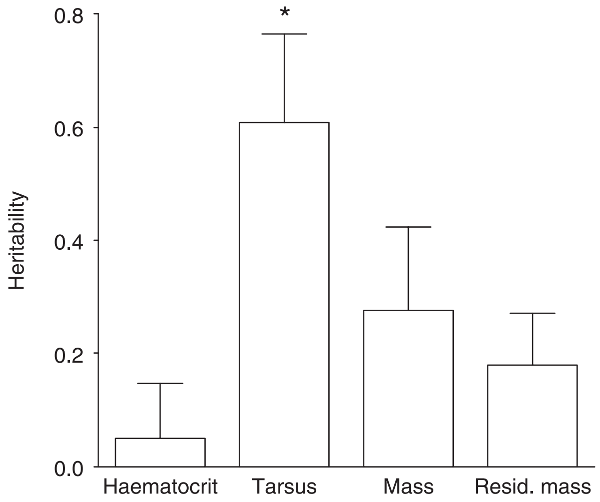

Figure 1. Heritability (+ se) of haematocrit, tarsus length, body mass and residual body mass (body mass relative to body size) in nestling Barn Swallows. An asterisk indicates heritability significantly different from zero.

DISCUSSION

One of the most interesting findings of this study was that residual body mass (an estimate of condition)

Table 2. Results from a mixed-model nested ANCOVA with haematocrit of nestling Barn Swallows as the dependent variable, nest of rearing and nest of origin (both nested within dyad) as factors, and residual body mass (body mass relative to body size) as a covariate. The original model also included the interaction between nest of origin and nest of rearing. All effects were considered as random except that of residual body mass. Factors that did not explain a significant amount of variance were removed from the model using a backward stepwise procedure, with the exception of nest of rearing, which explained a marginally non-significant amount of variance.

	MS/error	*df*	*F*	*P*
Residual body mass	102.3/14.3	1, 137	7.16	0.0084
Dyad	73.0/22.8	20, 21.4	3.21	0.0050
Nest of rearing (dyad)	22.9/14.3	21, 137	1.60	0.057
Model	53.2/14.3	42, 137	3.72	< 0.001

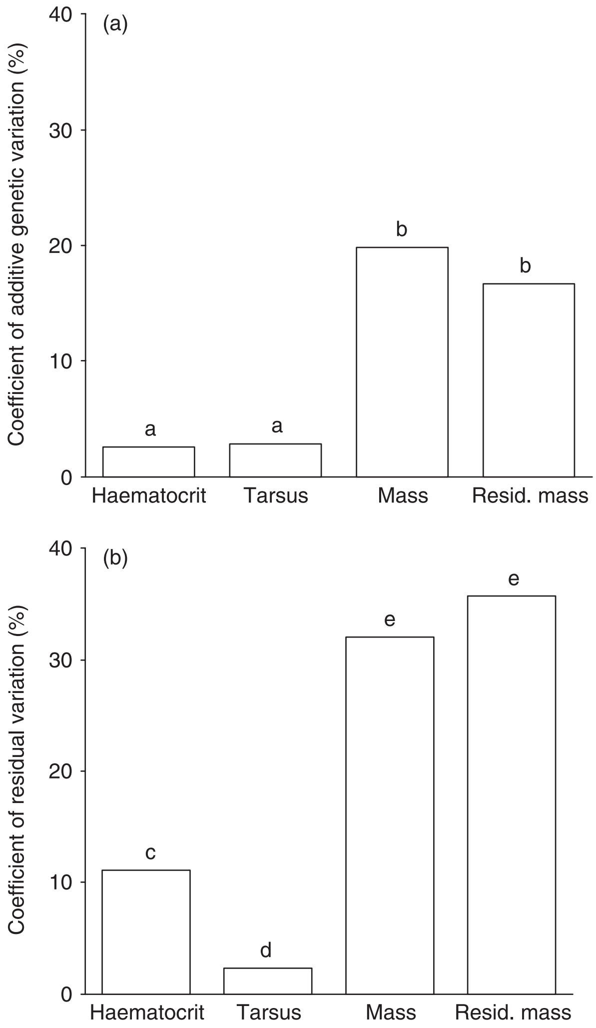

Figure 2. Coefficients of (a) additive genetic variation and (b) residual variation for haematocrit, tarsus length, body mass and residual body mass (body mass relative to body size) of nestling Barn Swallows. Different letters mean that the difference is statistically significant ($n_1 = n_2 = 21$, $Z \geq 4.14$, $P < 0.001$ in the nine cases). The same letter means that the difference is not significant ($n_1 = n_2 = 21$, $Z \leq 0.76$, $P > 0.4$ in the three cases).

was significantly related to haematocrit in nestling Barn Swallows, even when environmental effects were controlled for. This result supports the idea frequently assumed in avian studies that haematocrit reflects condition in birds (see Introduction). Our second approach to the problem, the comparison of residual variation (CV_R) between traits, can help to clarify this relationship. As expected, CV_R of traits closely related to condition (and therefore to fitness), such as body mass or body mass relative to body size, were significantly larger than CV_R of traits that presumably are not associated with condition or fitness (e.g. tarsus length). Most importantly, CV_R of haematocrit was larger than that of tarsus length, again supporting the link between haematocrit and condition. If haematocrit was unrelated to condition or fitness, we would expect similar CV_R for haematocrit and tarsus length, but that was not the case in our study. However, the CV_R of haematocrit was significantly smaller than those of body mass and body mass relative to body size, suggesting that the relationship between haematocrit and fitness was weaker than the relationship between body mass (absolute or relative) and fitness.

All these results taken together suggest that haematocrit is indeed related to condition in nestling Barn Swallows. However, this relationship is probably weak, which may help to explain the contradictory results found in the literature (see Introduction). A weak relationship would be consistent with some studies actually finding a significant relationship between haematocrit and estimates of condition or fitness, and other studies failing to find such a relationship. If this interpretation is correct, haematocrit would not be a suitable estimate of condition, and other phenotypic characters (e.g. body mass) should be preferred. Only if the sample size and/or variation in condition are large would the use of haematocrit as an estimate of condition be justified, preferably in addition to other estimates of condition. This conclusion could easily be tested in future studies, for example assessing the relationship between haematocrit and condition before and after experimentally manipulating variation in condition, preferably with large sample sizes, which would also allow comparisons between results with the full set or a random subset of data.

The study of additive genetic variation (CV_A) and heritability in haematocrit, tarsus length and body mass of nestling Barn Swallows also yielded interesting results. Haematocrit showed non-significant heritability and, consistent with Fisher's (1930) fundamental theorem, also showed relatively small CV_A. Moreover, haematocrit did not show very large CV_R, suggesting that low heritability was mostly due to small V_A. By contrast, tarsus length was the only trait with significant heritability, and, according to Fisher's theorem, a large genetic variation for that trait would be expected. Contrary to expectation, CV_A for tarsus length was as small as that of haematocrit, and smaller than those of body mass and residual body mass. High heritability of tarsus length was probably due to small V_R, i.e. to small environmental variation. This result is very similar to results from previous studies with Barn Swallows (Cadée 2000) and other bird species (Merilä & Sheldon 2000) also showing small CV_R and CV_A for tarsus length. The relationship between heritability and genetic variation in the traits investigated in this study did not seem to be straightforward as different patterns were found for different traits. The relationship between heritability and residual variation was not clear either, but the only trait showing significant heritability (tarsus length) had the smallest CV_R.

As pointed out in the Methods section, our estimates of additive genetic variance also included maternal effects and might be affected by extra-pair paternity. Consequently, our estimates of additive genetic variance, heritability and residual variance might be either overestimated or underestimated, depending on the strength of the different effects. If maternal effects were similar for all studied traits, the conclusions reached above would still apply, given that they were based on relative comparisons among traits. However, different levels of maternal effects for different traits cannot be ruled out. On the other hand, heritabilities were not compared with each other but checked for significant differences from zero. If heritability values were overestimated, the significant heritability of tarsus length would be a candidate result for such an effect, although tarsus length has been found to be significantly heritable in most avian studies (e.g. Merilä & Fry 1998, Gosler & Harper 2000, Merilä & Sheldon 2000), including Barn Swallows (Cadée 2000). If heritability values were underestimated, the marginally non-significant heritabilities of body mass and residual body mass might be actually significant, but this would not substantially affect our conclusions, as haematocrit would still remain far from heritable.

The low heritability found in this study for nestling haematocrit agrees with a previous study in Pied Flycatchers (Potti *et al.* 1999). However, a study of House Martins *Delichon urbica* found significant heritable variation for this trait (Christe *et al.* 2000). It has been suggested that environmental conditions might affect heritability, especially for morphological traits, with lower heritability in poor than in good environmental conditions (Merilä 1997, Charmantier *et al.* 2004). However, other studies have not found this pattern (Merilä & Fry 1998), and the relationship is even less clear for non-morphological traits (Hoffman & Parsons 1991). The three studies investigating heritability of haematocrit in birds (Potti *et al.* 1999, Christe *et al.* 2000, this study) were performed not only in different localities and years, but also in different species, making any comparison among them more difficult. Moreover, in the study by Christe *et al.* (2000), environmental conditions were experimentally manipulated, thus potentially increasing environmental variance components relative to the other two studies, and making heritability comparisons even less appropriate. Consequently, more studies are needed before drawing any general conclusion about the heritability of haematocrit in birds. It is also important to point out that the sample size in the current study is rather small (21 families), and, consequently, estimates of heritability should be considered with caution.

In summary, nestling Barn Swallows showed a significant relationship between haematocrit and body mass relative to body size, an estimate of condition, suggesting that haematocrit reflects condition in this species. However, the fact that CV_R of haematocrit was smaller than those of body mass or body mass relative to body size might indicate that the relationship between haematocrit and condition (and, ultimately, fitness) is weak. Consequently, the use of haematocrit as an estimate of condition in birds is not recommended. Finally, heritability was significant only for tarsus length, the trait with the smallest residual (i.e. environmental) variation, but there was no clear relationship between heritability and genetic variation.

We thank Juan Soler for his help with statistics and Tim Mousseau and two anonymous referees for comments that greatly helped to improve the manuscript. J.J.C. was supported by a postdoctoral grant from the European Union and the Spanish Ministry of Education and Science (BOS2001-1717) and F.d.L. by the Spanish Ministry of Education and Science (BOS2003-1713).

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Received 11 October 2005;
revision accepted 13 June 2006.