Variation in the hematocrit of a passerine bird across life stages is mainly of environmental origin

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The heritability of the hematocrit in adult, breeding pied flycatchers *Ficedula hypoleuca* is examined in a southern European population across seven years to see the consistency, or lack thereof, of patterns found with the trait at fledgling age where no significant heritability could be detected. While the across-years repeatability of the trait in adult, breeding birds was low but significant, heritabilities based on adult parent-adult offspring regressions controlling for assortative mating and full-sibling comparisons did not differ significantly from zero. Neither were heritability estimates affected by selection on fledgling hematocrit, as it was unrelated to local recruitment. There was no relationship between fledgling and adult hematocrit, suggesting that both behave as phenotypically plastic, different traits. This is the first study testing for the heritability of a physiological trait in birds with data from adult, free-ranging individuals.

Field studies on birds have provided information on variation in genetic parameters in natural populations, most dealing with heritability of morphological or life history traits (Merilä and Sheldon 2001, Artacho et al. 2005). Few studies have addressed genetic variation in physiological traits in adult individuals (Boulinier et al. 1997, Potti and Merino 1995). In evolutionary biology, a necessary condition for a trait to evolve is that it should be heritable, i.e. it should retain additive genetic variance (Lynch and Walsh 1998).

Hematocrit, packed cell volume in relation to the volume of whole blood, is a complex trait which has received increased attention in avian ecophysiology in the context of breeding (Hurtrez-Boussès et al. 1997, Potti et al. 1999, Cuervo et al. 2007). Causes and effects of variation in the hematocrit of growing and breeding birds have proven difficult to disentangle, some studies arguing for a direct relationship with body condition, and thus fitness (Brown 1996, Hurtrez-Boussès et al. 1997, Potti et al. 1999), and others implying that such a relationship is absent (Dawson and Bortolotti 1997, Cuervo et al. 2007). Larger hematocrit levels may indicate a faster rate of metabolism or an increased workload rather than

overall condition. Although little is known about the degree of genetic determination of hematocrit in wild bird populations, it is likely that it is under partial genetic control (e.g. Pravenec et al. 1997). For instance, in humans there is significant evidence for linkage of hematocrit to area 6q23–24 in chromosome 6, with heritability in healthy humans being estimated at 40–65% (Lin et al. 2005). Evidence for a significant additive genetic component to hematocrit in avian systems derives mostly from highly controlled, artificial poultry breeding systems, where heritabilities up to 80% have been reported (Shlosberg et al. 1996, 1998, but see Christe et al. 2000).

Here I address the quantitative genetic basis of hematocrit assessed in the wild in adult breeding birds of a migrant bird species, the pied flycatcher *Ficedula hypoleuca*. In a previous paper (Potti et al. 1999) we experimentally showed that the additive genetic component of fledgling hematocrit in a southern European population of pied flycatchers was minimal. Most of the variation in fledgling hematocrit was explained by the environment where the bird was reared, not by their nest of origin. Unrelated nestlings reared in the same nest had similar hematocrits. The data in this original

report were insufficient to test for a relationship between hematocrit, as measured in a fledgling, and its value in the same individual at adult. Here I test for this relationship and examine whether additive genetic variation in hematocrit is apparent in pied flycatchers as adults.

Methods

A pied flycatcher population breeding in nest boxes in an oak *Quercus pyrenaica* forest in central Spain was studied between 1993 and 2001 (see Potti et al. 1999). Only unmanipulated nests were included in analyses, as studies in the same population have shown significant effects of experimental treatments on fledgling hematocrit (e.g., Merino and Potti 1998). Adults were captured inside their nestboxes while incubating (females) or feeding nestlings aged 7–13 d and bled by veinpuncture of the wing vein. To obtain hematocrit, a capillary tube was immediately centrifuged at 11,500 rpm for 8 min in a portable centrifuge (Compur 1101, Bayer diagnostics Ltd., Germany). On day 13 fledglings were ringed and bled to determine their hematocrit, which for surviving birds was compared with that taken at later years. Ectoparasitic nest-mites *Dermanyssus* spp. significantly affect fledgling hematocrit (Potti et al. 1999) and their abundances were therefore controlled for in the ontogenetic analysis of hematocrit. Mite abundances in nests were visually estimated as low or high on the day the measurements of fledglings were made. These scores are highly predictive indices of the intensities of mite infestations, as shown by mite counts in Berlese funnels (Merino and Potti 1995). The exact age of many breeding adults was known due to natal philopatry (Potti and Montalvo 1991a), or inferred as one-year old or older based on plumage characteristics (Potti and Montalvo 1991b); for analyses, bird age was categorized into these two latter classes as using the full age range (1 to 8 years old) did not alter conclusions but decreased statistical power. No data on hematocrits were available for males in 1993 or for any adult bird in 1997.

To standardize for individual and sex variation in the hematocrit along the study period I performed a mixed-model ANOVA (SAS Institute 2004) of hematocrit data with bird identity as a random factor and year, sex and sex by year interaction as fixed factors. Results showed a significant effect of year ($F_{6, 185} = 12.58$, $P < 0.001$) and a significant sex by year interaction ($F_{5, 185} = 8.57$, $P < 0.001$). Therefore, for heritability analyses I standardized the data by subtracting from each value the yearly within-sex average. As some individuals had up to four records of their hematocrit, in different years, their data were averaged. This was then compared between parents and their offspring by

least square regression. Use of single-sex offspring to calculate heritabilities gave no additional information to that presented with both sexes grouped. Using data for offspring and one parent, the doubled regression coefficient (b) estimates the heritability ($h^2 = 2b$), while b estimates heritability ($h^2 = b$) when the mid-parent value is used. I also tested for assortative mating that can bias single-parent heritability estimates. To correct for this, I used the expression $h^2 / (1 \pm r)$, where r is the phenotypic correlation between parental values (Merilä and Sheldon 2001).

The stability or consistency of the hematocrit within adult birds as they age was estimated by the repeatability (R , the intraclass correlation coefficient), i.e. the ratio of the among-individuals variance to the sum of both the among-individuals and within-individuals variances, expressed as a proportion (Boag and van Noordwijk 1987, Lessells and Boag 1987, Merilä and Sheldon 2001). Similar rationale may be used to estimate heritability from familial data on hematocrit of siblings at the adult age. With data on full-sibs, $h^2 = \frac{1}{2} R$. Data on half-sibs were insufficient to make a meaningful analysis. The standard error of R was calculated with the approximation of Becker (1984); in Merilä and Sheldon 2001). Means are given ± 1 SD.

Results

My data set consists of 704 readings of hematocrit in 236 males and 271 females. I obtained a single hematocrit measurement for 170 males and 187 females, and 2 to 4 measurements, in different years, for an additional 66 males and 84 females.

A restricted maximum likelihood, mixed-model ANOVA with bird identity as a random factor and age and sex as fixed factors showed that the hematocrit did not vary with age ($F_{1,194} = 0.42$, $P = 0.52$), sex ($F_{1,194} = 0.08$, $P = 0.78$; males, mean = 48.11 ± 3.45 , $n = 236$; females, mean = 47.60 ± 3.63 , $n = 271$), or their interaction ($F_{1,194} = 1.30$, $P = 0.26$). In both sexes, therefore, the across-years repeatability of the hematocrit was, though low, significant or nearly so (females: $R = 0.23$, $SE = 0.09$, $n_0 = 2.33$, $F_{83,197} = 1.71$, $P = 0.004$; males: $R = 0.17$, $SE = 0.11$, $n_0 = 2.21$, $F_{65,147} = 1.46$, $P = 0.053$).

Male and female pied flycatchers paired assortatively with respect to their hematocrit ($r = 0.20$, $n = 230$, $P = 0.003$). Contrary to the strong effect that the abundance of ectoparasitic mites has on fledgling hematocrit (Potti et al. 1999), those of adult breeding males and females were not affected by the abundances of mites in the nests they were caring for (GLMs controlling for year effects; males: $F_{1,5,47} = 0.53$, $P = 0.49$; females: $F_{1,6,47} = 1.80$, $P = 0.22$).

Within-individual variation in the hematocrit: from fledging to adulthood

For 53 males and 36 females I had data on their hematocrit at both fledging and as an adult. Adult hematocrit was consistently higher than values at fledging (Potti et al. 1999). Fledgling hematocrit did not differ between the sexes ($F_{1,87}=0.0$, $P=0.99$). The hematocrit for adults was not predicted by the value at fledging (GLM, $F_{1,86}=0.18$, $P=0.68$) and this held irrespective of an individual's sex ($F_{1,86}=2.91$, $P=0.09$), the abundance of ectoparasitic mites in its nest of birth ($F_{1,81}=0.45$, $P=0.50$) or the interaction of sex with mite abundance ($F_{1,81}=0.79$, $P=0.38$). There was no relationship between the hematocrit measured at fledging age and the probability that the bird recruited locally as a breeder up to three years later (separate logistic regressions for cohorts born in 1994 to 1998, with survival being checked until 2001; total sample size, $n=155$ recovered fledglings and $n=1287$ non-recovered fledglings; $P>0.12$ in all regressions).

Heritability of adult hematocrit

Table 1 summarizes means and variances of the adult hematocrit in the parent and offspring generations in both sexes. Heritabilities of the adult hematocrit calculated by parent-offspring regressions and ANOVAs on families of full siblings are summarized in Table 2. All the comparisons resulted in non-significant heritabilities.

Discussion

Hematocrit of adult pied flycatchers seems to retain little or no genetic variation, as also occurs with hematocrit at fledging age (Potti et al. 1999). However, this could not be predicted as the expression of genetic variance may change across the ontogeny (e.g. Charmantier et al. 2005, Wilson et al. 2005) so that it cannot be safely assumed that absence of heritability at one life stage (Potti et al. 1999) translates into lack of heritability across an individual's lifetime. Despite

Table 1. Statistics for the sample of adult hematocrit data of parent and offspring pied flycatchers used in the heritability analyses.

	n	Mean	Range
SD All offspring	109	47.67	38–56
3.18			
Female offspring	59	47.60	38–55
Male offspring	50	47.75	42–56
Mothers	82	48.06	38–55
Fathers	80	49.16	40–59

Table 2. Heritabilities ($h^2 \pm 1$ SE) of the adult hematocrit in pied flycatchers- n is the number of families in each analysis. Single-parent estimates are corrected for assortative mating.

Pairs of relatives	$h^2 \pm 1$ SE	n	P
Father-offspring	0.1890.14	80	0.35
Mother-offspring	0.0390.22	82	0.68
Midparent-offspring	0.0890.20	53	0.57
Full-siblings	0.1490.11	17	0.12

moderately large sample sizes, I could not detect significant resemblance in the hematocrit of parent and offspring pied flycatchers measured at the same, adult stage. Consequently, none of the estimates of heritability were significantly different from zero. In addition, no evidence for selection on fledgling hematocrit, a potential bias to explain differences in heritability among life stages, was observed. The pattern found in this study recalls those found earlier in the same population with hematocrit near fledgling age: genetic variation could not be detected and, in addition, there was no correlation between adult and fledgling mid-offspring values (Potti et al. 1999). The latter relationship could have been confounded by the fact that the adult hematocrit was substantially higher than in fledglings. This problem was obviated here, however, as all genetic analyses were done with breeding adults and the repeatability analysis showed that adult hematocrit has already stabilized.

Earlier observational and experimental work in my study population has shown that nutrition and parasites are major environmental influences on the hematocrit value of pied flycatcher fledglings (Merino and Potti 1998, Potti et al. 1999). However, the ontogenetic comparison showed that nestlings that survive high mite infestations in their nest of rearing have recovered from their early-life handicap by the time they become breeding adults, i.e. are capable to overcome their severe anemia once they leave the mite-infested nests. Hematocrit thus seems to be sensitive to environmental conditions (Potti et al. 1999, Williams et al. 2004, Simon et al. 2005) and is a rapidly changing trait in early life, so that temporary deficits do not impose a permanent handicap on the individual. The adaptive flexibility of hematocrit, which could itself be inherited, coupled with high environmental variance, may obscure the detection of its underlying additive genetic variation.

The apparent absence of heritability in hematocrit contrasts with the significant repeatability of the trait present in both male and female adult pied flycatchers. Under certain conditions (Dohm 2002), e.g. absence of genotype by environment interactions or operation of maternal effects, repeatability may set an upper bound to heritability estimates (Falconer and Mackay 1996). If

these conditions are met, the heritability of hematocrit,

taking into account the standard error of the repeatability estimate (Merilä and Sheldon 2001), would not exceed about 0.30. A field study on house martin *Delichon urbica* fledglings found the same heritability estimate of hematocrit (0.14) as I have reported for adult full-siblings, but their estimate was significant in contrast to that presented here (Christe et al. 2000). Two other studies in blue tits *Parus caeruleus* (Simon et al. 2005) and barn swallows *Hirundo rustica* (Cuervo et al. 2007) did not find evidence for heritability of hematocrit at fledging.

It is conceivable that a trait such as the adult hematocrit has been subjected to stabilizing selection related to the maintenance of body homeostasis. For example, low hematocrit entails difficulties in oxygen transport, while high hematocrit may be associated with disorders such as the ascites syndrome, a problem in the poultry industry (e.g. Pakdel et al. 2002). Thus, the ability to regulate the hematocrit within certain boundaries could be a component of individual quality. The data in my pied flycatcher population suggest, however, that this component retains little additive genetic variance at both the fledgling and adult ages and thus seems not to be a direct target of natural selection under recent conditions.

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