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Experimental manipulation of tail ornament size affects the hematocrit of male barn swallows (*Hirundo rustica*)

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Abstract Ornamental tail feathers of male barn swallows (*Hirundo rustica*) confer an advantage in sexual selection because long-tailed males are preferred by females. However, the size of tail ornaments exceeds the natural selection optimum and males are predicted to pay an energetic cost for flying, directly related to tail length. An increase in hematocrit is an adaptive response to enhance oxygen uptake, for example during periods of intense locomotory activity. In this study, we analyzed the effect of experimental manipulation of tail length on the hematocrit of male barn swallows from an Italian and a Spanish population. We predicted that the natural decrease in hematocrit during the breeding season would be reduced by experimental elongation and enhanced by experimental shortening of tail ornaments. The results showed that the decrease in hematocrit was significantly different among tail treatments, and tail-elongated males had the smallest hematocrit reduction. In Italy, the hematocrit of tail-elongated males did not change after tail manipulation, while that of two control groups and tail-shortened males decreased. A comparatively high hematocrit in males with experimentally enlarged tail ornaments may be a response to increased energetic requirements and, hence, to oxygen demands for flying imposed by their tail morphology.

Key words Hematocrit · *Hirundo rustica* · Secondary sexual characters

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Introduction

Advertisement models of sexual selection propose that ornamental male characters serve the function of 'honest' inter-sexual signals of male phenotypic or genetic quality, and that 'honesty' is enforced on the signal by the costs they impose on the signaller (Zahavi 1975, 1977; Heywood 1989; Grafen 1990). Male ornaments preferred by females may be costly to produce because of the anabolic energy demands for their development or otherwise negative effects of hormones which ultimately control their growth (Beckage 1983; Grossman 1985; Lawrence 1991; Folstad and Karter 1992). Larger ornaments may also impose higher costs for their maintenance than smaller ones. Large ornaments may render their bearer more conspicuous to predators than small ones, or they may impinge on locomotion and foraging efficiency. Ornamental tails of many bird species, for example, may increase the aerodynamic costs of flight or ground locomotion and reduce manoeuvrability, thus affecting foraging performance on aerial prey or anti-predatory behavior.

The energy costs and metabolic rates associated with various bird activities have been measured by a variety of techniques (e.g., Hails 1979; Vehrencamp et al. 1989; Andersson and Gustafsson 1995). Some recent studies have focused on the aerodynamic properties of bird ornamental tails (Thomas 1993; Norberg 1994), but direct or indirect measures of the physiological costs imposed by sexually selected traits on flight activity are scarce.

The hematocrit, the proportion of blood volume occupied by packed red blood cells, influences the extent and efficiency of oxygen uptake and transfer to tissues, and has also been regarded as an index of metabolic activity during a period of days to weeks preceding the date of blood sampling (Carpenter 1975). Birds are known to tune their individual hematocrit in response to a variety of factors. Increased oxygen demands for thermogenesis or intense locomotory activity, experimen-

tally induced hypoxia and low oxygen partial pressures cause an increase in the hematocrit through erythropoiesis (Sealander 1962; Jaeger and McGrath 1974; Weathers and Snyder 1974; Carey and Morton 1976; Palomeque and Planas 1978; Clemens 1990). In migratory populations of birds, the hematocrit declines after the end of migration (Jones 1983; Morton 1994). Hence, birds seem to raise their hematocrit as an acclimation to intense activity regimes or exogenous factors which enhance oxygen demands or reduce its availability. A change in the hematocrit resulting from polycythemia is a homeostatic response for temporary adaptations (Black et al. 1978). From a functional point of view, raised hematocrit levels can thus be considered a physiological response that enhances the efficiency of oxygen uptake and transfer by the blood stream during spells of intense oxidative metabolic activity (Gessaman et al. 1986).

The aim of this study was to evaluate the effect of manipulation of the size of the ornamental outermost tail feathers on the hematocrit of male barn swallows (*Hirundo rustica*) during the breeding season. The barn swallow is a socially monogamous, migratory passerine which feeds on the wing on flying insects. Males start arriving from the wintering quarters in southern and central Africa to the breeding areas in southern Europe in February–March and establish a small nesting territory where they try to attract prospecting females by singing and displaying their tail ornaments. Sexual dimorphism in most morphological characters is small, but it is pronounced for the outermost tail feathers. Females prefer males with naturally long or experimentally elongated outermost tail feathers, and fitness benefits accrue to well-ornamented males from female mate choice (Møller 1994). Since the outermost tail feathers of males exceed the theoretical optimal length for flying (Thomas 1993), they may impose a cost both in terms of energy expenditure and foraging efficiency. Indeed, males with comparatively long outermost tail feathers have lower maneuverability and capture smaller insect prey (Møller and de Lope 1994; Møller et al. 1995a) than those with short ones. The hematocrit of both males and females declines after arrival in the breeding grounds, and this trend has been found consistently in populations from northern and southern parts of the European breeding range (Saino et al., in preparation; see also Results). The hematocrit of males measured shortly after arrival at the breeding sites is positively correlated with the length of the outermost tail feathers (Saino et al., in preparation).

If the outermost tail feathers of males enhance the energy costs of flying, this should produce a positive relationship between tail length and, for example, metabolic rate or oxygen consumption. Hence, we predicted that the hematocrit of males would decrease less after experimental elongation of tail feathers than that of unmanipulated males and, conversely, that the hematocrit of tail-shortened males would decrease more than that of unmanipulated controls.

Materials and methods

This study was carried out in two areas located in northern Italy (45°30' N, 9°20' E) and western Spain (38°50' N, 6°59' W) during 1994 (Italy) and 1995 (Italy and Spain). In both study areas, barn swallow colonies were located in rural buildings, mainly cow stables, and just a few bred solitarily. Barn swallows considered in this study were captured during the day or at dawn by mist-nets starting on 28 March in Italy and 16 February in Spain. At the time of first capture, we measured a number of morphological characters including length of the left and right outermost tail feathers, length of the central tail feathers, left and right flattened wing length, bill length, and keel and right tarsus length. Tail length was expressed as the mean value of the left and right outermost tail feathers. In Italy, usually at the time of first capture, males were assigned sequentially to one of four experimental groups, i.e., the first, second, third, and fourth males to be captured were assigned to the first, second, third, or fourth group according to the order of capture; the fifth male to be captured was assigned to the first group and so forth with the subsequent males. The experimental groups were: (1) no tail manipulation (control I), (2) tail cut at approximately 10 mm from the base and then reglued (control II), (3) tail shortened by removal of 20 mm of outermost tail feathers on both sides at 10 mm from the base of the feathers, and (4) tail elongated by 20 mm by insertion of a piece of outermost tail feather 10 mm from the base. In Spain, we established only groups 1, 3, and 4 and, hence, we had no control group for treatment. Hence, the tail length manipulation procedure was identical to that adopted in previous studies of the barn swallow (e.g., Møller 1988).

At the time of first capture and, for a large proportion of individuals, at the time of subsequent recapture(s), we took a blood sample in 1–3 capillary tubes after puncture of the ulnar vein. The blood samples averaged 160 μ l in the two populations. Centrifugation conditions for the capillaries were as follows: 4,000 rpm for 10 min in Italy and 11,500 rpm for 7 min in Spain, always referring to a radius of the centrifuge rotor of 94 mm. The hematocrit was measured in each capillary tube as the ratio between the length of the capillary occupied by blood cells and the total length of the capillary occupied by blood components. Variability between two randomly chosen capillaries from each of 500 individuals was significantly larger than the variability among individuals (one-way analysis of variance, $F = 22.0$, $df = 498,499$, $P < 0.0001$). The repeatability index computed according to Falconer (1989) was high (0.91), indicating that different capillaries from the same individual gave highly consistent measures of its hematocrit. We therefore expressed the hematocrit of a male as the mean of values recorded for the capillaries available for that male. It must be emphasised that differences in centrifugation time and angular speed between the samples from the two populations did not qualitatively affect our results because population was included as a factor in the analysis of covariance (ANCOVA) in which data from the two populations were entered simultaneously (see Results), and no comparisons of treatment effect between the two populations are performed.

We define a decrease in the hematocrit of an individual as the signed difference between the hematocrit value observed at the time of tail manipulation or, for males of control group I, at the time (hereafter, for simplicity, 'time of first capture') of the inclusion of the individual in the experiment and the hematocrit recorded at the last recapture. We excluded from the analysis all individuals for which less than 15 days had elapsed from first capture to recapture under the assumption that before 15 days from first capture, males had not completed their response to the novel flying conditions imposed by tail manipulation. This assumption is based on the observation that a change in the hematocrit determined by ecological conditions in other bird species can take weeks to evolve (see Morton 1994).

Results and discussion

To check if the assignment procedure of males produced homogeneous experimental groups, we compared the within-group means of the morphological variables by one-way analysis of variance. None of the variables showed significant differences among groups in the Italian or the Spanish samples (one-way analysis of variance, F values always associated with P values larger than 0.05), indicating that the experimental groups were homogeneous with respect to morphology. Hematocrit values at first capture did not differ between tail-shortened, tail-elongated and tail-unmanipulated males in Spain ($F = 0.47$, $df = 2,71$, n.s.) or in Italy ($F = 2.11$, $df = 2,253$, n.s.). We excluded from this analysis males in Italy that had their tail cut and reglued (control II) because they were not included in the analyses of the effect of tail treatment. Data collected in 1994 and 1995 in Italy were pooled because no significant difference in mean hematocrit values was observed between the two years ($F = 1.34$, $df = 1,285$, n.s.). No statistically significant difference among the groups was observed for the date of first capture (Italy: $F = 2.22$, $df = 2,253$, n.s.; Spain: $F = 0.84$, $df = 2,71$, n.s.) or inter-capture time (Italy: $F = 1.55$, $df = 2,253$, n.s.; Spain: $F = 0.71$, $df = 2,71$, n.s.). Mean (SE) inter-capture time (in days) in Italy and Spain, respectively, was 39.1 (1.71) and 103.2 (3.09) for control group I, 42.7 (2.88) and 104.9 (4.30) for tail-shortened males, and 45.3 (3.39) and 109.3 (3.68) for tail-elongated males. In Italy, the mean time to recapture of control II males was 47.4 (4.06).

An ANCOVA of the decrease in hematocrit in which treatment was entered as factor and time to recapture as covariate showed a significant positive relationship between inter-capture time and hematocrit decrease in both the Italian and the Spanish samples (Italy: $F = 7.33$, $df = 1,252$, $P = 0.006$; Spain: $F = 7.02$, $df = 1,70$, $P = 0.01$). Hence, the decrease in hematocrit was larger for males that were recaptured after a comparatively long time had elapsed from first capture. When the quadratic term of time to recapture was also entered in the ANCOVA model, it did not significantly correlate with the hematocrit decrease (Italy: $F = 0.03$, $df = 1,251$, n.s.; Spain: $F = 0.24$, $df = 1,69$, n.s.). These analyses show that the hematocrit declined linearly as time from manipulation elapsed. We could therefore express the decrease in hematocrit, while correcting for inter-capture time, as the ratio between the decrease in hematocrit and the number of days elapsed between first capture and recapture (hereafter 'daily decrease of hematocrit').

We tested the hypothesis of no daily decrease of hematocrit for tail-shortened males of each population against the alternative hypothesis that the daily decrease of hematocrit was larger than 0, by a one-tailed t -test. Use of a directional test for this group was justified by the fact that we already knew that the hematocrit declines with time in unmanipulated birds and reduced cost of flying by shortening the outermost tail feathers

should have emphasized the natural decline in hematocrit values. Since the predicted effect of tail elongation was opposite to the natural decline in hematocrit, we could make no prediction about the sign of the change of hematocrit in tail-elongated males. For tail-elongated males, the daily decrease in hematocrit was therefore tested by a two-tailed test against the null hypothesis H_0 : daily decrease of hematocrit = 0. Italian and Spanish males with unmanipulated tails (control I) showed a significant daily hematocrit decrease (Italy: $t = 5.43$, $df = 194$, two-tailed $P < 0.0001$; Spain: $t = 5.47$, $df = 38$, two-tailed $P < 0.0001$). Similarly, in Italy, the hematocrit of males that had had their tail cut and reglued showed a significant daily decrease (control II; $t = 2.26$, $df = 30$, two-tailed $P = 0.032$). In both populations, the daily decrease in hematocrit of tail-shortened males was significantly larger than 0 (Italy: $t = 1.80$, $df = 28$, one-tailed $P = 0.041$; Spain: $t = 4.29$, $df = 20$, one-tailed $P < 0.0005$). In Italy, the daily decrease in hematocrit of tail-elongated males was not significantly different from 0 ($t = -0.58$, $df = 31$, two-tailed test, n.s.) and its sign was opposite to that observed for the other three groups (Fig. 1). In Spain, the daily decrease in hematocrit was smaller than that of the other groups but was significantly larger than 0 ($t = 2.26$, $df = 14$, two-tailed $P = 0.04$; Fig. 2). However, the predicted larger change in hematocrit of tail-shortened males than in the other groups was observed for the Spanish swallows, but not for the Italian ones. When we repeated the above analyses using the difference between hematocrit at first capture and recapture (not divided by inter-capture time), we obtained qualitatively similar results. Moreover, P values were smaller for five of the six statistically significant t -tests.

Daily changes in hematocrit in the experimental groups were compared by analysis of covariance in

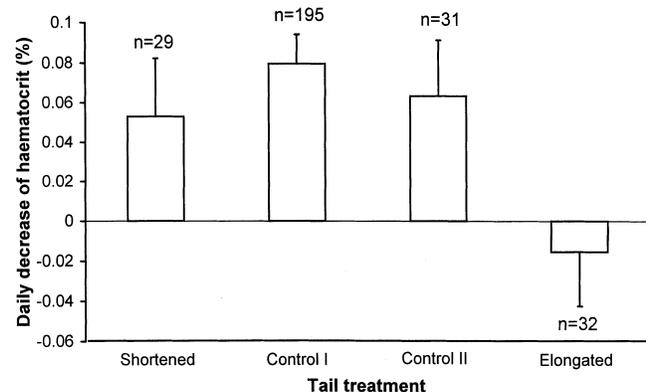


Fig. 1 Percent daily decrease in hematocrit (mean and standard error) measured as the ratio between the decrease in hematocrit (hematocrit at first capture – hematocrit at last recapture) and inter-capture time (in days) for Italian barn swallow males that had had their tail shortened by 20 mm (*Shortened*), unmanipulated (*Control I*), cut and reglued (*Control II*), or elongated by 20 mm (*Elongated*). Numbers represent sample sizes

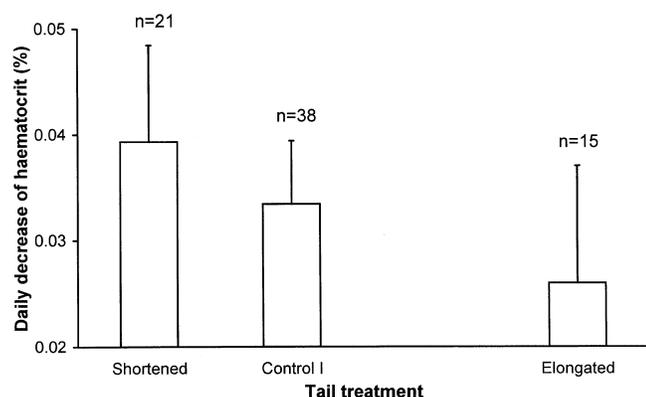


Fig. 2 Percent daily decrease in hematocrit (mean and standard error) measured as the ratio between the decrease in hematocrit (hematocrit at first capture – hematocrit at last recapture) and inter-capture time (in days) for Spanish barn swallow males that had their tail shortened by 20 mm (*Shortened*), unmanipulated (*Control I*), or elongated by 20 mm (*Elongated*). Numbers represent sample sizes

which pre-treatment tail length was entered as covariate, and treatment and population as factors (Table 1). We excluded from this analysis the group of males in Italy that had their tails cut and reglued (control II) because this group was not represented in the Spanish sample. Population and pre-treatment tail length had no significant effect on the change in hematocrit, while the effect of tail manipulation was statistically significant (Table 1).

This is one of the first studies to test for physiological costs of secondary sexual characters and, to our knowledge, is the first in which a causal relationship between morphology of a sexually selected character and hematocrit is demonstrated. Consistent with our prediction, experimental elongation of tail feathers exerted an effect on the hematocrit opposite to the natural decline of hematocrit after migration. The decrease in hematocrit of tail-elongated males in both populations was smaller than that of control and tail-shortened males, and in Italy was not statistically different from 0. Our interpretation of this finding is that elongation of the tail enhances the energy costs of flying. Increased oxygen demands for oxidative metabolism are then accomplished by raised hematocrit levels and, thus, larger oxygen uptake. This interpretation is clearly supported by the fact that elongation of outermost tail feathers of

male barn swallows enhances the deviation of this ornamental character from the natural selection optimal size (Thomas 1993), thus increasing the energetic cost of flight. Moreover, an increased hematocrit is a commonly observed physiological response to prolonged muscular activity. Contrary to our prediction, however, in the Italian population, shortening of tail feathers did not apparently result in a decrease in hematocrit larger than in the control groups. If the cost of these characters increases disproportionately with ornament size, then the absolute effect of tail shortening is presumably much smaller than that of tail elongation. This might have obscured the precise pattern of change in the hematocrit of tail-shortened males relative to control males, such that tail shortening showed no effect. Alternatively, tail shortening might also increase the costs of flying. According to aerodynamical considerations (Thomas 1993), the optimal shape of a forked tail is a triangular one when the tail is fanned at 120°. Any tail element protruding from the optimal profile would generate drag and no lift. In order to achieve the optimal profile, outermost tail feathers should be approximately twice as long as innermost tail feathers. If shortening by 20 mm produced outermost tail feathers whose length was below optimum, then tail-shortening might also have increased the costs of flying. It seems unlikely, however, that this explanation is correct because in Italy shortening by 20 mm rectrices whose original mean length (109.5 mm) was approximately 20 mm larger than the theoretical optimal length (Møller et al. 1995b) should have produced an optimal tail shape. In Spain, on the other hand, where shortening by 20 mm of feathers whose original mean length was 101.2 mm should have produced a sub-optimal tail profile, the ranking of experimental groups according to the decrease in hematocrit was exactly as predicted.

What is the relevance of our findings to current knowledge about the evolution of male morphological ornamental traits? Advertisement models of sexual selection propose that choosy females prefer males with large ornaments because they are honestly signalling their ability to withstand the costs imposed by production or maintenance of the ornaments and, thus, their phenotypic or genetic quality. In order to serve the function of honest ‘handicaps,’ the size of morphological ornaments should deviate from the natural selection optimum and the costs they impose should increase more than proportionally with ornament size.

Since erythroptosis, resulting in an increase in the hematocrit, can be considered as a costly physiological process, we conclude that the causal, positive relationship we have shown in the present study between tail length and hematocrit demonstrates that an energetic cost is associated with the condition of possessing sexually attractive ornamental characters and that this cost is larger for large ornaments. A simple physiological variable such as hematocrit can thus be used to test hypotheses about costs of sexual selection.

Table 1 Effects of population and tail treatment (factors) and pre-treatment tail length (covariate) on the daily decrease in hematocrit of Italian and Spanish barn swallow males, in an ANCOVA. In this analysis only control group I, tail-shortened and tail-elongated males were included because control group II was established only in Italy

	Mean square	df	F	P
Population	0.013	1	0.43	n.s.
Tail treatment	0.099	2	3.31	< 0.04
Pre-manipulation of tail length	0.023	1	0.76	n.s.

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