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Effects of experimental tail shortening on the phenotypic condition of barn swallows *Hirundo rustica*: implications for tail-length evolution

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Abstract

Some studies have suggested that tail streamers in the barn swallow (*Hirundo rustica*) may have been elongated 10-12 mm by sexual selection, but according to other studies, the length of these feathers is at the aerodynamic optimum or very close to it. To shed light on this issue, outermost tail feathers were experimentally shortened in male and female barn swallows by 1, 11 or 21 mm. Changes in four physiological parameters commonly used to estimate phenotypic condition in birds (weight, erythrocyte sedimentation rate, blood leukocyte concentration and heterophil/lymphocyte ratio) were checked one month later. Health improved (blood leukocyte concentration decreased) in the group of birds with tails shortened by 11 mm (both males and females), but body condition deteriorated (weight decreased) compared to the other two experimental groups. There was no significant effect of tail-length manipulation on the other two physiological parameters. These contradictory results suggest trade-offs between components of phenotypic condition. Possible negative relationships between condition-related traits imply that using one or very few physiological parameters to estimate phenotypic condition might not be appropriate. The most plausible explanation for the turning point in phenotypic condition when streamers were shortened by 11 mm is that these feathers are 7-15 mm longer than the aerodynamic optimum in both sexes. Therefore, our results are consistent with the hypothesis that tail streamers have been elongated 10-12 mm by sexual selection. This conclusion disagrees with a previous study on the effect of experimental tail shortening on haematocrit, but the complexity of interpreting changes in haematocrit might account for this discrepancy.

Introduction

Sexual selection and other forms of natural selection very often have opposite effects on the evolution of phenotypes. This happens because the trait expression that optimizes mating success usually does not optimize other fitness components, e.g. survival (Darwin 1871, Andersson 1994). However, in some cases, at least during certain period of time, both sexual and natural non-sexual selection might produce the same kind of evolutionary change in a trait. In these cases, it is difficult to elucidate what proportion of the evolutionary change is due to each kind of selection, or even if both sexual and natural non-sexual selection or only one of them is responsible for that change. A classic example illustrating this difficulty is the evolution of neck length in the giraffe (*Giraffa camelopardalis*), that was traditionally attributed to competition for food, but might be also explained by sexual selection, specifically by competition among males for access to females (Simmons and Scheepers 1996). According to theoretical models, when a trait is displaced from the natural non-sexual selection optimum by sexual selection, the expression of this trait inevitably entails costs, generally viability costs (Fisher 1930, Zahavi 1975, Iwasa et al. 1991, Pomiankowski et al. 1991). Many studies in different taxa have indeed found that secondary sexual characters, both ornaments and weapons, are costly to produce and/or maintain (e.g. Grether 1997, Basolo and Alcaraz 2003, Allen and Levinton 2007; but see McCullough and Emlen 2013). Therefore, looking for costs may provide useful information to determine whether sexual selection has been really involved in the evolution of putative sexual traits.

A bird species in which sexual versus natural non-sexual selection effects have been extensively studied during recent decades is the barn swallow (*Hirundo rustica*), a small passerine with outermost tail feathers much longer than the rest of the tail and longer in males than in females (Cramp 1988, Møller 1994). A number of studies have concluded that these feathers have evolved through sexual selection because long feathers make the bearer a more attractive sexual partner (Møller 1988, 1992), although they entail viability costs (Møller 1989, Møller and de Lope 1994). However, other studies have suggested that these feathers might have evolved solely through natural non-sexual selection because they improve flight performance, specifically in tight turns (Norberg 1994). This gave rise to an intense debate with arguments for and against the two hypotheses and the different ways tails could be manipulated to test them (Evans and Thomas 1997, Thomas and Rowe 1997, Evans 1998, Møller et al. 1998, Barbosa and Møller 1999, Hedenström and Møller 1999), and the debate, far from being over, has recently been revived (Bro-Jørgensen et al. 2007, Aparicio and Møller 2012, Evans et al. 2012).

In two studies in Scotland (Buchanan and Evans 2000, Rowe et al. 2001) on the function of the outermost barn swallow tail feathers, the apical part of the feathers (the streamer) was shortened and its effect on flight parameters was checked. The rationale for these experiments was that there is an aerodynamically optimal tail length and any further lengthening caused by sexual selection would impair flight performance. Therefore, if tail length is at an aerodynamic optimum, any experimental shortening would affect flight performance negatively. On the contrary, if tail feathers have been elongated by sexual selection beyond the aerodynamic optimum, moderate tail shortening (up to the length elongated by sexual selection) would improve flight performance. It should be noted that severe tail shortening (beyond the length elongated by sexual selection, if in fact this had an effect on tail length) would always affect flight performance negatively. Both studies concluded that only 10-12 mm of tail length may be attributed to sexual selection, and the remaining length has evolved through natural non-sexual selection, not only in males, but also in females. Thus both sexual and natural (non-sexual) selection would be responsible for the long outermost tail feathers of male and female barn swallows.

The abovementioned studies in Scotland concentrated on flight performance during particular manoeuvres, specifically when barn swallows entered or left the buildings housing their nests (Buchanan and Evans 2000) or when negotiating a maze (Rowe et al. 2001). A possible problem with this approach is that the aerodynamically optimal tail length varies depending on the type of flight. For example, long tail streamers may have an aerodynamic function in tight turns (Norberg 1994), but cause only drag in level flight (Thomas 1993, Thomas and Balmford 1995). Therefore, assessment of the aerodynamically optimal tail length would require estimating the optimal length for each type of flight and combining these lengths in such a way that the relative importance of each type of flight is taken into account. An alternative to this procedure might be the use of physiological parameters that integrate costs and benefits of any type of flight. One example of such parameters is the haematocrit, the proportion of blood occupied by red blood cells. Other factors remaining unchanged (e.g. dehydration, altitude, nutrition or parasitism), an increase in haematocrit may be considered an adaptive physiological response to high levels of oxygen demand (Birchard 1997, Fair 2007). The most important cause of increased oxygen demand is usually intense muscular activity, and in birds, muscular activity involved in flight (Balasch et al. 1974, Viscor et al. 1985, Hõrak et al. 1998). This is particularly evident in species that feed on the wing and migrate long distances, such as the barn swallow (Cramp 1988, Turner 2006).

A study in southern Spain (Cuervo and de Ayala 2005) on the function of barn swallow tail streamers checked for changes in haematocrit as a consequence of experimental tail shortening. These changes require some background information to be understood. Barn swallows arrive at their breeding grounds in Europe after a long journey of several thousand kilometres from their sub-Saharan winter quarters (Cramp 1988). Due to the intense effort required for such a long flight, barn swallow haematocrit levels are very high when they arrive at their breeding grounds, and gradually decrease during the breeding season (Saino et al. 1997a). If tail length were experimentally manipulated at the beginning of the breeding season, a stronger decrease in haematocrit would be expected in birds with tail length closer to the aerodynamic optimum. This is because it would take less effort for birds with aerodynamically optimal tails to fly, thus consuming less oxygen in muscular activity and achieving the most efficient oxygen uptake with lower haematocrit levels (for the rationale of this experiment see also Saino et al. 1997b). The results of the study carried out in southern Spain (Cuervo and Ayala 2005) did not agree with those found in Scotland and were instead consistent with the tail length of southern Spanish swallows already being at the aerodynamic optimum or very close to it. Inter-population differences in tail length (the outermost tail feathers are around 10 mm longer in Scotland than in southern Spain) might help explain the different results found in these two populations (Cuervo and de Ayala 2005). One possible problem using haematocrit to estimate flight effort is the assumption that other factors that may be affecting haematocrit (e.g. dehydration or nutrition; Fair 2007) remain constant. This assumption might not hold in this case (see an example for possible changes in nutrition below), so the results should be interpreted with caution, and other physiological parameters might be better suited than haematocrit for such a study.

It has been suggested that experimental tail shortening in the barn swallow affects flight performance (Buchanan and Evans 2000, Rowe et al. 2001) and flight effort (Cuervo and de Ayala 2005). However, tail length manipulation might also affect the phenotypic condition of the birds. The two examples below illustrate how this could happen. First, barn swallows show a preference for feeding on large insects, which are the most profitable prey, but are also more difficult and energetically expensive to catch (Turner 1982). Therefore, any deterioration in flight performance would decrease the amount of food caught or the proportion of the most profitable prey in the diet (Møller et al. 1995). In turn, a reduction in food quantity or quality might affect body condition negatively, with a decrease, for example, in energy reserves. Second, tail length manipulation could affect phenotypic condition through trade-offs between resources devoted to different physiological processes. If birds had to make a stronger effort to fly due to tail manipulation, flight muscle activity would consume valuable resources (e.g. energy) that would not then be available for other processes such as immune response (Ots et al. 2001, Eraud et al. 2005). The immune system is crucial to fight against pathogens and parasites (Roitt et al. 1998), and is thus decisive for individual survival (Møller and Saino 2004).

In this study, we used data on phenotypic condition from a previous experiment (Cuervo and de Ayala 2005) to re-examine the function of tail streamers in the barn swallow. The experiment consisted of shortening the streamers by 1, 11 or 21 mm, and checking the phenotypic condition of the birds immediately before and around one month after manipulation. If streamers have been elongated 10-12 mm by sexual selection, as previously suggested (Buchanan and Evans 2000, Rowe et al. 2001), we predicted that shortening tail length by 11 mm would improve (or deteriorate less) phenotypic condition compared to the other two treatments. The argument behind this prediction is that a tail shortened by 11 mm would be closer to the aerodynamic optimum and would thus enhance the bird's flight performance more than the other two treatments. As a result, barn swallows with tails shortened by 11 mm would catch more or better food or use less energy to fly (see above), with the consequent improvement in phenotypic condition. However, if streamer length was at the aerodynamic optimum or very close to it, as suggested by other studies (Cuervo and de Ayala 2005), we predicted that shortening tail length by 1 mm would deteriorate phenotypic condition less than the other two treatments. The difference should be particularly pronounced between birds with tail shortened by 1 and 21 mm, because these two treatments would cause the smallest and largest displacements from the aerodynamically optimal tail length.

Phenotypic condition may be defined as “the ability to acquire and allocate resources efficiently and effectively to activities that enhance survival and reproduction (i.e. […] evolutionary fitness)” (Thornhill and Gangestad 1999). Phenotypic condition cannot be measured directly, only estimated through the assessment of phenotypic variables related to fitness. In theory, the more fitness-related variables are measured, the better the estimate of the phenotypic condition of an organism. As explained above, the aerodynamically optimal tail length in barn swallows might differ from tail length that optimizes fitness, because tail feathers may have been elongated by sexual selection to increase mating (and thus reproductive) success (Møller 1988, 1989), even if this elongation impairs survival (Møller and de Lope 1994). Consequently, birds with the aerodynamically optimal tail length might not show greater reproductive success or increased fitness, but we assume they would show improved survival. This is why the use of phenotypic condition in this study is restrictive and refers exclusively to survival-related variables. Specifically, we studied phenotypic variables providing information on body condition, health, and physiological stress.

Methods

Field procedures

Detailed description of fieldwork methods and experimental tail-length manipulation may be found elsewhere (Cuervo and de Ayala 2005), so only the most relevant information is given here. The study was carried out in Almería, south-eastern Spain (36°45'-37°05'N, 2°09'-2°31'W), during April-July (spring-summer, i.e., during mating and breeding) of 2002. Barn swallows were caught using mist nets placed at dawn across windows and doors in the farmhouses where they spent the night. At first capture, every individual was weighed (with a Pesola spring balance to the nearest 0.25 g), ringed with metal and coloured plastic bands, and sexed according to tail length (tail is usually longer in males) and presence of a brood patch (only females incubate). The length of right and left outermost tail feathers was measured with a ruler (to the nearest 0.5 mm), taking the mean of the two measurements as the tail length. A blood sample was taken from the brachial vein in a 75-μl capillary tube and one drop was used to make a blood smear which was air dried and fixed in methanol. Then the capillary tube with the remaining blood was sealed with wax, stored horizontally in ice in a cooler and taken to the laboratory. As many birds as possible were recaptured 30-36 days after first capture (hereafter second capture). But in this second capture, other birds were captured for the first time, and then recaptured (second capture for these birds) around one month later. Thus, some birds were captured for the first time early in the breeding season while other birds were captured for the first time late in the breeding season. In the second capture, every bird was weighed again and another blood sample was taken. All nests were surveyed every second day to identify the parents attending the nest and to determine brood size and dates of hatching and fledging.

When captured for the first time, barn swallows were randomly assigned to one of the three experimental treatments. The first treatment consisted of shortening tail length by 11 mm in an attempt to make the tail length as close as possible to the aerodynamic optimum if tail streamers have been elongated 10-12 mm by sexual selection (Buchanan and Evans 2000, Rowe et al. 2001). A second experimental treatment consisted of shortening tail length by only 1 mm to keep tail length very close to the original length, i.e., to the aerodynamic optimum if tail streamers have not been elongated by sexual selection (Cuervo and de Ayala 2005), while still affecting the integrity of the feather, as in the previous treatment. The third experimental treatment consisted of shortening tail length by 21 mm, so the difference in tail length reduction between birds with tail shortened by 11 mm and the other two experimental groups was the same. Manipulation in all treatments consisted of cutting the tip of the outermost tail feathers (by 1, 11 or 21 mm) and trimming the streamer with scissors to imitate its naturally rounded end. Very often, both birds in a pair were manipulated, either with the same or different experimental treatments. All birds were freed immediately after manipulation in the same place where they had been caught. No birds with broken, missing or moulting tail feathers in either the first or second captures were included in the study.

Assessment of phenotypic condition

Phenotypic condition was assessed as temporal changes in four physiological (or physiology-related) traits commonly used to estimate body condition, health or stress in birds: weight, erythrocyte sedimentation rate (sedimentation rate hereafter), blood leukocyte concentration, and heterophil/lymphocyte ratio (H/L). The first trait, weight, was used as an estimate of body condition, i.e., of underlying energy reserves, because it has been shown that weight increases adult survival in a number of bird species (e.g. Morrison et al. 2007, Benson and Bednarz 2010, Harding et al. 2011).

Sedimentation rate was used as an index of health status. This parameter reflects the amount of protein in the blood, including fibrinogen and immunoglobulin, which are produced as a response to inflammatory and infectious diseases. Many authors consider high sedimentation rate an indicator of poor health status (e.g. Masello and Quillfeldt 2004, Garamszegi et al. 2005, Hoi et al. 2012). When capillary tubes with barn swallow blood were taken to the laboratory, they were stored vertically at 4°C for four hours. After that, the length of the cellular and plasma portions of the tube were measured with a digital calliper (to the nearest 0.01 mm) and the ratio of plasma to total blood was used as our preliminary sedimentation rate estimate. Then capillary tubes were centrifuged at 7700 r.p.m. for 10 minutes and haematocrit was calculated (see Cuervo and de Ayala 2005). Sedimentation rate is known to depend on haematocrit, and this was indeed the case in our sample (linear regression; first captures: $F_{1,54} = 34.51$, $p < 0.001$, slope (SE) = -0.981 (0.167); second captures: $F_{1,54} = 17.21$, $p < 0.001$, slope (SE) = -0.784 (0.189)). Moreover, the relationship between the two variables was similar in both captures (ANCOVA including first and second captures; interaction capture x haematocrit: $F_{1,108} = 0.60$, $p = 0.44$; Linear Mixed Model including also the individual as a random factor; interaction capture x haematocrit: $F_{1,53} = 0.22$, $p = 0.64$). Consequently, sedimentation rate was regressed on haematocrit combining first and second captures (i.e., every bird was counted twice), and residuals from this regression were used as estimates of sedimentation rate in subsequent analyses.

The last two physiological traits were calculated from blood smears, which were stained by the May-Grunwald-Giemsa method in the lab and scanned at 630x magnification following standard routines. Leukocytes (classified as lymphocytes, monocytes, eosinophils, heterophils, and basophils) and erythrocytes were counted in a number of microscopic fields to a total of 100 leukocytes per sample. The third parameter, blood leukocyte concentration, was used as another index of health status, because the number of leukocytes in the blood increases with infectious diseases (Saino et al. 1998, Szép and Møller 1999, Apanius et al. 2000). Leukocyte concentration was assessed as the number of leukocytes per 100 erythrocytes (i.e., 10^4 divided by erythrocyte count) multiplied by haematocrit. This is a relative concentration and has no units. Finally, H/L is widely considered a reliable indicator of physiological stress in birds (Maxwell and Robertson 1998, Moreno et al. 2002), and stress in turn is also widely accepted as a factor affecting survival (Blas et al. 2007, MacDougall-

Shackleton et al. 2009). H/L was calculated as simply the relative heterophil count divided by the relative lymphocyte count in each sample.

Variation in phenotypic condition parameters between first and second captures (parameter in second capture minus parameter in first capture) was divided by the number of days elapsed between the two captures (30-36 days) to find the change in phenotypic condition parameters per day for every individual.

Statistical methods

A total of 56 barn swallows were included in the study: 21 with tail shortened by 1 mm (8 males, 13 females), 19 with tail shortened by 11 mm (10 males, 9 females), and 16 with tail shortened by 21 mm (9 males, 7 females). Individuals assigned to the three experimental groups did not differ significantly in morphological traits or in reproductive parameters, with the exception of tail length after manipulation (Cuervo and de Ayala 2005). At first capture, the three experimental groups did not differ significantly in either haematocrit (Cuervo and de Ayala 2005) or in the four traits used to assess phenotypic condition (one-way ANOVAs, $F_{2,53} \leq 1.52$, $p \geq 0.23$).

To examine any effect of experimental treatment on phenotypic condition, we used daily change in a phenotypic condition trait as the dependent variable and experimental treatment and five other variables (two continuous and three categorical) that might influence phenotypic condition as independent variables. The continuous variables were original tail length and parental effort. The parental effort index was calculated as brood size multiplied by the percentage of days with nestlings or fledglings in the period between the two blood samplings (on average adults continue feeding their offspring for six days after fledgling;

review in Turner 2006, p. 145), and multiplied by the mean daily relative feeding rate during the period attending nestlings or fledglings, considering that one-day-old broods received one feeding visit per day, six-day-old broods received three feeding visits per day and ten-day-old or older broods received five feeding visits per day. These feeding rates were based on real figures six, 17 and 29 feeding visits per hour, respectively (Turner 2006, p. 140), and relative feeding rates at other ages were calculated by interpolation. The categorical variables were sex, mate's experimental treatment (tail unmanipulated or shortened by 1, 11 or 21 mm) and date of capture (whether captured for the first time early or late in the breeding season). With all these variables and the two-way interactions between categorical variables we performed backward stepwise General Linear Models, eliminating all variables and interactions associated with p-values ≥ 0.10.

Length of original outermost tail feathers and H/L were $\log_{10}$-transformed and the index of parental effort was squared-root transformed to obtain normal distributions, but all other variables followed a normal distribution without transformation (Kolmogorov-Smirnov test, $p > 0.20$). All statistical tests were two-tailed, and the level of significance was set at 0.05.

Results

The only variable significantly related to weight change in barn swallows was experimental treatment (Table 1), mainly because weight decreased in barn swallows with tails shortened by 11 mm but did not change significantly in the other two experimental groups (Fig. 1a). Weight change in the experimental group with tails shortened by 21 mm was intermediate between the other two groups (Fig. 1a). Barn swallows with tails shortened by 11 mm did not show the most improvement (or the least deterioration) in body condition, as predicted if streamers have been elongated 10-12 mm by sexual selection, but quite the opposite.

The final model explaining changes in blood leukocyte concentration included experimental treatment, sex, date of capture (early or late in the season) and the index of parental effort, although the last variable did not explain a significant amount of the variance (Table 1). Change in leukocyte concentration differed among the three experimental groups, mainly because leukocyte concentration decreased (i.e., health improved) in barn swallows with tail shortened by 11 mm, but did not change significantly in the other two groups (Fig. 1b). In this case, barn swallows with tail shortened by 11 mm showed the most improvement in health, as predicted if streamers have been elongated 10-12 mm by sexual selection. Leukocyte concentration was also significantly related to sex and date of capture (Table 1), decreasing in males but not in females, and in birds captured late in the season, but increasing in birds captured early (least-square means (SE) of daily variation in leukocyte concentration; males, -0.124 (0.049); females, 0.023 (0.048); early in the season, 0.056 (0.031); late in the season, -0.158 (0.072)).

Neither the experimental treatment nor the other variables that might influence phenotypic condition explained a significant amount of variance in change in sedimentation rate or H/L (Table 1). Sedimentation rate increased in birds with tails shortened by 21 mm, but did not change significantly in the other two groups (Fig. 1c). H/L, however, did not change significantly in any of the experimental groups (Fig. 1d).

Discussion

As explained in the Introduction, if streamers have been elongated 10-12 mm by sexual selection (Buchanan and Evans 2000, Rowe et al. 2001), we predicted an improvement in phenotypic condition in the group of birds with tails shortened by 11 mm over birds with tail shortened by 1 or 21 mm. This was indeed the case according to changes in blood leukocyte concentration, an index of health status (Fig. 1b). However, changes in weight suggest the opposite, as body condition deteriorated in birds with tails shortened by 11 mm compared to the other two experimental groups, or at least to birds with tail shortened by 1 mm (Fig. 1a). On the other hand, if streamer length was at the aerodynamic optimum or very close to it (Cuervo and de Ayala 2005), we predicted the least deterioration in phenotypic condition in birds with tails shortened by 1 mm compared to the other two experimental groups, but mainly to birds with tail shortened by 21 mm. However, tail shortening by 1 and 21 mm had a similar effect on phenotypic condition (Fig. 1). Experimental tail length manipulation did not significantly affect sedimentation rate (another index of health status) or H/L (an index of physiological stress) (Table 1). It should be noted that changes in phenotypic condition were checked only once, around one month after tail length manipulation, so longer-term effects could not be assessed in this study and, therefore, cannot be ruled out. At first sight, some results are consistent with tail streamers being 10-12 mm longer than the aerodynamic optimum while others contradict it, so this study would not allow us to reach any conclusion. A closer examination, however, may reveal a possible explanation for these findings and shed some light on tail-length evolution (see below).

One of the most interesting results of this study was that health of birds with tails shortened by 11 mm improved, but body condition deteriorated, as a consequence of the experimental treatment. One possible explanation for this apparently paradoxical result is the existence of trade-offs between different components of phenotypic condition. It is often intuitively assumed that all estimates of phenotypic condition should be positively related to one another, but this would occur only in ideal circumstances. For example, different traits may reflect completely different aspects of phenotypic condition that do not need to be related at all. Moreover, organisms in natural conditions usually have access to limited resources, and devoting these resources to one function may be detrimental to others, giving rise to trade-offs. For example, trade-offs between nestling growth rate and immune response (Soler et al. 2003, Tschirren and Richner 2006) or between immune system components (Johnsen and Zuk 1999, Buchanan et al. 2003, Arriero 2009) have been suggested. In any case, the relationships between different estimates of phenotypic condition are often complex and condition or environment-dependent (Johnsen and Zuk 1999, Alonso-Alvarez and Tella 2001, Arriero 2009). It should be noted that a negative correlation between two phenotypic traits does not necessarily imply a trade-off, but when both traits are positively related to fitness, a trade-off is the most plausible explanation (see review in Agrawal et al. 2010).

If trade-offs between different condition-related systems within an organism are possible, negative relationships between different estimates of phenotypic condition should also be possible (see references above). In this case, certain traits used to estimate phenotypic condition might be negatively and not positively related to condition. Therefore, unless we know which traits and in what circumstances may have their relationship with overall phenotypic condition reversed, any assessment of condition based on one or very few traits might be uninformative or even misleading. Depending on the particular traits studied, one result, the opposite, or even both (contradictory results from different traits, as is the case in our study) might be found. As explained in the Introduction, we studied physiological traits presumably related to the probability of survival, a fitness component that was expected to deteriorate when tail length was displaced from the aerodynamic optimum. However, owing to possible trade-offs between different components of survival, a more appropriate approach would have been to study survival directly, for example, by checking which birds returned to the breeding colonies the following year (see Møller 1989, Møller and de Lope 1994). Unfortunately, direct survival information was not available.

Although survival was not studied directly, and despite the possible trade-off between the immune function and body condition, our study can still provide information on tail length evolution. Interestingly enough, our results suggest a turning point for some parameters used to estimate phenotypic condition when the tail was shortened by 11 mm (Fig. 1). This means that shortening tails more (21 mm) or less (1 mm) had similar effects on flight performance, but both effects differed (they might be either beneficial or detrimental) from the effect of shortening the tail by 11 mm. The most plausible explanation for this turning point is that overall phenotypic condition improved in the group of birds with tails shortened by 11 mm more than in the other two groups, as shown by the decrease in blood leukocyte concentration (Fig. 1b). The decrease in weight when the tail was shortened by 11 mm (Fig. 1a) might be the consequence of a trade-off between immune function and body condition. This explanation implies that tail length was closer to the aerodynamic optimum when the tail was shortened by 11 mm than in the other two treatments and, consequently, streamers would have been elongated approximately between 7 and 15 mm by sexual selection. This conclusion is consistent with the hypothesis that tail streamers in the barn swallow have been elongated 10-12 mm by sexual selection (Buchanan and Evans 2000, Rowe et al. 2001), but not with the hypothesis that tail length is at the aerodynamic optimum or very close to it (Cuervo and de Ayala 2005).

An alternative explanation for the turning point is that overall phenotypic condition deteriorated in the group of birds with tail shortened by 11 mm more than in the other two groups, as shown by the decrease in weight (Fig. 1a). The decrease in blood leukocyte concentration when the tail was shortened by 11 mm (Fig. 1b) might be the consequence of a trade-off (see above). However, we do not think this explanation is probable, because we cannot think of any scenario in which tail shortening by 11 mm could be more detrimental to flight performance than the other two experimental treatments. First, if shortening tails by 11 mm made tail length closer to the aerodynamic optimum than the other two treatments, phenotypic condition in this group of birds would improve rather than deteriorate. Second, if shortening tails by 21 mm made tail length closer to the aerodynamic optimum than the other two treatments, an improvement in the phenotypic condition of birds with tails shortened by 1 mm over birds with tails shortened by 11 mm would not be expected. And third, if shortening tails by 1 mm made tail length closer to the aerodynamic optimum than the other two treatments, an improvement in the phenotypic condition of birds with tails shortened by 21 mm over birds with tails shortened by 11 mm would not be expected either.

The decrease in blood leukocyte concentration in the group of birds with tails shortened by 11 mm was interpreted as an improvement in health, because the number of leukocytes generally increases with infectious diseases (Saino et al. 1998, Szép and Møller 1999, Apanius et al. 2000). Nevertheless, it may be argued that birds in very poor health might be unable to produce leukocytes fast enough to compensate for their use in fighting infection, thus resulting in a decrease in blood leukocyte concentration. In fact, low numbers of leukocytes are often found in birds with severe infections (Rosskopf et al. 1981, Bienzle et al. 1997, Schoemaker et al. 2000). In these circumstances, a decrease in leukocyte concentration would be associated with deterioration in health instead of improvement. However, in our study, this explanation does not seem probable, because the group of birds with tails shortened by 11 mm were not in particularly poor health. All birds apparently bred normally and reproductive performance did not differ significantly among the experimental groups (Cuervo and de Ayala 2005). Moreover, severe deterioration in health should also have affected other parameters indicating health status, such as sedimentation rate, but this variable was not significantly affected by the experimental treatments (Table 1).

Experimental tail length manipulation had an effect on phenotypic condition, but interestingly, this effect was similar in males and females, as suggested by the non-significant interactions between treatment and sex (Table 1). This result agrees with previous studies that found no sexual differences in the effect of tail-length manipulation on flight performance (Buchanan and Evans 2000, Rowe et al. 2001). Similar results in males and females suggest that outermost tail feathers are longer than the aerodynamic optimum not only in male but also in female barn swallows, and this might be explained by at least two non-exclusive mechanisms. First, tail length would be under sexual selection in males because of female preference to mate with long-tailed males (Møller 1988), and a strong genetic correlation between the sexes for tail length (Møller 1993) would cause tail lengthening also in females. Second, tail length would be under direct sexual selection in females because long tails would provide direct benefits to females in terms of mate acquisition, as is the case with female feather ornaments in other bird species (e.g. Jones and Hunter 1993). Current evidence, however, does not support a mating function for tail feathers in female barn swallows (Cuervo et al. 1996). Sexual dimorphism in tail length with longer tails in males than in females (Cramp 1988, Møller 1994) has often been explained as a consequence of sexual selection (e.g. Hedenström and Møller 1999). However, similar effects of experimental tail length manipulation on males and females might indicate that sexual dimorphism is caused by natural rather than sexual selection. As suggested by Rowe et al. (2001), the risk of streamer breakage during incubation might be higher in females than in males (only females incubate in the European subspecies), and thus shorter streamers in females would decrease that risk.

Experimental tail length manipulation in barn swallows led us to different conclusions with regard to the portion of the streamer that has been elongated by sexual selection, depending on whether phenotypic condition (this study) or flight effort (Cuervo and de Ayala 2005) was considered. Variation in phenotypic condition, mainly in health status estimated as blood leukocyte concentration, suggested that streamers have been elongated around 7-15 mm by sexual selection (see above). However, variation in flight effort, estimated as changes in haematocrit, suggested that streamers have not been elongated (or very little) by sexual selection (Cuervo and de Ayala 2005). One possible explanation for this discrepancy is the potential complexity of interpreting changes in haematocrit. First, haematocrit might be affected not only by changes in flight effort (specifically in oxygen demand), as previously assumed (Cuervo and de Ayala 2005), but also by changes in phenotypic condition. Whether haematocrit is a good estimate of condition in birds is under debate (e.g. Dawson and Bortolotti 1997), but malnutrition and disease often affect haematocrit negatively (e.g. Hurtrez-Boussès et al. 1997, Hoi-Leitner et al. 2001). If haematocrit reflects both flight effort and condition, changes in haematocrit will depend on the relative strength of each effect. Second, different treatments might affect flight effort and condition in different ways, depending on the behavioural response to each experimental treatment. At least in theory, manipulated birds might keep flight effort constant, with small or no change in haematocrit, but change quantity or quality of food. Or, on the contrary, manipulated birds might keep quantity and quality of food constant, but change flight effort, thus resulting in a strong change in haematocrit. Obviously, any intermediate strategy changing both flight effort and food caught is also possible. But most importantly, the behavioural response might differ among experimental treatments. Third, there might be a trade-off between different components of phenotypic condition (see references above) giving rise to negative relationships between haematocrit and physiological traits commonly used to estimate condition. And fourth, haematocrit in birds is affected by a large number of factors (review in Fair et al. 2007), and not only by phenotypic condition and oxygen demand.

To summarize, experimental tail shortening in barn swallows caused changes in weight and blood leukocyte concentration that suggest the existence of trade-offs between different components of phenotypic condition. These trade-offs advise against the use of one or very few physiological parameters to estimate phenotypic condition. The turning point for phenotypic condition when the tail was shortened by 11 mm suggests that tail streamers in both male and female barn swallows have been elongated 7-15 mm by sexual selection. This finding is consistent with previous studies suggesting that tail streamers have been elongated 10-12 mm by sexual selection.

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Table 1. Final models after backward stepwise General Linear Models explaining daily variation in four phenotypic condition parameters. In the case of sedimentation rate and H/L, none of the independent variables was retained in final models, but the effect of experimental treatment is shown for information. For a list of all independent variables considered, see section Statistical methods. H/L = heterophil/lymphocyte ratio, IPE = index of parental effort. Final models had the statistics: daily variation in weight, multiple $r^2 = 0.126$; daily variation in sedimentation rate, multiple $r^2 = 0.053$; daily variation in leukocyte concentration, $F_{5,50} = 6.72$, multiple $r^2 = 0.402$, $p < 0.0001$; daily variation in H/L, multiple $r^2 = 0.005$.

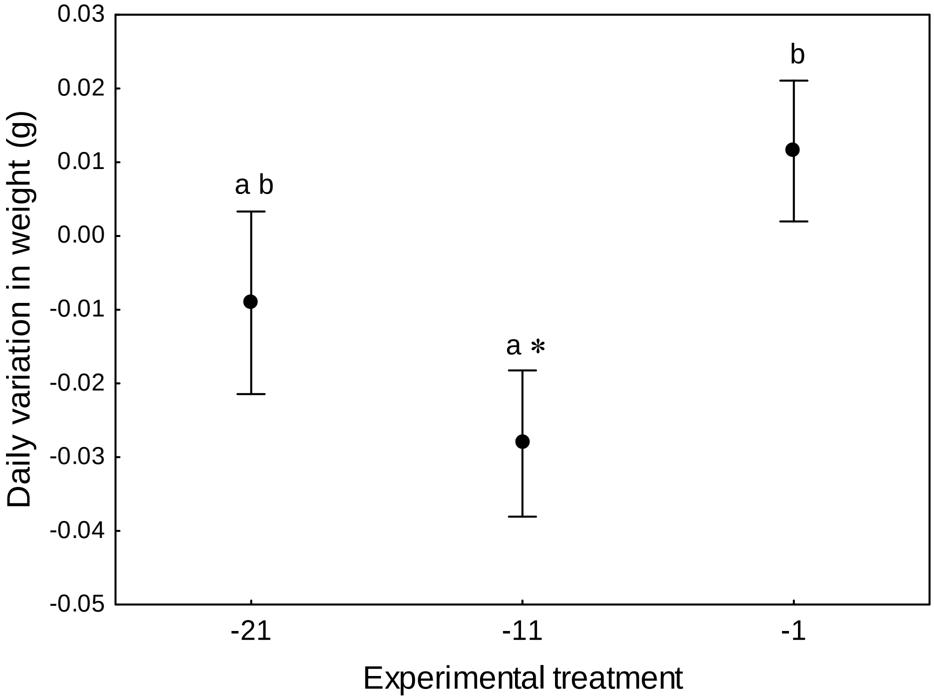
Dependent variable	Independent variable	Sum of squares	df	F	p
Weight	Treatment	0.0157	2	3.83	0.028
	Error	0.1089	53		
Sedimentation rate	Treatment	0.0564	2	1.47	0.24
	Error	1.0141	53		
Leukocytes	Treatment	0.6351	2	7.02	0.0021
	Sex	0.2870	1	6.34	0.015
	Date of capture	0.3394	1	7.50	0.0085
	IPE	0.1609	1	3.56	0.065
	Error	2.2623	50		
H/L	Treatment	37×10^{-6}	2	0.12	0.89
	Error	0.0081	53		

Figure legend

Figure 1. Means ± SE for daily variation in (a) weight, (b) relative blood leukocyte concentration (least square means from a General Linear Model including treatment, sex, date of capture and parental effort index), (c) sedimentation rate, and (d) heterophil/lymphocyte ratio (H/L) in barn swallows with tail shortened by 1, 11 or 21 mm. Although experimental treatment did not significantly affect daily variation in sedimentation rate or H/L, these parameters are also shown for illustration purposes. Different letters denote statistically significant differences between experimental groups in Tukey post-hoc tests. An asterisk (*) denotes a significant change (daily variation different from zero in a one-sample t-test) in that particular phenotypic parameter and experimental group.

Figure 1

(a)


0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
-0.05
Daily variation in weight (g)
a b
a *
b
-21
-11
-1
Experimental treatment

(b)

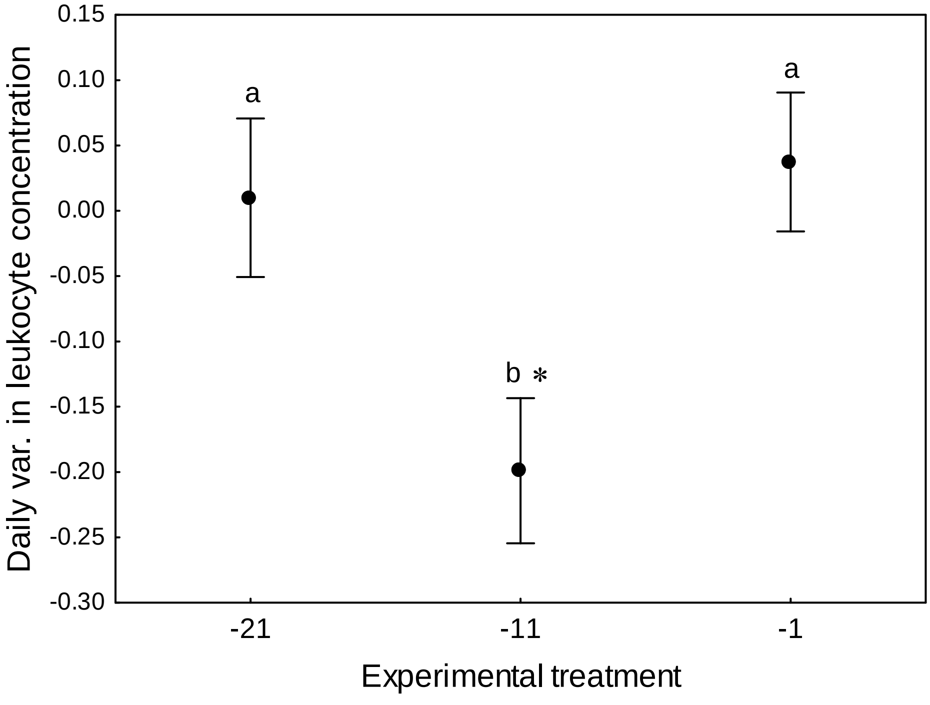
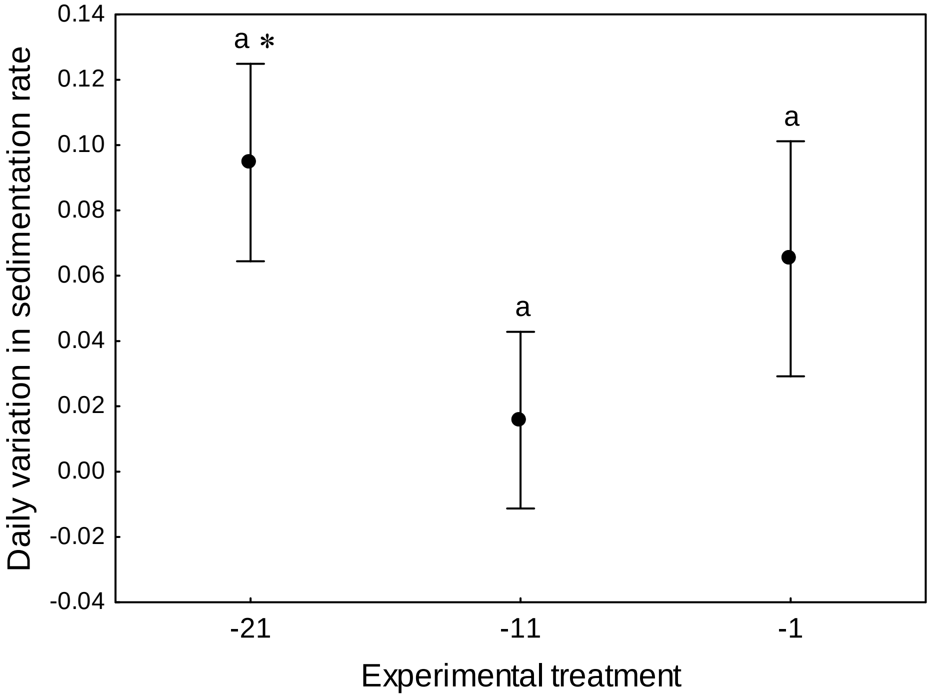

0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.30
Daily var. in leukocyte concentration
a
b *
a
-21
-11
-1
Experimental treatment

Figure 1 (continuation)

(c)



(d)