

## MELANIN-BASED COLORATION IS A NONDIRECTIONALLY SELECTED SEX-SPECIFIC SIGNAL OF OFFSPRING DEVELOPMENT IN THE ALPINE SWIFT

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**Abstract.**—Two mutually exclusive hypotheses have been put forward to explain the evolution and adaptive function of melanin-based color traits. According to sexual selection theory melanism is a directionally selected signal of individual quality, whereas theory on the maintenance of genetic polymorphism proposes that alternative melanin-based variants achieve equal fitness. Alpine swift (*Apus melba*) males and females have a conspicuous patch of white feathers on the breast with their rachis varying continuously from white to black, and hence the breast varies from white to striated. If this trait is a sexually selected signal of quality, its expression should be condition dependent and the degree of melanism directionally selected. If variation in melanism is a polymorphism, its expression should be genetically determined and fitness of melanin-based variants equal. We experimentally tested these predictions by exchanging eggs or hatchlings between randomly chosen nests and by estimating survival and reproduction in relation to melanism. We found that breast melanism is heritable and that the environment and body condition do not significantly influence its expression. Between 5 and 50 days of age nestlings were heavier and their wings longer when breast feathers of their biological father were blacker, and they also fledged at a younger age. This shows that aspects of offspring quality covary positively with the degree of melanism. However, this did not result in directional selection because nestling survival and recruitment in the local breeding population were not associated with father breast melanism. Furthermore, adult survival, age at first reproduction and probability of skipping reproduction did not covary with the degree of melanism. Genetic variation in breast melanism is therefore maintained either because nonmelanic males achieve fitness similar to melanic males via a different route than producing fast-growing offspring, or because the advantage of producing fast-growing offspring is not sufficiently pronounced to result in directional selection.

**Key words.**—Alpine swift, directional selection, growth rate, melanin-based coloration, sexual selection, signaling.

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Two mutually exclusive mechanisms have been proposed to explain the adaptive function of animal coloration. According to sexual selection theory, conspicuous ornaments are honest signals of individual quality because only the best individuals can afford to pay the costs of producing and wearing such traits. Because ornamented individuals are of higher quality and gain a mating advantage from displaying a bright color trait, coloration should be directionally selected (Andersson 1994). However, in many animals the expression of different color patterns is under strong genetic control, and the maintenance of genetic variation implies that color morphs are not directionally selected but either selectively neutral or under disruptive, balancing, frequency- or density-dependent selection (e.g., Jones et al. 1977; Sinervo and Liveley 1996; Losey et al. 1997; Day 2000; Hoffman and Blouin 2000; Roulin 2004; Olendorf et al. 2006). Morphs may therefore be alternative but equally fit strategies to cope with fluctuating environmental factors or social situations. Knowledge of the mechanism underlying the expression of coloration can provide useful insight to predict the type of selection exerted on coloration.

In vertebrates, several types of pigments are responsible for the coloration of skin, hair, eyes, and feathers. These pigments are either synthesized de novo by animals, such as melanins that are responsible for black eumelanic and reddish-brown pheomelanic colors, or obtained through the diet, such as carotenoids responsible for yellow to red. Carotenoid-based colors have received attention from evolutionary ecologists because they are assumed to be present in limited

quantity in the environment implying that these colors can reliably signal individual foraging quality (Olson and Owens 1998), and also because carotenoids have antioxidant properties implying that carotenoid-based colors can be traded off against other physiological processes like immunity and reproduction (McGraw et al. 2005; Biard et al. 2005). For these reasons, sexual selection on carotenoid-based colors is expected to be directional, a prediction supported by field data (review in Hill 2006).

In contrast, melanin synthesis has been demonstrated to be under strong genetic control in vertebrates (Hearing and Tsukamoto 1991; Roulin and Dijkstra 2003; Majerus and Mundy 2003). Hence, continuous variation in melanin-based coloration until recently was not expected to reflect individual quality and thereby not expected to be directionally sexually selected (Badyaev and Hill 2000). Several empirical studies do not support this proposition with both eumelanin-based and pheomelanin-based color traits indicating aspects of individual quality and playing a role in mate choice in several species (e.g., Jawor and Breitwisch 2003; Roulin 2004; Hill 2006). For example, in the eastern bluebird (*Sialia sialis*) males with larger chestnut breast patches provision nestlings more often, fledge heavier offspring, and mate with females that nest earlier (Siefferman and Hill 2003). In feral cats (*Felis catus*) orange males are heavier than nonorange males, whereas the reverse is true in females (Pontier et al. 1998). In the barn owl (*Tyto alba*) cross-fostering experiments have shown that the size of black spots displayed by biological mothers covaries positively with parasite resistance, humoral

immunocompetence, and developmental homeostasis of their cross-fostered offspring (Roulin et al. 2000, 2001, 2003), and males appear to assess this trait to choose a mate and adjust parental effort (Roulin 1999). In the common yellowthroat (*Geothlypis trichas*), the size of black facial mask predicts both male-male competition and female mate choice (Tarof et al. 2005). From an ultimate point of view, these covariations between melanin-based coloration and phenotypic quality could evolve either because melanin-based coloration is sexually selected with the degree of melanism honestly signaling several aspects of quality or because melanin-based morphs are associated with different aspects of quality in such a way that the fitness of each morph is equal.

From a proximate point of view, two hypotheses can explain why melanin-based ornaments covary with fitness components. First, the expression, display, maintenance, or wearing of melanin-based ornaments entails fitness costs so that lesser quality individuals cannot express the trait to the same degree as higher quality individuals (Zahavi 1975). Production of melanin pigments may be costly in terms of energy, acquisition of melanin precursors (Griffith 2000; Poston et al. 2005), or dietary access to scarce minerals such as Ca and Zn involved in melanogenesis (McGraw 2003; Niecke et al. 2003). However, in birds, empirical evidence for these costs is not significant or inconsistent between studies (Hill and Brawner 1998; Roulin et al. 1998, 2006; Gonzalez et al. 1999; Griffith et al. 1999; McGraw et al. 2002; Roulin and Dijkstra 2003; Poston et al. 2005; Siefferman and Hill 2005), whereas in fish, color expression can be sensitive to temperature (Horth 2003). Even though condition dependence in terms of melanin production is still hypothetical at least in birds (few experimental studies have been carried out in other wild vertebrates), displaying melanin-based colors seems to entail significant costs in male house sparrows (*Passer domesticus*). In this species, the full display of the black bib increases with the abrasion of the white tip of melanic feathers, a process that social interactions accelerate. As a consequence, because testosterone mediates aggressive behaviors, individuals with a high level of circulating androgens tend to show a larger black bib because their feathers abrade more rapidly (Gonzales et al. 2001). The general applicability of the mechanism demonstrated in house sparrows is still unknown (but see Rohwer 1975). Since directional sexual selection is usually exerted on condition-dependent ornaments (Andersson 1994), further studies are needed to determine whether body condition and the environment influence the expression, display, and wearing of melanin-based ornaments.

Under the second hypothesis, melanin-based ornaments are not condition dependent but they covary with phenotypic attributes either because coloration plays a role in social interactions or prey-predator relationships or because genes determining the production of melanin pigments also regulate other traits. In vertebrates, the binding of  $\alpha$ -MSH,  $\beta$ -MSH, and ACTH on the melanocortin-1 receptor (Mc1-R) induces the production of eumelanin at the expense of pheomelanin (Eberle 1988; Slominski et al. 2004; Mundy 2006), and these hormones affect many physiological processes (e.g., Wikberg et al. 2000). In this case, genetic variation in melanin-based coloration is maintained if genes responsible for different levels of melanism provide the same fitness benefits under

frequency-dependent or disruptive selection (Maynard-Smith 1982; Spichtig and Kawecki 2004). However, because most studies on the adaptive function of melanin-based colors are correlative (Jawor and Breitwisch 2003; Roulin 2004), it remains unclear whether covariation between melanin-based colors and individual quality arises because such color traits are condition dependent or because their underlying genes pleiotropically alter other phenotypic attributes. It is therefore important to examine whether melanin-based colors are directionally selected or not.

Our aim here is to test experimentally whether the expression of melanin-based coloration is condition dependent or mainly under genetic control, whether this color trait signals aspects of individual quality, and whether this results in directional selection. For this purpose, we considered the colonial alpine swift (*Apus melba*) in which males and females vary in the extent to which the rachis of white feathers of the breast is black. We cross-fostered eggs or hatchlings between randomly chosen nests to test whether this color trait is heritable. For a subset of nests, we also manipulated brood size to investigate whether the expression of melanic breast feathers is sensitive to sibling condition (in nestlings) and reproductive effort (in parents). Cross-fostering experiments were particularly useful to examine the relationship between breast melanism of the biological and foster parents and both nestling body mass and wing length growth as well as age at fledging, a parameter that determines the length of the rearing period and, in turn, residual reproductive value of the parents (Bize et al. 2004). Finally, using a dataset of six years we investigated whether the probability for parents of recruiting offspring into the local breeding population is associated with the degree of melanism of breast feathers. We applied capture-recapture models to assess whether survival prospect, probability of skipping reproduction, and age at first reproduction differ between individuals displaying different levels of melanism.

## MATERIALS AND METHODS

### *Model Organism*

The alpine swift is a migrant colonial apodiform bird that breeds in holes of cliffs and under the roof of tall buildings. It feeds on insects caught exclusively in flight. It is socially monogamous and reproduces in colonies counting a few (<5 pairs) to several hundred breeding pairs. Paternity analyses have not yet been carried out in the alpine swift but in the related colonial common swift (*A. apus*) extrapair paternity is rare with only four chicks (4.5%) of 88 being extrapair young (Martins et al. 2002). A single clutch is produced per year with one to four eggs being laid at one-day intervals. Both parents incubate the eggs for 20 days and feed the offspring up to fledging, which takes place at 50 to 69 days of age. Parental care stops after offspring have taken their first flight (Bize et al. 2004). Adults are sexually mature at an age of two or three years and the oldest recaptured bird reported was 26 years old (Swiss Ornithological Station, pers. comm.). Male and female nestlings are similar in size and in reflectance spectra measured on the white parts of the breast and brown back feathers (Bize et al. 2005). Natal and breeding dispersal is limited with birds breeding usually in the



FIG. 1. (A) Nonmelanic (color score 0) and (B) strongly melanized breast (color score 3) in *Apus melba*.

colony where they were born (P. Bize, pers. obs.), implying that survival probabilities estimated with capture-recapture analyses are not severely biased by emigration.

#### General Procedure

The fieldwork was conducted over six breeding seasons between 2000 and 2005 in two Swiss colonies located in clock towers in Bienne (about 100 breeding pairs) and Solothurn (about 50 breeding pairs). The two cities are located 21 km from each other. Each year, nests were regularly visited to determine laying date, clutch size, and egg volume calculated with the formula  $V = LB^2\pi/6$  (Bize et al. 2002),  $L$  and  $B$  referring to egg length and breadth measured to the nearest 0.1 mm. Because we visited colonies every day, we could determine hatching date of all nestlings, which were individually recognized with a nontoxic permanent color mark before being ringed at 10 days with an aluminum numbered band. In each nest, we recorded the number of nestlings that died before taking their first flight. Each year, adults were captured while sitting on their eggs or hatchlings. P. Bize weighed all adults to the nearest 0.1 g and measured the length of the left wing, tail, and fork to the nearest mm and sternum and beak to the nearest 0.1 mm. Age of the adults was known with precision because many of them had been banded as nestlings. A drop of blood was collected from the foot of each adult and from the wing of each nestling to determine sex from blood cell DNA using molecular techniques (Bize et al. 2005).

#### Assessment of Breast Melanism

Alpine swifts have a conspicuous white patch on the breast, but the rachis of breast feathers vary in the amount of black. Between 2001 and 2005, P. Bize assessed breast plumage

melanism in a discrete way, although variation in feather blackness is continuous. It is difficult to obtain a quantitative measure of breast melanism (Fig. 1). He assigned the score 0 if the rachis of all breast feathers was not melanized, 1 if few breast feathers had a weakly melanized rachis, 2 if most breast feathers were melanized to an intermediate level, and 3 if all breast feathers had a strongly melanized rachis (strongly melanic individuals have still a conspicuous white breast patch since only the rachis is black; Fig. 1). This method proved reliable and P. Bize assigned similar scores to individuals caught in successive years (repeatability,  $r = 0.87$ ,  $F_{674,733} = 14.56$ ,  $P < 0.0001$ ). Because individuals obtained similar scores in different years, for individuals captured in 2000 we gave them their average score assigned along the four years of the study.

#### Cross-Fostering Experiments

To experimentally investigate whether the degree of breast melanism is heritable, and to determine whether nestling growth rate and age at fledging covary with breast melanism of their biological parents, we carried out cross-fostering experiments in Bienne and Solothurn. Between pairs of nests we either cross-fostered the complete clutches within 48 h after clutch completion (so-called full cross-fostering experiment) or only one or two two-day-old hatchlings while their siblings remained in the nest of origin (so-called partial cross-fostering experiment; Table 1). Therefore, nests used in partial cross-fostering experiments contained chicks of two origins. Nests of each pair had similar laying date (Pearson correlation,  $r = 0.96$ ,  $n = 172$  pairs of nests;  $P < 0.0001$ ), clutch size ( $r = 0.54$ ,  $n = 172$ ;  $P < 0.0001$ ) and brood size at hatching ( $r = 0.38$ ,  $n = 172$ ;  $P < 0.0001$ ). Biological and foster parents did not resemble each other with respect to

TABLE 1. Year and colony where cross-fostering experiments were performed. Full cross-fostering experiments refer to the exchange of clutches between pairs of nests, whereas partial cross-fostering experiments refer to the exchange of approximately half of two-day-old hatchlings between pairs of nests. Number of nests used in cross-fostering experiments is indicated. Final statistical analyses were restricted to broods that successfully fledged offspring.

Full cross-fostering			Partial cross-fostering		
Year	Colony	Number of nests	Year	Colony	Number of nests
2000	Bienne	42	2002	Bienne	72
2004	Bienne	32	2002	Solothurn	40
2004	Solothurn	36	2003	Bienne	50
2005	Solothurn	40	2003	Solothurn	34
			2004	Bienne	28
Total		150			224

breast melanism (Pearson correlation biological mother  $\times$  foster mother:  $r = 0.02$ ,  $n = 326$ ,  $P = 0.75$ ; biological mother  $\times$  foster father:  $r = 0.05$ ,  $n = 322$ ,  $P = 0.42$ ; biological father  $\times$  foster mother:  $r = 0.06$ ,  $n = 320$ ,  $P = 0.28$ ) with the exception of biological fathers resembling foster fathers ( $r = 0.20$ ,  $n = 312$ ,  $P = 0.0004$ ). Coloration of both biological and foster parents was therefore entered in the statistical analyses to estimate their relative effect. Nestling body mass was measured to the nearest 0.1 g and wing length to the nearest mm at day 5, 10, 20, 30, 40, 50, and until they fledged at around  $55.5 \pm 3.0$  days of age (mean  $\pm$  SD). To investigate the relationship between nestling body mass and wing length and the degree of parental breast melanism, we considered 649 cross-fostered nestlings from 339 experimental nests that survived at least until fledging.

#### Brood Size Manipulation Experiment

We experimentally investigated the effect of sibling competition and parental effort on the expression of breast melanism in 2003 and 2004 in Bienne by enlarging or reducing brood sizes in 16 and 14 pairs of nests, respectively. Nests with a similar clutch size (Pearson correlation,  $r = 0.76$ ,  $n = 30$ ,  $P < 0.0001$ ), hatching date ( $r = 0.99$ ,  $n = 30$ ,  $P < 0.0001$ ), and brood size at hatching ( $r = 0.86$ ,  $n = 30$ ,  $P < 0.0001$ ) were matched in pairs, and within each pair of nests we exchanged two hatchlings from one of the nest (after the manipulation this nest had a reduced brood size) against one hatchling from the other nest (after the manipulation this nest had an enlarged brood size). This exchange was done with five-day-old nestlings in 2003 and with two-day-old nestlings in 2004. This design permitted us to perform simultaneously a brood size manipulation and partial cross-fostering experiment (however, these broods are not considered in Table 1). Prior to manipulation, each nest contained two to three nestlings, and thus brood size after the manipulation remained within the natural range (1–4 nestlings). At the time of cross-fostering, exchanged hatchlings were similar in size to their resident siblings (paired  $t$ -test, all  $P$ -values  $> 0.32$ ) and to their new nest-mates (paired  $t$ -test, all  $P$ -values  $> 0.41$ ), and enlarged and reduced broods did not differ in clutch size, hatching date, brood size at hatching, louse-fly load, and mean nestling body mass (Student  $t$ -test, all  $P$ -values  $> 0.50$ ). Because experimental brood size enlargement significantly

decreased offspring growth rate (Bize and Roulin 2006), this design was useful to analyze its effect on the expression of breast melanism in offspring but also on the between-year change in the degree of breast melanism in parents (therefore, we considered melanin score assigned in each single year and not the average score as in most other analyses). For these analyses, we took into account only cross-fostered nestlings. Because breast melanism becomes apparent only at adulthood, the effect of brood size manipulation on the expression of breast melanism was analyzed on individuals reared in enlarged ( $n = 11$  nestlings) and reduced broods ( $n = 18$ ) that were subsequently recaptured in the colony at the first or second year of age.

#### Statistical Procedure

Statistical analyses were performed with the software package SAS (SAS Institute 1996), except for capture-mark-recapture analyses. All tests were two-tailed, and  $P$ -values smaller than 0.05 were considered significant. To investigate the relationship between parent breast melanism and nestling body mass and wing length, we used a mixed-model ANCOVA for repeated measures (proc mixed, SAS 1996; Littell et al. 1996), with nestling body mass or wing length as dependent variable; breast melanism of biological and foster mothers and fathers, nestling age, and age<sup>2</sup> as covariates; and year as a categorical variable. We included nestling age<sup>2</sup> because there is a quadratic relationship between body mass and age; swifts show adaptive body mass loss before fledging (Bize et al. 2003; Wright et al. 2006). The identities of parents (i.e., ring numbers) were included in the model as random factors since most of them appeared more than once in the data file (many individuals bred in more than one year). Nestling identity given by ring number was entered as repeats, permitting us to take into account the fact that time intervals between each measure of body mass are unequal.

Survival was estimated with capture-mark-recapture (CMR) methods (Lebreton et al. 1992) implemented in program MARK 4.2 (White and Burnham 1999). These methods take into account the possibility that not every individual was encountered on every occasion and allow estimating the recapture rate (probability of capturing an individual given that it is alive and in the population) independently of the survival rate. Even if we were mainly interested in finding possible differences in life history between individuals displaying different degrees of breast melanism, we considered several other factors that may cause variation in survival or recapture. The factors were age (four age classes: 1, 2, 3, or 4 years and older), sex, colony (Bienne and Solothurn), and breeding status (nonbreeder and breeder). In a preliminary analysis, we found no evidence for an effect of colony or sex on survival and recapture, and therefore we concentrate our analyses on the remaining factors. Our dataset consisted of 2011 captures of 483 adults between 2001 and 2005.

We modeled breeding status as two states (breeder and nonbreeder) within a multistate CMR model (Nichols et al. 1994). In addition to estimating survival and recapture of breeders and nonbreeders, this model also allowed us to estimate the probability of changing breeding status from one year to the next. The models thus consisted of six parts:

TABLE 2. Model selection for multistate capture-mark-recapture analysis. The models consist of six components: survival of nonbreeders ( $S_n$ ) and breeders ( $S_b$ ), recapture of nonbreeders ( $P_n$ ) and breeders ( $P_b$ ), the transition rate from nonbreeder to breeder ( $\Psi_{nb}$ ), and the transition rate from breeder to nonbreeder ( $\Psi_{bn}$ ). We considered year ( $y$ ) and age as factors potentially affecting these model components, and examined the effect of the covariate melanism (mel). Asterisk indicates that all interactions between the factors were included in the model, “+” indicates additive effects, and “=” indicates that two model components were set equal. The models were evaluated by Akaike’s information criterion (AIC).  $\Delta$  AIC is the difference between the current model and the best one, and Akaike weights give the relative support for a given model compared to the other models.  $K$  is the number of parameters.

Model	AIC	$\Delta$ AIC	Akaike weight	$K$	Deviance
1) $S_n(y) = S_b(y)P_n()P_b()\Psi_{nb}(\text{age})\Psi_{bn}()$	1443.497	0.000	0.272	10	1423.242
2) $S_n(\text{age} + y)S_b(y)P_n()P_b()\Psi_{nb}(\text{age})\Psi_{bn}()$	1444.188	0.691	0.193	14	1415.699
3) $S_n(y)S_b(y)P_n()P_b()\Psi_{nb}(\text{age})\Psi_{bn}()$	1444.560	1.063	0.160	12	1420.197
4) $S_n(y) = S_b(y)P_n()P_b()\Psi_{nb}(\text{age})\Psi_{bn}(\text{mel})$	1444.665	1.169	0.152	11	1422.359
5) $S_n(y + \text{mel}) = S_b(y + \text{mel})P_n()P_b()\Psi_{nb}(\text{age})\Psi_{bn}()$	1445.218	1.721	0.115	11	1422.912
6) $S_n(y) = S_b(y)P_n()P_b()\Psi_{nb}(\text{mel} + \text{age})\Psi_{bn}()$	1445.548	2.051	0.098	11	1423.241
7) $S_n() = S_b()P_n()P_b()\Psi_{nb}(\text{age})\Psi_{bn}()$	1450.026	6.530	0.010	8	1433.860
8) $S_n(\text{age}*y*\text{mel})S_b(y)P_n()P_b()\Psi_{nb}(\text{age}*\text{mel})\Psi_{bn}()$	1455.345	11.848	0.001	29	1395.283
9) $S_n(\text{age}*y*\text{mel})S_b(y*\text{mel})P_n(\text{mel})P_b(\text{mel})\Psi_{nb}(\text{age}*\text{mel})\Psi_{bn}(\text{mel})$	1463.217	19.720	0.000	34	1392.380

survival of nonbreeders and breeders, recapture of nonbreeders and breeders, transition from nonbreeder to breeder, and transition from breeder to nonbreeder. Our most general model included interactive effects of the degree of breast melanism, age, and year on nonbreeder survival, and breast melanism and year on breeder survival (we did not consider age effects on breeder survival because the majority of them was in the oldest age class, four years and older). We allowed the recapture rate to differ between breeders and nonbreeders, and between individuals with different melanistic scores. We considered the effects of breast melanism and age on the transition probability from nonbreeder to breeder. Finally, we allowed the transition probability from breeder to nonbreeder to differ between individuals for which breast feathers were differently melanized. The model including all these factors was the starting model for model selection (model 9, Table 2). We tested the goodness-of-fit for this model using the median  $c$ -hat approach in program MARK (10 simulations each at 10 levels of overdispersion ranging from  $c$ -hat = 1

to  $c$ -hat = 1.5). This test showed no overdispersion and thus a good fit of our general model to the data (estimated  $c$ -hat = 1.029, SE = 0.015).

We based model selection on the small sample size adjusted Akaike’s information criterion (AIC), where a lower value indicates a better model. Models differing in AIC from the best model by less than two are considered as having substantial support by the data (Burnham and Anderson 2002). In the case of nested models, it is also informative to observe changes in deviance when a factor is added to the model. A small change indicates that this factor does not improve the model, even though the difference in AIC may be relatively small because adding a factor with one parameter can increase AIC by a maximum of 2 even if this factor has no effect whatsoever.

## RESULTS

### *Interannual Change and Sexual Dimorphism in Breast Melanism, and Its Genetic and Environmental Components*

The final level of breast melanism is reached at one year of age because at fledging feathers are never melanistic and because the degree of breast melanism did not change after the age of one year (mixed model ANCOVA, age:  $F_{1,505} = 0.40$ ,  $P = 0.52$ , individual identity:  $Z = 14.30$ ,  $P < 0.0001$ ). Breast melanism is not sexually dimorphic, the average melanistic score being similar in male ( $1.28 \pm 0.06$ ) and female adults ( $1.38 \pm 0.07$ ) (Student’s  $t$ -test,  $t_{622} = 1.10$ ,  $P = 0.27$ ; Fig. 2).

Breast melanism is heritable, as shown by the significant regression between midvalue of biological parents and midvalue of their cross-fostered offspring measured at adulthood (Falconer 1989;  $F_{1,62} = 28.45$ ,  $P < 0.0001$ ,  $h^2 = 0.78 \pm 0.15$ ; Fig. 3A). A similar regression between midvalue of foster parents and midvalue of their foster offspring was not significant ( $F_{1,62} = 0.96$ ,  $P = 0.33$ ,  $h^2 = 0.16 \pm 0.17$ ; Fig. 3B) indicating that the rearing environment did not inflate the resemblance between related individuals.

The average degree of breast melanism at adulthood in individuals raised in enlarged and reduced broods was similar, as shown in an ANCOVA with the degree of offspring

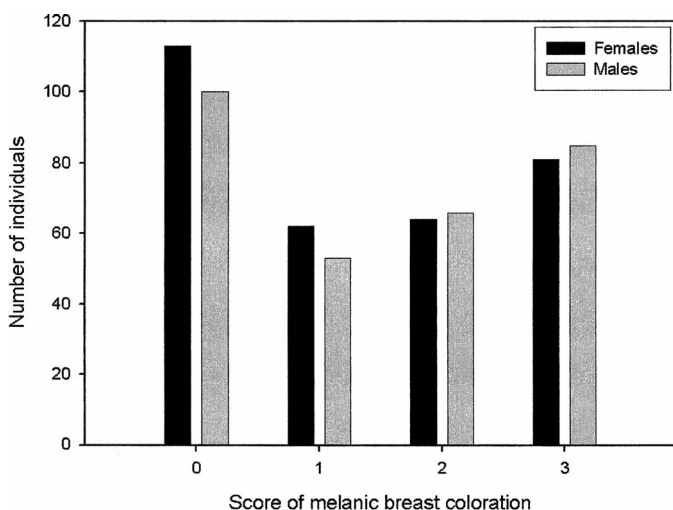


FIG. 2. Frequency distribution of breast melanism scores in male and female adults. Each individual appears only once. If individuals were captured in more than one year, we considered the most frequently assigned color score.

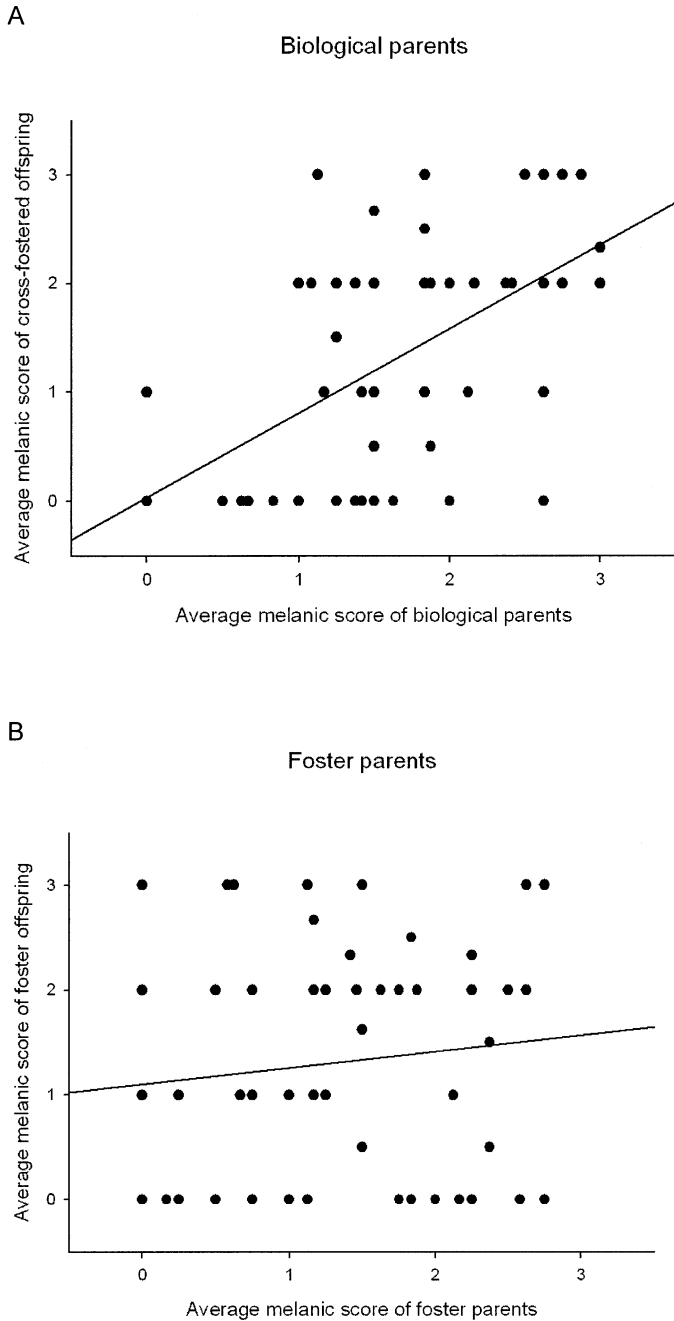


FIG. 3. Relationship between breast melanism of cross-fostered offspring and both the (A) biological and (B) foster parents. Sibling values were averaged, as were values of male and female partners.

breast melanism as the dependent variable, with the mean breast melanism score of the biological parents ( $F_{1,23} = 19.32$ ,  $P = 0.0002$ ) as the first independent variable, and with brood size treatment ( $F_{1,23} = 0.78$ ,  $P = 0.39$ ) as a second variable. The interaction between the two independent variables was not significant ( $F_{1,22} = 0.14$ ,  $P = 0.71$ ). This analysis indicates that the level of sibling competition encountered at the nestling stage does not influence to a detectable level the expression of breast melanism at adulthood. Using the same brood size manipulation experiment, we test-

ed whether the degree of breast melanism changes the year after in parents that tended an enlarged or a reduced brood. For this test, we considered color score assigned in each year and not the average score as in most other statistical analyses. The change in breast melanism of parents having raised an enlarged or a reduced brood was similar in a logistic regression with the change of breast melanism as the dependent variable ( $-1$  when it decreased from one year to the next,  $0$  when it remained the same,  $1$  when it increased) and, as independent variables, brood size treatment ( $\chi^2 = 1.48$ ,  $n = 84$ ,  $P = 0.22$ ), sex ( $\chi^2 = 0.62$ ,  $n = 84$ ,  $P = 0.43$ ) and interaction between sex and brood size treatment ( $\chi^2 = 3.21$ ,  $n = 84$ ,  $P = 0.07$ ). Hence, in adults the expression of breast melanism was not significantly influenced by parental effort (i.e., brood size treatment) in the previous year.

*Relationship between Nestling Body Mass, Size, Age at Fledging, and Breast Melanism of Biological and Foster Parents*

Nestlings were heavier when the rachis of breast feathers of their biological father was melanized to a larger extent (Table 3; Fig. 4A). Interaction between the degree of breast melanism and nestling age was not significant; therefore, we removed it from the final statistical model. The absence of a significant interaction indicates that cross-fostered offspring born from melanistic biological fathers were heavier than offspring born from nonmelanistic biological fathers throughout the rearing period. Sex of nestlings, colony, and the degree of breast melanism of biological mother as well as of foster father and mother were not associated with body mass of cross-fostered nestlings (all  $P$ -values  $> 0.16$ ); hence, we removed these factors from the final model presented in Table 3. In these analyses, we considered nestlings that were cross-fostered at the egg and hatchling stage. If we restrict the analyses to nestlings that were cross-fostered at the egg stage, we obtain similar results (similar model as in Table 3, breast melanism of biological father:  $F_{1,764} = 5.59$ ;  $P = 0.02$ ).

The degree of biological father breast melanism was positively associated with wing length of cross-fostered offspring, a relationship that was age dependent (interaction of father melanism by nestling age in Table 3; Fig. 4B). There was no significant relationship between nestling wing length and sex; colony; or breast melanism of biological mother, foster father, and foster mother also in interaction with age (all  $P$ -values  $> 0.09$ ); hence, these variables were removed from the final model presented in Table 3. If we restrict the analyses to nestlings that were cross-fostered at the egg stage, biological father breast melanism was no longer associated with nestling wing length alone or in interaction (similar model as in Table 3, all  $P$ -values  $> 0.09$ ).

Age at fledging of cross-fostered nestlings was negatively correlated with body mass at fledging (Fig. 5) and with biological father breast melanism (mixed-model ANCOVA, nestling body mass:  $F_{1,29} = 9.65$ ,  $P = 0.004$ ; nestling wing length:  $F_{1,29} = 0.73$ ,  $P = 0.40$ ; biological father breast melanism:  $F_{1,29} = 5.45$ ,  $P = 0.03$ ; year:  $F_{2,29} = 5.69$ ,  $P = 0.008$ ; father identity:  $Z = 0.54$ ,  $P = 0.29$ , mother identity:  $Z = 0.93$ ,  $P = 0.18$ ).

TABLE 3. Mixed-model ANCOVA with nestling body mass (or wing length) as the dependent variable; nestling sex as factor (it was not significant and hence removed from the model); breast melanism of biological father, nestling age, and age<sup>2</sup> as covariates; year as a categorical variable; and their interactions. Nonsignificant interactions were removed step by step from the model (all *P*-values > 0.09). *F*-values are given for fixed effects and Wald *Z*-values for random effects.

Source	Body mass			Wing length		
	<i>F</i> or Wald <i>Z</i>	df	<i>P</i>	<i>F</i> or Wald <i>Z</i>	df	<i>P</i>
<b>Fixed effects</b>						
Biological father breast melanism	5.27	1,1852	0.02	0.75	1,1870	0.39
Nestling age	1749.39	1,1852	<0.0001	2,8240.70	1,1870	<0.0001
Nestling age <sup>2</sup>	310.80	1,1852	<0.0001	—	—	—
Year	4.67	4,1852	0.0009	16.34	4,1870	<0.0001
Interaction age by year	27.19	4,1852	<0.0001	28.05	4,1870	<0.0001
Interaction age <sup>2</sup> by year	37.61	4,1852	<0.0001	—	—	—
Interaction father melanism × nestling age	—	—	—	9.23	1,1870	0.002
<b>Random effects, identities of:</b>						
Biological fathers	1.02		0.15	0.00		1.0
Biological mothers	1.36		0.09	2.19		0.01
Foster fathers	0.68		0.25	2.97		0.002
Foster mothers	1.24		0.11	1.07		0.14
Nestlings	11.25		<0.0001	11.06		<0.0001

#### *Relationship between Nestling Survival, Recruitment, and Breast Melanism of Biological Father*

The higher body mass and wing length of offspring born from fathers displaying melanic breast feathers did not translate into a higher nestling survival (logistic regression with survival as dependent variable (0/1), biological father breast melanism:  $\chi^2 = 1.41$ ,  $P = 0.24$ ,  $n = 675$  among which 71 died before fledging). The probability that at least one cross-fostered offspring per breeding attempt was recruited in the local breeding population was not associated with biological father breast melanism in each year (logistic regression with recruitment success as dependent variable (0/1), 2000:  $\chi^2 = 0.37$ ,  $n = 25$ ,  $P = 0.54$ ; 2002:  $\chi^2 = 0.21$ ,  $n = 109$ ,  $P = 0.65$ ; 2003:  $\chi^2 = 0.49$ ,  $n = 77$ ,  $P = 0.48$ ; 2004:  $\chi^2 = 2.02$ ,  $n = 85$ ,  $P = 0.16$ ; there was not enough data in 2001 for reliable testing).

#### *Potential Mechanisms Accounting for the Relationship between Father Breast Melanism and Nestling Body Mass and Size*

The degree of breast melanism of breeding males was associated with neither clutch size (mixed-model ANCOVA with breast melanism of biological father:  $F_{1,161} = 0.61$ ,  $P = 0.43$ , controlling for year:  $F_{4,161} = 1.51$ ,  $P = 0.20$ ; father identity:  $Z = 1.77$ ,  $P = 0.04$ ; mother identity:  $Z = 2.89$ ,  $P = 0.002$ ), hatching date (breast melanism of biological father  $F_{1,158} = 0.62$ ,  $P = 0.43$ ; year:  $F_{4,158} = 24.23$ ,  $P < 0.0001$ ; father identity:  $Z = 3.01$ ,  $P = 0.001$ ; mother identity:  $Z = 2.96$ ,  $P = 0.002$ ), nor mean egg volume (mixed-model ANCOVA: breast melanism of breeding males:  $F_{1,157} = 0.13$ ,  $P = 0.72$ ; year:  $F_{4,157} = 2.74$ ,  $P = 0.03$ ; father identity:  $Z = 1.67$ ,  $P = 0.05$ ; mother identity:  $Z = 7.83$ ,  $P < 0.0001$ ). Therefore, these three factors cannot explain why father breast melanism covaried with offspring body mass and wing length.

If fathers pass on to offspring genes coding for breast melanism and simultaneously genes coding for a large body size, the degree of melanism should covary with body mass not

only at the nestling stage but also at adulthood. However, this was not the case because among adults wing length, tail length, fork and sternum size, and body mass were not correlated with breast melanism; beak size was negatively (and not positively) correlated with breast melanism (Table 4).

In each year, pairing with respect to breast melanism was random (Pearson correlation, all  $P$ -values > 0.36). The degree of male breast melanism was not correlated with body mass (mixed-model ANCOVA: breast melanism of breeding males:  $F_{1,74} = 0.23$ ,  $P = 0.63$ ; year:  $F_{4,74} = 5.03$ ,  $P = 0.001$ ; father identity:  $Z = 1.04$ ,  $P = 0.15$ ; mother identity:  $Z = 1.69$ ,  $P = 0.05$ ), wing length (breast melanism of breeding males:  $F_{1,76} = 0.48$ ,  $P = 0.49$ ; year:  $F_{4,76} = 3.86$ ,  $P = 0.007$ ; father identity:  $Z = 0.00$ ,  $P = 1.0$ , mother identity:  $Z = 8.13$ ,  $P < 0.0001$ ), and sternum length of their female partner (breast melanism of breeding males:  $F_{1,71} = 0.00$ ,  $P = 0.95$ ; year:  $F_{3,71} = 11.71$ ,  $P < 0.0001$ ; father identity:  $Z = 1.57$ ,  $P = 0.06$ ; mother identity:  $Z = 8.32$ ,  $P < 0.0001$ ). This suggests that offspring did not simultaneously receive genes coding for melanic breast feathers from their father and genes coding for a large body mass and size from their mother.

#### *Survival, Breeding Probability, and Age at First Reproduction in Relation to Breast Melanism*

The best capture-recapture model included year-dependent survival, different recapture rates for nonbreeders and breeders, age-dependent transition rate from nonbreeder to breeder, and constant transition rate from breeder to nonbreeder (model 1, Table 2). There was no evidence for a relationship between the degree of breast melanism and survival, recapture, or the transitions between nonbreeder and breeder status because all models including such effects had similar deviance and higher AIC values. Note that the difference in AIC between such nested models differing only by one parameter must necessarily be  $\leq 2$  if melanism had no effect (see also Materials and Methods). These results thus suggest that individuals with different levels of breast melanism are similar in the basic life-history traits survival and age at first repro-

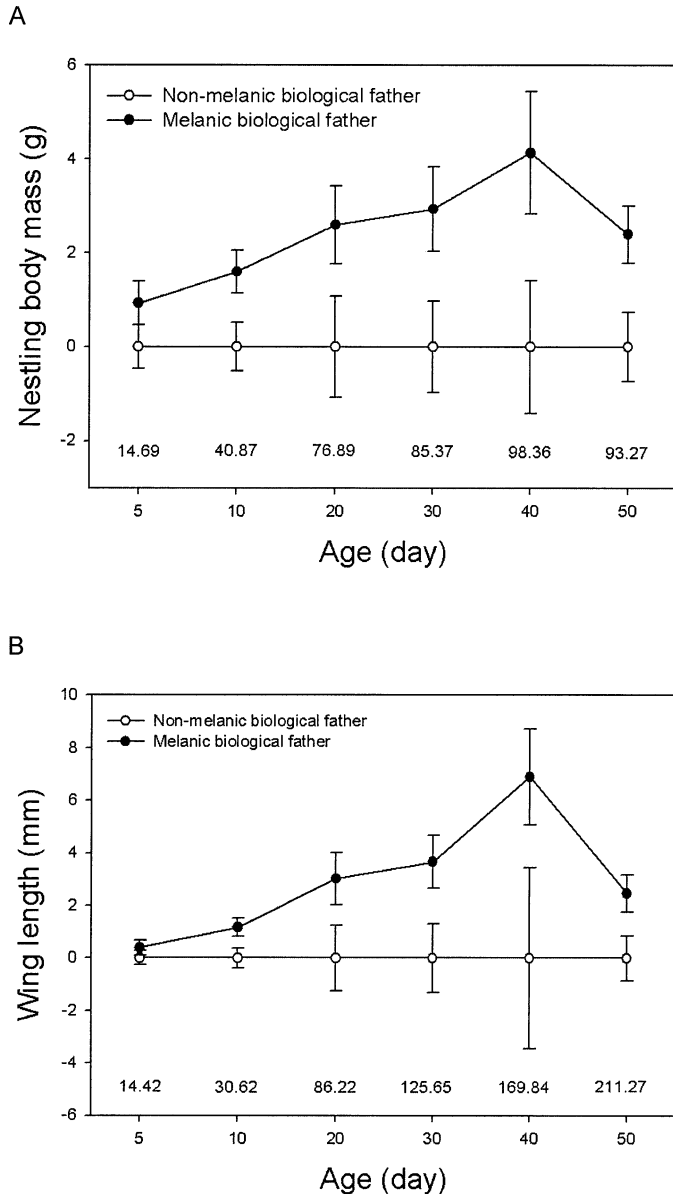


FIG. 4. Mean difference in (A) body mass and (B) wing length ( $\pm 1$  SE) of cross-fostered nestlings in relation to their age and breast melanism of their biological father. Open and closed symbols correspond to nonmelanic (scores 0 and 1 pooled) and melanic biological fathers (scores 2 and 3 pooled), respectively. Numbers above the x-axis indicate mean body mass (A) and mean wing length (B) of nestlings born from nonmelanic biological fathers. For example, at five days of age, nestlings born from nonmelanic biological fathers had a mean body mass of 14.69 g and nestlings born from melanic biological fathers were 0.93 g heavier.

duction. Close competitors were the models with lower survival of nonbreeders than breeders, and with age-specific survival of nonbreeders (models 3 and 2, Table 2).

The best model (model 1, Table 2) estimated survival to vary between 0.70 (SE = 0.03) and 0.84 (SE = 0.03) over the years of the study (mean = 0.77, among-year variance = 0.0024, estimates taking year as random effect). The recapture rates were 0.37 (SE = 0.05) for nonbreeders and 1.0 (SE = 0.003) for breeders. The probability to start breeding

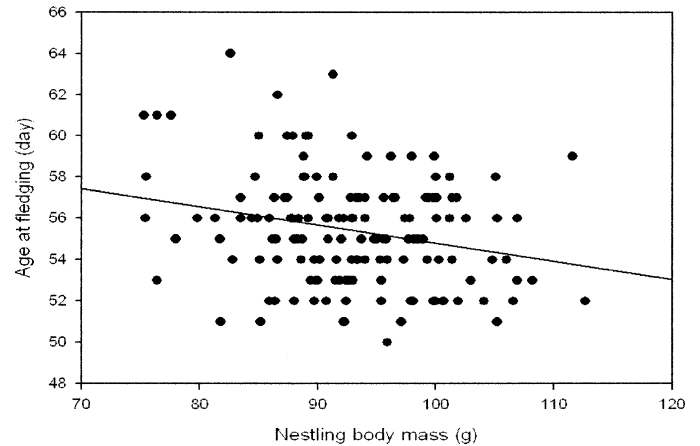


FIG. 5. Relationship between nestling body mass at fledging and age at fledging.

for nonbreeders was 0.22 (SE = 0.06) for birds aged 1, 0.70 (SE = 0.07) for birds aged 2, 0.89 (SE = 0.10) for birds aged 3, and 0.91 (SE = 0.08) for older birds. The probability to stop breeding was 0.08 (SE = 0.01).

## DISCUSSION

### *Genetic and Environmental Components of Breast Melanism*

In the present study, we performed cross-fostering experiments to investigate whether the expression of melanin-based coloration in the alpine swift is heritable or sensitive to environmental factors. Breast melanism is not expressed at the nestling stage, and individuals reach their final breast coloration at one year of age, which is after their first molt in the winter quarters. In line with previous similar cross-fostering experiments in the barn owl, this color trait appeared to be strongly heritable (Roulin et al. 1998; Roulin and Dijkstra 2003). Additionally, melanin expression was neither sensitive to the rearing environment experienced as a nestling nor to body condition. This result is concordant

TABLE 4. Within-individual phenotypic correlation between body size and breast melanism in adult alpine swifts. ANCOVAs were applied with the degree of breast melanism as covariate and sex as an independent factor. Interactions between sex and breast melanism on body size traits were not significant and therefore removed from the final models. Sample sizes are unequal owing to missing measures in some individuals. Significant sex effects indicate that females were smaller than males.

Trait	Factor	F	df	P
Wing length	breast melanism	2.98	1,621	0.09
	sex	55.85	1,621	<0.0001
Tail length	breast melanism	0.60	1,621	0.83
	sex	64.52	1,621	<0.0001
Fork length	breast melanism	0.05	1,621	0.83
	sex	88.13	1,621	<0.0001
Beak size	breast melanism	4.06	1,620	0.044
	sex	19.44	1,620	<0.0001
Sternum size	breast melanism	2.11	1,570	0.15
	sex	47.64	1,570	<0.0001
Body mass	breast melanism	0.45	1,620	0.50
	sex	0.44	1,620	0.51

with studies performed in male house sparrows (*Passer domesticus*; McGraw et al. 2002; Gonzales et al. 1999; Badyaev and Young 2004; but see Griffith et al. 1999), in the house finch (*Carpodacus mexicanus*; Hill and Brawner 1998), eastern bluebird (*Sialia sialis*; Siefferman and Hill 2005), pied flycatcher (*Ficedula hypoleuca*; Dale et al. 1999), and barn owl (*Tyto alba*; Roulin et al. 1998) but not in the mosquitofish (*Gambusia holbrooki*; Horth 2003). This provides further arguments to the hypothesis that in birds the expression of melanin-based colors can be under strong genetic control (Roulin 2004). The brood size manipulation experiment in the alpine swift also showed that the production of melanin breast feathers is not influenced by reproductive effort (in adults) in the previous year. Similar experiments in the sexually dimorphic collared flycatcher (*Ficedula albicollis*; Gustafsson et al. 1995) and house sparrow (*Passer domesticus*; Griffith 2000) showed that the size of a feather ornament increases and decreases when reproductive effort is experimentally reduced and increased, respectively. The discrepancy in the effect of brood size manipulation on adult coloration in different species might be explained by directional sexual selection being exerted in the collared flycatcher and house sparrow but not in the non-sexually dimorphic alpine swift. This may explain why heritability of plumage coloration is high in swifts ( $h^2 = 0.78$ ) whereas in the collared flycatcher it varies between 0.65 in good years and 0.19 in poor years when additive genetic variation cannot be fully expressed (Qvarnström 1999).

#### *Potential Mechanisms Underlying Covariation between Biological Father Breast Melanism and Nestling Body Size*

Cross-fostering experiments are widely used to partition phenotypic variance into genetic and environmental components (e.g., Gebhardt-Henrich and van Noordwijk 1991). In our study, this approach permitted us to conclude that covariation between offspring body mass and father breast melanism is due neither to incubation efficiency nor to post-hatching parental effects. In addition, we showed that within adults body mass and size were not correlated with breast melanism, suggesting that covariation between nestling body mass and biological father breast melanism is not caused by paternally expressed genes (i.e., genomic imprinting) having pleiotropic effects on breast melanism and body mass and wing length. Furthermore, the degree of male breast melanism was not correlated with size of female partners, suggesting that offspring did not simultaneously receive genes from their father to develop melanin breast feathers and genes from their mother to be heavier and larger (i.e., linkage disequilibrium; e.g., Roulin 2006). Therefore, genomic imprinting and linkage disequilibrium are unlikely to underlie the covariation between melanin-based coloration and offspring body condition.

Covariation between father breast melanism and offspring body mass may also be due to nongenetic maternal effects. Females are known to incorporate in the yolk of their eggs significant concentrations of a number of steroid hormones, of which androgens and in particular testosterone has beneficial effects on nestling growth and development (Grootuis et al. 2005). Our results could therefore be due to differential

female investment of specific components into their eggs in such a way that, when mated with males displaying melanin breast feathers, their offspring become heavier and larger. This effect may not persist for long periods of time since within adults breast melanism was not significantly associated with body mass and size. The finding that egg volume was not correlated with biological father breast melanism proves that melanin males did not have heavier nestlings because they had bigger eggs. This is consistent with the hypothesis that females deposit different amounts of high quality components in their egg yolk with respect to the degree of breast melanism displayed by their mate rather than dissimilar yolk quantities. Gil et al. (2004) already reported such a pattern in canaries (*Serinus canaria*), with females depositing higher concentrations of testosterone in their eggs when they heard attractive than nonattractive male songs, without any difference in the weight of eggs with respect to male attractiveness (see Cunningham and Russel 2000 for such an example).

#### *No Evidence that Breast Melanism Is Directionally Selected*

We found that male alpine swifts with deeply melanized breast feathers produce offspring that were on average 4 g heavier and had 6.5 mm longer wings than those born from nonmelanic fathers (Fig. 4). These two differences were apparently not sufficient to reduce nestling mortality and increase offspring recruitment in the local breeding population, but large enough to reduce the length of the rearing period by two days. Based on a previous study showing that an increase in the rearing period by only 4.3% strongly impaired future reproductive success of parents (Bize et al. 2004), we predicted that pairs for which the male displays melanin breast feathers enjoy a higher residual reproductive value and in turn higher future reproductive success. However, we were unable to detect any relationship between adult survival probability and the degree of breast melanism in the capture-recapture analyses. The high (0.78) heritability of breast melanism and the apparent absence of directional selection are consistent with the hypothesis that the degree of breast melanism is a genetic polymorphism.

The absence of detectable directional selection in the alpine swift can be explained by the following two mechanisms. First, variation in the degree of breast melanism is maintained because the advantage for melanin males to produce offspring that are heavier and larger compared to nonmelanic fathers is not large enough to induce directional selection. Covariation between the degree of melanism and offspring body condition may be due to genes responsible for variation in coloration having pleiotropic effects on other phenotypic traits. The evolution of such a covariation may therefore be a by-product of melanin synthesis rather than the result of natural or sexual selection. Second, nonmelanic fathers may derive another advantage to equalize fitness with melanin males than to produce heavy offspring. For example, genes involved in the synthesis of melanin may have some detrimental effects so that the balance between the benefits and costs of displaying melanin rachs is close to zero. To test which of these two hypotheses is the most likely, we intend to investigate whether the degree of breast melanism covaries

positively or negatively with immunocompetence and resistance to parasites since in the alpine swift the louse-fly *Craeterna melbae* incurs significant developmental and reproductive costs (Bize et al. 2003, 2004, 2005).

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