MUTATION AND SEXUAL SELECTION: A TEST USING BARN SWALLOWS FROM CHERNOBYL

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Abstract.—Secondary sexual characters have been hypothesized to be particularly susceptible to the deleterious effects of mutation because the expression of such characters is usually influenced by many more metabolic pathways than are ordinary morphological characters. We tested this hypothesis using the elevated mutation rates in the barn swallow (*Hirundo rustica*) of the Chernobyl region of Ukraine as a model system. A great deal is known about the relative importance of different characters for male mating success in this species. The importance of phenotypic characters for male mating success was quantified based on a long-term study of a Danish breeding population, by expressing phenotypic differences between mated and unmated males as the difference between log-transformed mean values. For field samples from Ukraine we likewise expressed the difference in male phenotype between individuals living in a relatively uncontaminated area and individuals from the Chernobyl region as the difference between log-transformed mean values. The standardized difference in male phenotype between the two regions in Ukraine for the 41 different characters was strongly positively correlated with the standardized difference in male phenotype between mated and unmated males from Denmark. The standardized difference in male phenotype between the two regions in Ukraine was significantly positively associated with sexual size dimorphism. However, the standardized difference in male phenotype between mated and unmated males was a much better predictor of standardized difference in male phenotype between the two regions in Ukraine than was the standardized difference in sexual size dimorphism, expressed as the difference between log-transformed mean values for males and females. These findings are consistent with the hypothesis that traits most important for sexual selection are particularly susceptible to the effects of deleterious mutations.

Key words.—Antioxidants, Chernobyl, condition dependence, *Hirundo rustica*, mate choice, mutation, sexual selection.

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The advantages of strong mate preferences in sexual selection remain largely undetermined (Andersson 1994). There is good evidence of direct, material benefits of mate choice in many taxa, but others show direct benefits generally being of little or no importance (reviewed in Møller and Jennions 2001). Although there is ample evidence for females obtaining genetic benefits for their offspring (reviewed in Møller and Alatalo 1999), the mechanisms generating such benefits remain illusive. Although mutation-selection balance likely plays a role in maintaining additive genetic variance in fitness and the expression of secondary sexual characters, whether such effects are sufficient is unknown. A Fisherian mechanism of sexual selection could arise from biased mutations affecting the expression of male secondary sexual characters (Pomiankowski et al. 1991), allowing females to avoid mutants from inspection of male phenotype. Male secondary sexual characters are exaggerated traits that for this reason are likely to be affected by many different metabolic pathways. Therefore, mutations may more readily be assessed from such characters than from ordinary phenotypic traits that are primarily influenced by mutations through a single or few metabolic pathways. Similarly, biased mutation simultaneously differentially affecting viability and the expression of male traits may render continuous sexual selection feasible (Iwasa et al. 1991). Both these ideas are based on the untested assumption that deleterious mutations constantly reduce the expression of male traits and maximum viability. Estimates of the additive genetic variance in viability have suggested that deleterious mutations may reduce

viability by 20% or more (Charlesworth 1987; Kondrashov 1988; Rice 1988; Lynch et al. 1999). Such effects may account for the small, but highly significant heritability of fitness (Burt 1995) and fitness components (Mousseau and Roff 1987; Roff and Mousseau 1987).

We quantified the effects of mutation on phenotype by expressing the difference in phenotype between male barn swallows (*Hirundo rustica*) from a little-contaminated region in Ukraine (Kanev) and conspecifics from the Chernobyl region, which has high levels of radioactive contamination (mainly Cs 137 and Sr 90; Shestopalov 1996). Ellegren et al. (1997) reported an increase in germline mutation rates in barn swallow microsatellite DNA by a factor of two to 10 when comparing families from Kanev with families from Chernobyl. Barn swallows from Chernobyl have markedly increased frequency of partial albinism in the plumage (Ellegren et al. 1997; Møller and Mousseau 2001). Previous studies have shown significant parent-offspring resemblance in partial albinism, indicating that such albinism is partly of germline origin (Ellegren et al. 1997). Individuals with partial albinism are less likely to survive than other individuals (Ellegren et al. 1997), and they generally have a smaller but more variable phenotype (Møller and Mousseau 2001). These patterns of phenotypic variation are consistent with the hypothesis that radioactive contamination has increased the rate of mutations, and that such mutations have deleterious effects. Thus, this area has the potential for providing insights into the effects of mutation on sexual selection and other evolutionary phenomena.

2139

One aim of this study was to investigate the extent to which the phenotypic characters that vary in importance for male mating success are susceptible to the deleterious effects of mutation. We predicted that the standardized difference in male phenotype between mated and unmated males, reflected by the difference between log-transformed mean values, should represent an estimate of the importance of a character for sexual selection. Likewise, we expressed the standardized phenotypic difference between individuals from the contaminated and the control area. If secondary sexual characters were particularly susceptible to the effects of mutation, compared to other phenotypic characters, we would predict a positive association between standardized difference in male phenotype with respect to mutation and standardized difference in phenotype with respect to male mating success. A second aim of this study was to investigate the relationship between sexual size dimorphism and effects of radiation on phenotype. We standardized sexual size dimorphism as described above. Sexual dimorphism evolves over extremely long time periods (Lande and Arnold 1985), whereas females are supposed to assess phenotypic characters that currently reveal benefits from mate choice (Andersson 1994). Therefore, we can predict that the standardized difference in phenotype related to mating success is mainly related to the standardized difference in phenotype related to radiation, and much less to the difference in phenotype related to sexual size dimorphism.

MATERIALS AND METHODS

Study Species

The barn swallow is an approximately 20-g aerial insectivorous passerine bird (Møller 1994a). Males arrive before females from the African winter quarters to the breeding grounds and attract mates by aerial displays of their red throat patches, long outermost tail feathers, and elaborate song (Møller 1994a). After a male is mated, the pair builds a nest, where the female lays eggs and incubates. Both males and females feed nestlings and often raise a second brood before returning to Africa in September or October (Møller 1994a).

Barn swallows from Chernobyl have increased asymmetry in their outermost tail feathers compared to males from a control area and to males predating the Chernobyl accident (Møller 1993). Barn swallow males from Chernobyl also have much paler throat coloration than males from a control area, with long-tailed males in particular being very pale compared to long-tailed control males (Camplani et al. 1999). The frequency of partial albinism is elevated by a factor of 10 in the Chernobyl area compared to a control area (Møller and Mousseau 2001). Microsatellite mutation rates in barn swallows increased by a factor of two to 10 in the Chernobyl region compared to controls (Ellegren et al. 1997).

Field Procedures and Definition of Characters

We studied barn swallows just outside the exclusion zone of the Chernobyl area (Shestopalov 1996) by visiting villages and checking collective farms for the presence of barn swallows. When such a farm had been located, we recorded radiation levels and captured adult barn swallows for mea-

surements and subsequent banding. Our own field measurements of radiation at the ground level using a hand-held Inspector dosimeter (SE International, Inc., Summertown, TN) revealed average levels of radiation of 0.390 mR/h (SE = 0.317) at 14 breeding sites in the Chernobyl region. As a control area we used Kanev, about 220 km southwest of Kiev, which has a relatively low level of contamination. Mean levels of radiation were 0.025 mR/h (SE = 0.002) at five breeding sites. We studied barn swallows in the two study areas 10–29 June 1991 (A. P. Møller), 16–24 June 1996 (A. P. Møller), 6–12 June 2000 (A. P. Møller, T. A. Mousseau) and 4–11 June 2002 (A. P. Møller, T. A. Mousseau).

We captured barn swallows with mist nets across open doors and windows in farm buildings. This method is highly efficient during the main breeding season since mark-recapture studies have shown a capture probability of 98% (Møller and Szép 2002).

We measured a total of 41 phenotypic characters in adult males and females from the two samples. Twenty morphological characters (beak length, beak width, beak depth, wing length, wing asymmetry, tail length, tail asymmetry, length of central tail feathers, wingspan, wing area, wing loading, aspect ratio, tarsus length, keel length, feather tip width, feather base width, tip vane asymmetry, base vane asymmetry, difference in width between tip and base, and body mass) were measured with a ruler, a digital caliper, or a Pesola AG (Baar, Switzerland) spring balance as described in Møller (1994a) and Møller et al. (1995). Six color variables (chroma, hue, ultraviolet reflectance, lutein-based reflectance, visible light reflectance, and albinism) were measured as described in Camplani et al. (1999). Ten immunological and hematological variables (monocytes, heterophils, lymphocytes, eosinophils, basophils, heterophil:lymphocyte ratio, immunoglobulins, hematocrit, buffy coat, and spleen mass) were measured as described in Camplani et al. (1999). Three parasitological variables (abundance of chewing lice, louseflies, feather mites) were estimated as described in Møller (1994a). Testes mass was measured as described in Møller (1994b). Fault bars were quantified as described in Møller (1989). All these variables have high and statistically significant repeatabilities as recorded from double measurements of the same individuals during later recaptures the same season. All characters were not measured for all individuals, and sample sizes therefore differ considerably among characters. We see no reason why this should cause any bias in the analyses. The only effect would be that some estimates should be less reliable than others, obscuring any true relationships.

A second sample of barn swallows was captured, measured and banded in Denmark during the years 1984–2002, using the same procedures as in Ukraine. This sample consisted of adult barn swallows captured the first time, when aged one year. Thus, all mated and unmated males were of a similar age when first captured and assessed for phenotypic characters. Mating status was determined from the presence (mated) or absence (unmated) of a female in the territory of banded males in the breeding sites (for further information see Møller 1994a).

We classified phenotypic characters as feather characters produced during the annual molt in Africa, when the linear measurements included feathers. The justification for this dis-

tinction is that these characters are developed in the African winter quarters during the annual molt many months after having left the contaminated breeding grounds. Characters were classified as physiological if they were immune response variables, hematological variables, or variables associated with coloration. The latter variables have a physiological basis in terms of either color pigments or microstructure of feathers. Physiological characters may change on a short-term basis, and this may increase their susceptibility to rapid changes in environmental conditions such as radioactive contamination. Hence, if the differences in phenotype were mainly due to phenotypic plasticity rather than the effects of mutations, we should expect to see a stronger effect on physiological variables than on other characters. In contrast, we should expect to see a weaker effect on feather characters grown in Africa than on other characters if the differences in phenotype were mainly due to phenotypic plasticity rather than the effects of mutations.

Statistical Analyses

We based our analyses on standardized differences in phenotype expressed in terms of differences in log-transformed mean values for the two samples of the 41 phenotypic characters (40 characters in the analyses of sexual size dimorphism). Since characters differed dramatically in size and variance, we had to use standardization.

We quantified the importance of phenotypic characters for male mating success by quantifying the difference in mean phenotype between mated and unmated males in the Danish sample. This sample is based on the phenotypes of 1354 mated males and 99 unmated males from Denmark. The difference in phenotype was expressed as differences in log-transformed mean values. This standardization eliminated any covariance between standardized difference and mean or standard deviation of the variables under investigation.

We quantified the difference in phenotype between males from Kanev (154 males) and males from Chernobyl (206 males) as differences in log-transformed mean values.

Finally, sexual size dimorphism was quantified as the difference in log-transformed mean values between mean male phenotype and mean female phenotype in Denmark. Testes size had to be excluded from this analysis because the trait is only expressed in males. We calculated sexual size dimorphism for all characters, including characters that were not morphological. This is justified since differences in physiology, immunology, or hematology are likely to form the physiological basis for the morphological characters.

We conservatively assessed the association between pairs of standardized sets of variables using the phi rank correlation for a two-by-two table in which the two states for each character are the negative and positive standardized values, respectively (Siegel and Castellan 1988).

We tested for the effect of outliers on the robustness of the reported relationships using Kendall rank correlation analysis (Siegel and Castellan 1988).

We tested for a difference in relationships between pairs of standardized sets of characters separately for feather characters that are formed during the annual molt in Africa and other characters, using analysis of covariance. A statistically significant factor-by-covariate interaction would indicate that the relationships for the two kinds of characters are statistically different. We made a similar test for physiological and other characters.

RESULTS

Mean and standard errors for all samples are reported in Tables 1–3. The standardized difference in male phenotype between Kanev and Chernobyl was strongly positively related to the standardized difference in male phenotype between mated and unmated males (Fig. 1; linear regression: $F = 167.196$, df = 1,39, $r^2 = 0.811$, $P < 0.0001$, slope [SE] = 0.903 [0.070]). The slope did not differ significantly from one ($t = 1.386$, df = 39, nonsignificant). Since both dependent and independent variables were measured with error, a reduced major axis may provide a better estimate (Sokal and Rohlf 1995). The slope in this case is 1.083 (SE = 0.109), which still does not differ significantly from one ($t = 0.763$, df = 39, nonsignificant). The relationship between standardized difference in male phenotype with respect to radiation and the standardized difference in male phenotype with respect to mating was robust since a rank correlation gave a similar conclusion ($\tau = 0.644$, $z = 5.930$, $P < 0.0001$). Thus, effects of environmental conditions in Chernobyl on male phenotype closely matched differences in male phenotype associated with mating success.

The effects of environmental conditions around Chernobyl on male phenotype could be due to physiological effects on phenotype or to effects of mutations. We know that the frequency of partial albinism is increased in the Chernobyl region compared to the control area, and that this difference was absent before the nuclear accident in 1986 (Ellegren et al. 1997; Møller and Mousseau 2001). If mutations accounted for differences in male phenotype in Ukraine, we should also expect that feather characters formed during the annual molt in the African winter quarters (where birds from Ukraine mix and share a common environment) would be good predictors of the importance of male phenotype for mating success. Barn swallows are less likely to be affected by the direct physiological effects of radiation in Africa, several months after having left the Chernobyl region. Indeed, feather characters were as good predictors as all phenotypic characters with no significant difference between feather characters and other characters, and the covariate by factor interaction was not statistically significant (Fig. 1; Table 4). Although physiological characters were no better predictors of susceptibility to radiation than other characters, the covariate-by-factor interaction was statistically significant (Fig. 1; Table 5). This implies that the mating success effect was weaker for physiological characters than for other characters.

If the expression of male secondary sexual characters was particularly affected by mutations, we would predict a positive relationship between effects of mutations on phenotypic variables and sexual size dimorphism. However, this relationship was not significant (Fig. 2; linear regression: $F = 0.547$, df = 1,38, $r^2 = 0.014$, $P = 0.464$, slope (SE) = 0.466 [0.631]). However, a rank correlation gave a significant positive relationship ($\tau = 0.385$, $z = 3.495$, $P = 0.0005$).

A more conservative method to assess the association be-

TABLE 1. Mean (SD) phenotypic values for adult male barn swallows from Chernobyl and Kanev, Ukraine. *n,* sample size.

Character	Chernobyl			Kanev		
	Mean	SD	*n*	Mean	SD	*n*
Albinism	0.146	0.355	206	0.013	0.114	154
Aspect ratio	7.300	0.335	45	7.120	0.940	73
Base vane asymmetry	2.780	0.465	60	2.710	0.453	8
Base width	6.980	0.542	60	6.960	4.469	8
Basophils	0.002	0.003	21	0.001	0.002	17
Bill height	2.558	0.297	206	2.751	0.391	153
Bill length	7.928	0.553	206	8.010	0.550	153
Bill width	11.759	0.721	206	11.995	0.605	153
Body mass	18.554	1.220	206	18.835	1.305	154
Buffy coat	0.215	0.135	33	0.242	0.191	24
Chewing lice	14.092	11.841	206	17.112	12.773	152
Chroma	0.255	0.034	33	0.267	0.044	24
Eosinophils	0.045	0.031	21	0.028	0.025	17
Fault bars	0.116	0.495	189	0.125	0.419	128
Feather mites	31.327	28.610	153	50.289	36.797	83
H/L ratio	0.376	0.266	21	0.264	0.091	17
Heterophils	0.230	0.108	21	0.192	0.054	17
Hematocrit	54.600	4.159	33	54.352	3.170	24
Hue	35.776	2.171	33	35.407	2.930	24
Immunoglobulins	13.200	2.062	17	12.269	3.098	15
Keel length	21.006	0.890	205	21.320	0.808	153
Louseflies	0.017	0.057	205	0.015	0.173	153
Lutein color	1.111	0.144	33	1.159	0.162	24
Lymphocytes	0.686	0.116	21	0.744	0.066	17
Monocytes	0.031	0.020	21	0.028	0.022	17
Spleen mass	20.000	13.194	17	29.900	15.105	15
Short tail length	44.206	1.771	204	44.392	1.806	153
Tail length	110.097	9.607	190	116.687	9.004	140
Tail asymmetry	4.752	4.452	190	0.232	2.757	140
Tarsus length	11.287	0.942	204	11.079	0.653	153
Testes mass	0.434	0.100	51	0.502	0.150	8
Tip vane asymmetry	0.090	0.155	60	0.040	0.113	8
Tip width	1.860	0.232	60	1.787	0.611	8
Tip-base difference	5.120	0.542	60	5.397	0.481	8
Ultraviolet	3.116	0.138	33	3.063	0.201	24
Visible light	3.889	0.092	33	3.838	0.118	24
Wing length	124.975	3.289	141	126.289	2.928	123
Wing asymmetry	0.219	0.443	204	0.179	0.335	154
Wing area	0.015	0.001	45	0.015	0.007	73
Wing loading	12.200	0.939	45	12.020	0.940	73
Wingspan	330.747	6.899	204	331.039	6.900	154

tween effects of radiation on phenotype and the difference in phenotype between mated and unmated males is to calculate the rank correlation for a two-by-two table in which the two states for each character are the negative and positive values, respectively (Siegel and Castellan 1988). Although standardized variables may be affected both by mean and standard deviation values, we can be sure that the sign of the phenotypic measures does not change with standardization. Hence, a test for similarity in sign of two categories of variables provides a clear, but conservative test of covariation. The phi correlation coefficient for the association between effects of radiation on phenotype and the difference in phenotype between mated and unmated males was r (φ) = 0.664, χ^2 = 16.739, df = 1, $P < 0.001$. The coefficient for radiation and sexual dimorphism was r (φ) = 0.323, χ^2 = 2.947, df = 1, $P < 0.10$, which did not reach statistical significance.

If characters subject to sexual selection are particularly susceptible to mutations, we should expect the association between effects of radiation on phenotype and the difference in phenotype between mated and unmated males to be much

stronger than for the difference in phenotype related to sexual dimorphism. A multiple regression analysis revealed a highly significant model that explained 81% of the variance (Table 6). Whereas the partial regression coefficient for standardized difference in phenotype related to radiation was highly significant and accounted for 25.2% of the variance, the partial regression coefficient for standardized difference in phenotype related to sexual dimorphism was not significant and accounted for almost none of the variance.

DISCUSSION

We tested the hypothesis that phenotypic characters of the greatest importance in terms of sexual selection are the traits particularly susceptible to the effects of radiation. The test consisted of comparison of the phenotype of male barn swallows from Chernobyl, in which mutation rates of microsatellites are increased by a factor two to 10, with a sample of males from a less-contaminated area in Ukraine. The standardized difference in male phenotype between these two

TABLE 2. Mean (SD) phenotypic values for mated and unmated male barn swallows from Denmark. *n,* sample size.

Character	Mated male			Unmated male		
	Mean	SD	*n*	Mean	SD	*n*
Albinism	0.001	0.165	991	0.030	0.044	65
Aspect ratio	7.239	0.406	942	7.280	0.351	60
Base vane asymmetry	2.628	0.510	26	2.730	0.621	12
Base width	6.820	0.612	26	6.840	0.587	12
Basophils	0.001	0.002	25	0.002	0.002	6
Bill height	2.806	0.331	1351	2.778	0.298	99
Bill length	7.649	0.511	1351	7.683	0.497	99
Bill width	11.974	0.603	1351	11.978	0.587	99
Body mass	19.091	1.141	1354	18.190	1.085	99
Buffy coat	0.610	0.426	37	0.517	0.449	6
Chewing lice	10.667	10.660	1246	13.872	13.864	86
Chroma	0.286	0.039	43	0.275	0.040	6
Eosinophils	0.044	0.030	25	0.059	0.029	6
Fault bars	0.151	0.540	1141	0.276	0.663	76
Feather mites	48.740	46.711	724	40.125	39.055	56
H/L ratio	0.266	0.093	37	0.300	0.089	6
Heterophils	0.228	0.112	25	0.273	0.112	25
Hematocrit	52.820	4.315	352	52.980	4.540	18
Hue	33.767	2.636	43	34.474	2.421	6
Immunoglobulins	13.920	3.200	16	15.200	2.970	6
Keel length	21.556	0.726	861	21.448	0.768	59
Louseflies	0.015	0.147	1355	0.020	0.139	99
Lutein color	1.123	0.043	43	1.096	0.053	15
Lymphocytes	0.775	0.069	25	0.742	0.066	6
Monocytes	0.028	0.022	25	0.031	0.021	6
Spleen mass	33.600	18.800	16	24.000	7.967	3
Short tail length	44.028	1.959	1102	43.080	1.386	75
Tail length	108.786	8.858	1351	99.333	62.754	99
Tail asymmetry	0.298	4.849	885	2.259	0.198	58
Tarsus length	11.266	0.625	1351	11.310	0.617	99
Testes mass	0.502	0.100	51	0.436	0.149	8
Tip vane asymmetry	0.074	0.153	26	0.120	0.162	12
Tip width	1.797	0.510	26	1.950	0.479	12
Tip-base difference	5.374	0.969	26	4.890	0.929	12
Ultraviolet	3.028	0.118	43	3.081	0.106	6
Visible light	3.792	0.079	43	3.817	0.082	6
Wing length	126.533	3.017	1354	124.626	2.915	99
Wing asymmetry	0.216	0.413	980	0.175	0.873	63
Wing area	0.015	0.001	944	0.015	0.001	60
Wing loading	12.477	1.161	944	12.693	1.002	60
Wingspan	333.936	7.396	1150	333.280	5.451	77

areas was strongly positively correlated with the standardized difference in phenotype between mated and unmated males of the same age from a distant population in Denmark. The slope was not significantly different from one, and although the reduced major axis had a larger slope, it did not differ from one. This suggests that differences in phenotype between barn swallows from Chernobyl and a control area are as large as differences in phenotype between mated and unmated males. We found similarly strong relationships for morphological characters formed during the annual molt in Africa and for physiological characters. Sexual selection theory proposes that secondary sexual characters may be particularly susceptible to the negative effects of mutation because many different metabolic pathways contribute to their expression (Iwasa et al. 1991). Indeed, we found that characters with greater influence on male mating success were affected to a greater extent by mutation (Fig. 1), whereas the effects of radiation on phenotype were only weakly predicted by sexual size dimorphism (Fig. 2).

We found evidence of a strong positive relationship be-

tween the standardized difference in phenotype associated with radiation and the standardized difference in phenotype associated with mating success. We used a strict definition of sexual selection arising from social mating success in this socially monogamous bird species. Several studies have shown that the differences in phenotype between mated and unmated male barn swallows provide reliable information on the characters associated with sexual selection as verified by experiments (Møller 1994a). Although many other mechanisms of sexual selection exist, and many of these have been demonstrated in the barn swallow (reviewed in Møller 1994a), we do not have information on the relationship between sexual selection and phenotypic characters for more than a few traits. Thus, we were forced to use strict mating success as an estimate for sexual selection to maximize our sample sizes.

We used linear regression analyses to investigate how differences in phenotype related to male mating success were associated with differences in phenotype related to standardized difference in phenotype associated with radiation. Our
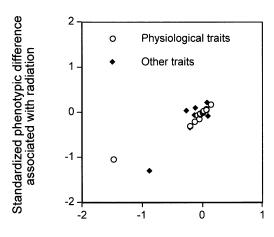
TABLE 3. Mean (SD) phenotypic values for adult male and female barn swallows from Denmark. *n,* sample size.

Character	Male			Female		
	Mean	SD	*n*	Mean	SD	*n*
Albinism	0.032	0.167	1113	0.037	0.203	1145
Aspect ratio	7.243	0.403	1008	7.153	0.412	1055
Base vane asymmetry	2.730	0.510	26	2.550	0.635	28
Base width	6.840	0.612	26	6.570	0.688	28
Basophils	0.001	0.002	31	0.002	0.003	34
Bill height	2.794	0.344	1507	2.763	0.348	1536
Bill length	7.655	0.501	1507	7.731	0.509	1536
Bill width	11.968	0.598	1507	11.831	0.589	1536
Body mass	19.068	1.131	1510	20.150	1.969	1539
Buffy coat	0.610	0.408	34	0.461	0.182	37
Chewing lice	11.035	11.032	1389	11.884	11.673	1504
Chroma	0.286	0.039	43	0.270	0.034	47
Eosinophils	0.047	0.030	31	0.065	0.031	34
Fault bars	0.163	0.535	1274	0.141	0.472	1321
Feather mites	46.574	45.547	831	42.837	49.184	843
H/L ratio	0.271	0.093	43	0.306	0.101	44
Heterophils	0.237	0.111	31	0.272	0.118	34
Hematocrit	52.899	4.366	370	51.404	5.208	351
Hue	34.626	2.131	43	33.767	2.756	47
Immunoglobulins	14.851	3.144	22	16.271	3.067	22
Keel length	21.535	0.737	971	20.771	0.766	982
Louseflies	0.019	0.155	1511	0.015	0.157	1541
Lutein color	1.080	0.105	43	1.048	0.110	47
Lymphocytes	0.769	0.068	31	0.737	0.066	34
Monocytes	0.029	0.022	31	0.033	0.022	34
Spleen mass	32.084	4.688	19	29.271	4.825	21
Short tail length	43.954	1.932	1234	44.557	1.934	1283
Tail length	108.129	9.003	1506	90.495	6.254	1528
Tail asymmetry	2.257	4.511	995	1.700	2.953	1053
Tarsus length	11.255	0.625	1507	11.285	0.635	1535
Tip vane asymmetry	0.120	0.153	26	0.180	0.159	28
Tip width	1.950	0.510	26	2.080	0.370	28
Tip-base difference	4.890	0.969	26	4.520	0.741	28
Ultraviolet	3.078	0.098	43	3.028	0.123	47
Visible color	3.809	0.079	43	3.792	0.082	47
Wing length	126.411	3.031	1510	124.347	2.941	1538
Wing asymmetry	0.545	0.388	1100	0.464	0.607	1138
Wing area	0.015	0.001	1010	0.015	0.001	1055
Wing loading	12.497	1.154	1010	13.443	1.627	1055
Wingspan	330.734	7.346	1284	326.022	6.738	1341

regression approach was verified in a second series of analyses based on conservative nonparametric correlations using the phi-correlation procedure. Even with that approach we found a very strong positive association for mating success, but not for sexual size dimorphism.

Previous studies of the barn swallow in Chernobyl and a control area have shown an increase in germline mutations of microsatellite loci by a factor two to 10 (Ellegren et al. 1997), similar to increases in mutation rates in wheat and humans (Dubrova et al. 1996; Kovalchuk et al. 2000). Likewise, studies of partial albinism in barn swallows have shown an increase in their frequency by a factor of ten when compared to control areas (Møller and Mousseau 2001). Partial albinism is caused by mutations, and barn swallows from Chernobyl showed that these were germline mutations (Ellegren et al. 1997). We do not have direct information on whether the expression of other phenotypic traits are affected by mutations, although we see no reason why that should not be the case. Indeed, we found very similar patterns for morphological traits produced in the winter quarters of the barn swallows in Africa during the annual molt and for other char-

acters (Fig. 1; Table 4). In fact, the patterns for physiological characters recorded during the breeding season in Ukraine and other characters differed significantly, with physiological characters showing weaker relationships for mating success as compared to other variables (Fig. 1; Table 5).

We compared the standardized difference in male phenotype between two areas in Ukraine and related these differences to standardized differences in phenotype between mated and unmated males in Denmark more than 2000 km away. This approach was adopted to ensure that the two datasets were independent. The effects of elevated mutation rates in Ukraine are unlikely to show up in Denmark, since natal and breeding dispersal distances are on average less than 5 km per generation (Møller 1994a). This approach also assumes that similar traits are important for sexual selection in different populations of the barn swallow. Indeed, long-term studies of populations of barn swallows in Denmark, Estonia, Hungary, Italy, and Spain have shown similarly strong effects of male tail length and red coloration of the plumage of the head on mating success (Møller 1994a; F. de Lope, M. Kose, A. P. Møller, P. Ninni, P. Pap, and N. Saino, unpubl. data).

FIG. 1. Relationship between standardized difference in phenotype between mated and unmated male barn swallows and standardized difference in phenotype between male barn swallows from Kanev and Chernobyl.

All data of the present study are comparable because the same person (A. P. Møller) measured all morphological characters in a standardized way, ensuring a high degree of repeatability (Møller 1994a). Likewise, other characters analyzed here showed a high repeatability based on repeat measurements (see Materials and Methods for references).

In our statistical analyses we treated the different characters as independent. We have already emphasized that such values cannot necessarily be considered statistically independent. Previous analyses of phenotypic correlations in the barn swallow have shown relatively weak positive associations for most characters, with correlation coefficients generally less than 0.5 (Møller 1994a). These associations are much weaker than usually found in studies of birds (e.g., Grant and Grant 1989). Thus, our conclusions are unlikely to be strongly affected by these weak phenotypic correlations. We also note that numerous other scientists have used estimates of different phenotypic traits as statistically independent observations in their analyses, even when phenotypic correlations are much stronger than in our case (e.g., Alatalo et al. 1988; Grant and Grant 1989; Houle 1992; Merilä and Sheldon 2000).

Sexual size dimorphism evolves over very long time scales (Lande and Arnold 1985), and the relationship between mating success and sexual size dimorphism is thus generally believed to be relatively weak (Andersson 1994). Female

TABLE 4. Analysis of covariance with standardized difference in phenotype between Chernobyl and Kanev as the dependent variable, feather character (F) as a factor, and standardized difference in phenotype between mated and unmated males (S) as a covariate. Model: $F = 54.636$, df $= 3,37$, $r^2 = 0.816$, $P < 0.0001$.
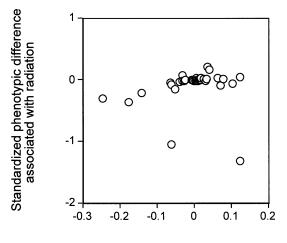
Source	Slope	SE	t	P
F	−0.029	0.040	0.74	0.465
S	1.164	0.342	3.40	0.0016
F × S	−0.283	0.350	0.81	0.423

TABLE 5. Analysis of covariance with standardized difference in phenotype between Chernobyl and Kanev as the dependent variable, physiological character (P) as a factor, and standardized difference in phenotype between mated and unmated males (S) as a covariate. Model: $F = 96.174$, df $= 3,37$, $r^2 = 0.886$, $P < 0.0001$.

Source	Slope	SE	t	P
P	−0.035	0.034	1.03	0.308
S	1.331	0.103	12.94	0.0001
P × S	−0.607	0.123	4.95	0.0001

mate preferences are supposed to have evolved toward benefits that can be accrued from current differences in male phenotype. If traits involved in sexual selection are particularly susceptible to mutation, we can expect that sexual size dimorphism should be a poorer predictor of mutation effects on phenotype than traits that have been directly shown to be associated with mating success. We tested this prediction in a multiple regression analysis (Table 6). The effects of radiation proved to be many times more important for the standardized difference in phenotype related to mating success than the standardized difference related to sexual size dimorphism, confirming our prediction. This observation suggests that characters that previously have been important in sexual selection, as evidenced from their current sexual size dimorphism, are less susceptible to the effects of mutation compared to characters that are currently of great importance for sexual selection. This also provides evidence of the dynamic nature of sexual selection, with sexually dimorphic traits that previously may have been of importance for sexual selection now showing less susceptibility to mutation than traits that differ in size between mated and unmated individuals.

Finally, we should consider whether the present results are of general interest, since the radiation levels in Chernobyl are perhaps extreme. Chernobyl barn swallows were used in the present study because we can assume with a high degree

FIG. 2. Relationship between standardized difference in phenotype between male barn swallows from Kanev and Chernobyl and standardized sexual size dimorphism.

TABLE 6. Multiple linear regression with standardized difference in phenotype between Chernobyl and Kanev as the dependent variable and the standardized difference in phenotype between mated and unmated males (S) and the standardized sexual size dimorphism (D) as independent variables. Model: $F = 78.748$, df = 2,37, $r^2 = 0.810$, $P < 0.0001$.

Variable	Slope	SE	*t*	*P*
S	0.901	0.072	12.439	0.0001
D	0.024	0.283	0.085	0.933

of certainty that mutation rates are considerably elevated when compared to other areas. Indeed, that was the case for microsatellites in the barn swallow and for several other organisms. Deleterious mutations are invariably occurring everywhere, although rates will vary depending on local conditions. The extent to which the phenotype of organisms is disrupted will depend on local mutation rates. However, there is no reason to believe that the positive relationships illustrated in Figures 1 and 2 will not be general. It is only the slope of these relationships that will differ among populations.

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