

## ALBINISM AND PHENOTYPE OF BARN SWALLOWS (*HIRUNDO RUSTICA*) FROM CHERNOBYL

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**Abstract.**—The effects of mutation on phenotypic expression are supposed to be mainly deleterious because mutations disrupt the expression of genes that function relatively well under current environmental conditions. Thus, mutations are assumed to give rise to deviant phenotypes that are generally selected against. Radioactive contamination in the Chernobyl region of Ukraine is associated with a significant increase by a factor two to 10 in mutation rate in microsatellite markers of the barn swallow, *Hirundo rustica*. Barn swallows from Chernobyl had a temporally constant, elevated frequency of partial albinism compared to the situation before radioactive contamination and compared to birds from a control area. Albinism disproportionately affected the carotenoid-based plumage of the head, suggesting that carotenoid metabolism is particularly susceptible to the effects of radiation. Individuals with partially albinistic plumage had, on average, lower mean phenotypic values than other birds, and this was particularly the case for males. Furthermore, differences in phenotypic variation, as determined using Levene's test, were significantly larger in partial albinos compared to nonalbinos in males, but not in females, even though the null expectation would be the opposite due to the lower mean phenotypic values of partial albinos. Although small phenotypes were commonly associated with germline mutations, there was no general decrease in overall body size during the period 1991–2000, implying that small individuals were selected against. Because partial albinism is disfavored by natural selection, the effects of mutations are deleterious, giving rise to a balance between mutation and selection.

**Key words.**—Albinism, carotenoids, mutation, phenotypic coefficient of variation, phenotypic correlation, radiation, selection.

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Mutation is a major factor contributing to the maintenance of genetic variation (Lynch et al. 1999). Most mutations are believed to be mildly deleterious with weakly negative effects on fitness (reviewed in Lynch et al. 1999) because any change in a DNA sequence will usually result in a reduction in the quality of the phenotype compared to what was produced by the original sequence. Any change in a coadapted gene sequence will generally produce a different protein that will result in the production of an inferior phenotype. Experimental studies in laboratory lines of *Drosophila* and *Caenorhabditis* have generally confirmed these effects (Lynch et al. 1999; Vassilieva et al. 2000). The decline in fitness per generation in such experiments is typically in the order of 0.1–1% per generation (Lynch et al. 1999; Vassilieva et al. 2000). However, it may be difficult to extrapolate these results from the laboratory to the field because of the generally more benign and stable environment in the laboratory.

There are very few studies of the effects of mutations on phenotypic expression and selection under field conditions. Of particular interest are studies finding increased mutation rates in humans and barn swallows (*Hirundo rustica*) from the radioactive contaminated area around Chernobyl in Ukraine (Dubrova et al. 1996; Ellegren et al. 1997). In studies of humans mutation rates of minisatellite DNA increased by a factor two (Dubrova et al. 1996), whereas Ellegren et al. (1997) reported an increase in mutation rates in barn swallow microsatellite DNA by a factor two to 10. Furthermore, the genome of somatic cells of mice exposed to radiation has been shown to remain fragile across generations, extending at least to the first generation (Dubrova et al. 2000). Indirect estimates of mutation rates based on the frequency of cells with aberrant micronuclei and chromosomes have shown that

similarly increased levels of cellular disruption exist in other organisms in contaminated areas around Chernobyl (Zakharov and Krysanov 1996).

Previous studies of the barn swallow in Chernobyl have shown that the level of fluctuating asymmetry in the outermost tail feathers of males increased by a factor seven in the Chernobyl area following 1986 compared to a less contaminated control area in Ukraine (Møller 1993) and museum samples from the same areas before that date (Møller 1993). A total of 15 different organisms have shown increased fluctuating asymmetry around Chernobyl when compared to samples from less contaminated areas (Møller 2001). Also, deformation of the tips of the outermost tail feathers has been recorded in low frequency from Chernobyl, but not from elsewhere in the range of the species (Møller 1993), and both malformation and asymmetry are associated with a delay in breeding date of male barn swallows (Møller 1993). This indicates that sexual selection is acting against males with malformed and asymmetric tails. Partial albinism is an uncommon plumage aberration of genetic origin in birds that rarely amounts to more than 1% of all individuals (Sage 1962; Dyck 1985). Barn swallows from Chernobyl have a highly elevated frequency of partial albinism, reaching 13–15% (Ellegren et al. 1997), and this albinism is of germline origin as determined from a significant parent-offspring resemblance (Ellegren et al. 1997). Field studies of house sparrows (*Passer domesticus*) in other areas have found that partial albinism is more common in cities (1–2%) and smaller towns than in the countryside, where the frequency of albinism is close to 0% (Il'enko 1960). This suggests that the factors associated with albinism are more prevalent in polluted, urban areas.

A disproportionate fraction of partial albinism in the barn



FIG. 1. A partially albinistic barn swallow from the Chernobyl region with a typical small number of white feathers in the red facial plumage and an individual with a normal phenotype (right). Photograph by T. A. Mousseau.

swallow occurs in the carotenoid-based facial plumage, but hardly any in the dark blue plumage of the neck, back, wings, and tail (Camplani et al. 1999). The coloration of carotenoid-based red plumage was significantly reduced in Chernobyl barn swallows, especially in males (Camplani et al. 1999). Whereas males with longer, and thus more attractive, outermost tail feathers generally have a brighter red coloration, long-tailed males from Chernobyl were no more bright red colored than short-tailed males (Camplani et al. 1999).

The aims of the present study were to test three predictions related to the phenotypic effects of mutations, using barn swallows from Chernobyl as a test case. First, we tested whether mean phenotypic values of morphological traits were

reduced in mutants compared to control individuals, using partial albinism as a phenotypic marker of mutations. Second, we tested whether the coefficient of variation for morphological traits differed between partial albinos and other individuals. Third, because the phenotypic characters of barn swallows have significant heritabilities (Møller 1994; Saino et al. 1997; A. P. Møller, F. de Lope, and N. Saino, unpubl. data) and the germline mutations giving rise to partial albinism are also associated with small phenotypes, we should expect an increase in the frequency of partial albinism and a decrease in overall size during the three sampling events in 1991, 1996, and 2000, unless albinistic and small individuals were selected against. Because annual survival rate is about 30% (Møller 1994), less than 1% of individuals in any given year would be present after four years, and differences in mean phenotype among the three sampling events should be due to a response to selection.

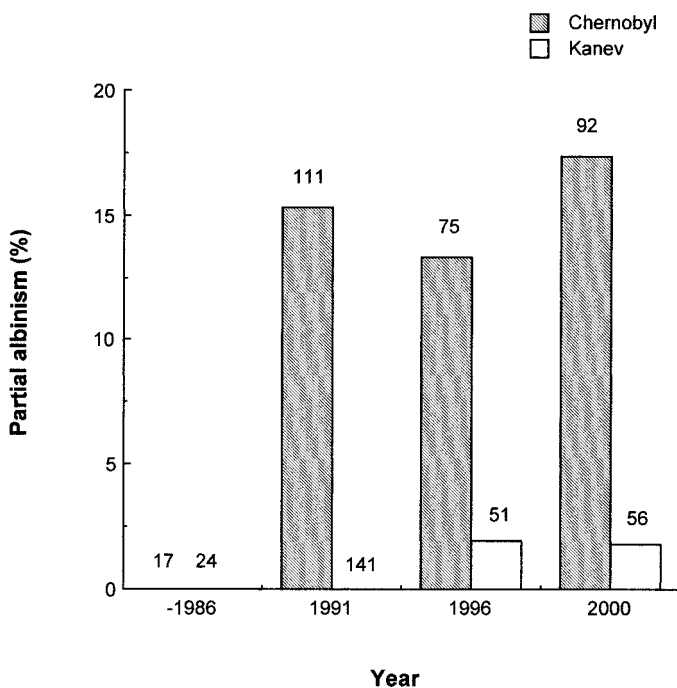


FIG. 2. Temporal pattern of the frequency of partial albinism in barn swallows from Chernobyl and Kanev, Ukraine. Numbers are sample sizes.

MATERIALS AND METHODS

Study Area

We studied barn swallows in parts of the southern and western sector just outside the exclusion zone of the Chernobyl area (Shestopalov 1996) during 10–29 June 1991 (APM), 16–24 June 1996 (APM), and 6–12 June 2000 (APM, TAM). We visited villages and stopped to check collective farms for the presence of barn swallows. If found, we attempted to capture as many adults as possible at windows and doors of farm buildings using mist nets. Based on our experience, this method usually results in the capture of more than three-quarters of all barn swallows during a single day. The villages visited and used for the study included Guda-Katyuzhanka, Kathyuskanka, Obukovichy, Phenevichy, Ivankov, Vakhovka, Glybovka, Pribirsk, Dytiatku, Pysky, Varonychi, Martynovichi, Bober, Vesniane, and Dymir. As a control area, we used Kanev (~220 km southwest of Kiev), which has a relatively low level of contamination. The low level of contamination was confirmed by our own measurements of radioactivity during the field season in 2000. The control villages visited and used for the study included Kanev, Stepatsy, and Stepanetske.

TABLE 1. Mean squares from three-way analyses of variance with sex, partial albinism, and year as factors for morphological traits of barn swallows from Chernobyl. The residual degrees of freedom are 254.

Variable	Sex (S)	Albinism (A)	S × A	Year (Y)	S × Y	A × Y	S × A × Y
Beak length (mm)	11.61	126.97*	3.91	589.37***	10.41	234.06***	1.68
Beak height (mm)	1.91	2.05	12.52	462.43***	0.74	6.33	3.21
Beak width (mm)	21.74	51.37	15.74	629.39***	69.28	53.97	11.62
Tarsus (mm)	4.12	305.03***	0.65	5402.03***	51.95	9.52	35.59
Keel (mm)	339.03*	65.19	352.34*	468.42**	20.16	55.20	59.06
Wing (mm)	5.36	44.28*	32.48	1.39	14.57	4.51	30.85*
Wingspan (mm)	85.31	208.78*	27.55	2.75	41.22	41.44	50.79
Tail (mm)	7175.43***	178.64	74.17	190.29*	17.92	116.92	27.78
Central tail (mm)	3.38	7.65	6.00	60.84***	1.67	0.81	3.25
Body mass (g)	234.22***	29.82	9.02	53.21*	53.77*	27.86	21.06
df	1	1	1	2	2	2	2

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

### Partial Albinism

When an adult barn swallow was captured, we carefully inspected its plumage for any signs of white feathers in the otherwise red and blue colored parts of the body. Any deviant plumage coloration was recorded, and individuals with one or more white feathers present in otherwise red or blue feather tracts were classified as partial albinos. Usually only a few feathers were white, but some individuals had a large number of white feathers (Fig. 1).

Upon capture, a large number of morphological traits were measured by APM in the same, standardized way. We measured the length of the beak to the feathering, the height of the beak at the distal end of the nares, and the width of the beak at the commissure to the nearest 0.01 mm with a digital caliper. Tarsus length and the length of the keel were measured likewise. The length of the right and the left flattened wing, the right and the left outermost tail feather, and the left and the right central tail feather were measured with a ruler to the nearest 1 mm. Tail and wing length were subsequently defined as the mean of the left and the right measurement. We measured wingspan by stretching the wings to the maximum possible and then recording the distance between the wing tips. Body mass was recorded with a Pesola balance to the nearest 0.1 g. All measurements were highly repeatable as demonstrated by repeat measurements of the same individuals (Møller 1994).

### Statistical Analyses

We tested for differences in the frequency of partial albinism among periods and study regions using  $G$ -tests (Sokal and Rohlf 1995). Three-way analysis of variance (ANOVA) was used to test for phenotypic differences among albinos and sexes and any interaction effects, while taking differences among years into account. Mean values for partially albinistic and nonalbinistic individuals of each sex were compared using a paired  $t$ -test on  $\log_{10}$ -transformed values. Furthermore, the difference in mean phenotype between albinistic and nonalbinistic individuals of each sex was compared with the null expectation of no difference using a one-sample  $t$ -test, and differences for males were compared with differences for females using a paired  $t$ -test.

We used a principal component analysis with the varimax procedure to extract the first five principal components from the 10 phenotypic variables. The scores were subsequently used for two-way ANOVAs to test whether they differed with respect to albinism, sex, and their interaction.

Comparison of phenotypic variation for partially albinistic and nonalbinistic individuals of each sex was made using Levene's test for unequal variances, after logarithmic transformation of the variables. Tests were made separately for each of the three periods for each sex. The null expectation in this case is that 5% of all tests are statistically significant and that equally many tests will have a greater variance for albinistic and nonalbinistic individuals.

TABLE 2. Mean (SE) phenotypic characters for partially albinistic and nonalbinistic male and female barn swallows from Chernobyl. Mean values with different superscripts are significantly different ( $P < 0.05$ ) according to Fisher's PLSD tests.

Variable	Male nonalbinos	Male albinos	Female nonalbinos	Female albinos
Beak length (mm)	7.84 (0.05) <sup>a</sup>	7.73 (0.15) <sup>b</sup>	7.93 (0.05) <sup>a</sup>	7.61 (0.13) <sup>b</sup>
Beak height (mm)	2.60 (0.03)	2.54 (0.09)	2.57 (0.03)	2.62 (0.07)
Beak width (mm)	11.73 (0.07)	11.70 (0.16)	11.72 (0.07)	11.51 (0.12)
Tarsus (mm)	11.57 (0.09) <sup>a</sup>	11.38 (0.24) <sup>b</sup>	11.63 (0.10) <sup>a</sup>	11.35 (0.21) <sup>b</sup>
Keel (mm)	21.05 (0.08)	20.73 (0.22)	20.40 (0.10)	20.58 (0.15)
Wing (mm)	125.21 (0.29) <sup>a</sup>	123.52 (0.80) <sup>b</sup>	123.80 (0.28)	123.50 (0.68)
Wingspan (mm)	331.41 (0.68)	329.10 (1.01)	328.87 (0.65)	327.14 (1.36)
Tail (mm)	109.31 (0.89) <sup>a</sup>	104.37 (1.94) <sup>b</sup>	91.98 (0.62)	91.28 (1.51)
Central tail (mm)	44.50 (0.16)	44.29 (0.63)	45.34 (0.20)	44.50 (0.60)
Body mass (g)	18.87 (1.16)	18.60 (1.86)	19.61 (1.45)	19.50 (3.37)
<i>N</i>	121	21	102	22

TABLE 3. Analysis of variance for the effect of year, area, and sex on phenotypic characters of male and female barn swallows from Chernobyl and Kanev. Mean values (SE) for different years with different superscripts are significantly different ( $P < 0.05$ ) according to Fisher's PLSD tests.

Variable	Year (Y)	Area (A)	Sex (S)	Y × A	Y × S	A × S	Y × A × S	Residual
Beak length (mm)	588.05***	111.79*	11.13	224.38***	9.31	8.64	3.78	24.26
Beak height (mm)	2029.96***	77.34***	0.17	34.44**	2.21	3.08	6.07	4.93
Beak width (mm)	708.71***	363.39**	11.97	592.14***	7.80	12.71	8.40	36.85
Tarsus (mm)	8048.50***	1761.20***	154.90	2463.34***	152.24*	89.94	60.48	49.20
Keel (mm)	1428.42***	35.08	4691.40***	153.28*	25.42	41.58	79.37	66.35
Wing (mm)	4.08	71.98**	244.37***	21.46	1.46	15.05	1.86	9.10
Wingspan (mm)	185.74*	33.79	1265.37***	243.44**	14.22	148.86	13.24	44.40
Tail (mm)	269.70*	147.06	32,864.96***	19.75	0.29	250.04	28.24	60.56
Central tail (mm)	101.45***	0.81	38.89***	28.73***	2.62	0.52	2.24	3.51
Body mass (g)	473.38***	319.10***	680.58***	750.72***	92.86**	4.71	30.22	17.11
PC1	97.37***	1.04	0.60	1.68	0.27	0.68	0.35	0.79
df	2	1	1	2	2	1	2	478

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

We tested for temporal trends in phenotype by investigating whether mean phenotypes differed among 1991, 1996, and 2000 using a three-way ANOVA with area, sex, and period as factors. The absence of partial albinos in some of the samples from the control area prevented us from including that factor into the ANOVA models.

## RESULTS

### *Patterns of Partial Albinism*

The percentage of individuals that were partial albinos was high in the Chernobyl area after the radioactive contamination in 1986, but not in museum samples predating that period (Fig. 2). For the control area, there was no such temporal trend, and the percentage of partial albinos was consistently low (Fig. 1). The difference between pre- and post-1986 samples for Chernobyl was statistically significant ( $G = 12.79$ ,  $df = 3$ ,  $P < 0.02$ ). Similarly, the difference between Chernobyl and Kanev post-1986 was statistically significant ( $G = 36.25$ ,  $df = 3$ ,  $P < 0.001$ ). There was no significant difference among years in the frequency of albinism in Chernobyl after 1986 ( $G = 0.53$ ,  $df = 2$ , ns). Among the 43 partially albinistic individuals, 42 had white feathers in the red facial plumage and one in the blue dorsal plumage. Given that the red plumage accounts for less than 10% of the body surface, this distribution was significantly different from a random expectation ( $G = 184.13$ ,  $df = 1$ ,  $P < 0.001$ ).

### *Mean Phenotype of Partial Albinos and Controls*

ANOVAs revealed significant effects of partial albinism for four of the 10 morphological characters (Table 1), with mean values for partially albinistic individuals being smaller than for nonalbinistic ones (Fisher's PLSD test,  $P < 0.05$ ). The sex by albinism interaction, the albinism × year interaction, and the sex × albinism × year interaction were each significant for a single character (Table 1). Mean values for the 10 morphological characters were significantly smaller for partial albinos than for nonalbinos in males (Table 2; paired  $t$ -test based on  $\log_{10}$ -transformed data:  $t = 4.05$ ,  $df = 9$ ,  $P = 0.003$ ), but not significantly so in females (Table 2, paired  $t$ -test:  $t = 1.73$ ,  $df = 9$ ,  $P = 0.12$ ). The difference in

mean value between partial albinos and nonalbinos for males did not differ significantly from that of females (paired  $t$ -test based on  $\log_{10}$ -transformed data:  $t = 0.86$ ,  $df = 9$ ,  $P = 0.41$ ).

A factor analysis using the orthogonal varimax approach extracted five principal component axes from the 10 variables. The first axis explained 24.3% of the variance, while the following axes each explained 8.6–18.9% of the variance. The first five axes explained in total 76.3% of the variance. The first axis was positively correlated with all 10 variables, with wingspan ( $r = 0.842$ ), keel length ( $r = 0.545$ ), wing length ( $r = 0.828$ ), and tail length ( $r = 0.567$ ) having large correlation coefficients. A two-way ANOVA on principal component 1 scores gave a significant effect for albinism ( $F = 4.421$ ,  $df = 1$ , 225,  $P = 0.037$ ) and sex ( $F = 4.384$ ,  $df = 1$ , 225,  $P = 0.037$ ), but not for the interaction ( $F = 1.116$ ,  $df = 1$ , 225,  $P = 0.298$ ). Mean score for albinistic birds was  $-0.335$  (SE = 0.160), whereas it was 0.072 (SE = 1.047) for nonalbinistic birds. None of the other principal component axes showed significant differences with respect to albinism.

### *Variance in Phenotype of Partial Albinos and Controls*

We compared variances for albinistic and nonalbinistic individuals for logarithmically transformed variables using Levene's test. For males we found significantly larger variances for partial albinos for five different characters (beak height 1991:  $F = 4.12$ ,  $df = 1$ , 61,  $P = 0.047$ ; central tail 1991:  $F = 7.85$ ,  $df = 1$ , 61,  $P = 0.0069$ ; keel 1996:  $F = 4.43$ ,  $df = 1$ , 35,  $P = 0.043$ ; tail 1996:  $F = 5.96$ ,  $df = 1$ , 35,  $P = 0.020$ ; central tail 1996:  $F = 6.14$ ,  $df = 1$ , 35,  $P = 0.018$ ). The observed number of significant tests of five exceeds the expected number of 1.5 (30 tests times 0.05) by a factor of three. Furthermore, all tests showed larger variance among partial albinos, whereas the predicted number was only half the tests. Among females, only two tests were statistically significant compared to the expected 1.5 tests (wing 1996:  $F = 5.18$ ,  $df = 1$ , 36,  $P = 0.029$ ; keel 2000:  $F = 5.66$ ,  $df = 1$ , 37,  $P = 0.023$ ). Whereas partial albinos had larger variance than nonalbinos for wing length in 1996, the pattern was the reverse for keel length in 2000.

TABLE 3. Extended

Variable	Chernobyl			Kanev		
	1991	1996	2000	1991	1996	2000
Beak length (mm)	7.76 (0.05) <sup>a</sup>	7.63 (0.05) <sup>a</sup>	8.12 (0.05) <sup>b</sup>	7.83 (0.04)	8.01 (0.07)	8.09 (0.08)
Beak height (mm)	2.91 (0.03) <sup>a</sup>	2.44 (0.02) <sup>b</sup>	2.36 (0.02) <sup>c</sup>	3.11 (0.02)	2.49 (0.03)	2.39 (0.03)
Beak width (mm)	11.38 (0.07) <sup>a</sup>	12.18 (0.07) <sup>b</sup>	11.68 (0.07) <sup>c</sup>	11.94 (0.05)	11.98 (0.09)	11.96 (0.07)
Tarsus (mm)	12.69 (0.05) <sup>a</sup>	11.01 (0.06) <sup>b</sup>	10.85 (0.05) <sup>c</sup>	11.42 (0.05)	11.05 (0.04)	10.75 (0.08)
Keel (mm)	20.99 (0.08) <sup>a</sup>	20.43 (0.13) <sup>b</sup>	20.74 (0.10) <sup>a</sup>	21.19 (0.07)	20.55 (0.12)	20.75 (0.12)
Wing (mm)	124.16 (0.28)	124.56 (0.39)	124.48 (0.36)	125.69 (0.20)	124.77 (0.38)	125.00 (0.44)
Wingspan (mm)	329.70 (0.62)	330.56 (0.81)	329.59 (0.80)	326.63 (0.62)	330.00 (0.80)	330.34 (1.03)
Tail (mm)	99.95 (1.32)	101.38 (1.36)	101.26 (1.21)	100.14 (1.10)	103.06 (1.81)	101.24 (1.92)
Central tail (mm)	45.13 (0.26) <sup>a</sup>	45.84 (0.13) <sup>b</sup>	43.62 (0.16) <sup>c</sup>	44.40 (0.17)	45.94 (0.18)	44.55 (0.25)
Body mass (g)	19.24 (0.17)	19.00 (0.13)	19.26 (0.14)	18.77 (0.12)	21.17 (0.18)	19.26 (0.18)
PC1	-0.10 (0.11)	0.07 (0.13)	0.04 (0.12)	-0.01 (0.09)	0.07 (0.14)	0.11 (0.17)
<i>N</i>	99	75	92	141	51	55

### Temporal Change in Phenotypes

We found statistically significant year by area interactions for eight of the 10 morphological characters (Table 3). Six variables showed significant sex effects, and two of the sex  $\times$  year interactions were statistically significant. In Chernobyl, only tarsus and beak height showed a consistently decreasing pattern during the three study years. For overall body size, estimated as the principal component 1 scores, there was no significant difference in mean phenotype among years (Table 3). There was also a decrease in phenotype in the control area for tarsus length and beak height, but these changes differed significantly between areas, as indicated by the highly significant area  $\times$  year interactions (Table 3).

### DISCUSSION

Partial albinism in birds arises as a consequence of somatic or germline mutations (Sage 1962; Dyck 1985). There is little information on the frequency of this phenomenon in other taxa and even less on the potential consequences (e.g., Laikre 1999; Bolker and Hill 2000). Previous studies of partial albinism in barn swallows from Chernobyl have shown that the frequency of partial albinism is elevated by a factor of 10 compared to before the radioactive contamination in 1986 and compared to a less contaminated control area (Ellegren et al. 1997). This finding was replicated in the present study (Fig. 2), suggesting that an elevated frequency of partially albinistic individuals still persists and that there is constancy in the frequency of partial albinos over time. Comparisons of the phenotype of parents and their offspring have revealed a significant resemblance with respect to partial albinism (Ellegren et al. 1997), suggesting that partial albinism is caused by germline mutations. The increase in the frequency of partial albinos in the Chernobyl region is of a magnitude similar to the increase in microsatellite mutation rates of barn swallows from this area (Ellegren et al. 1997). Thus, there is consistency between the frequency of partial albinos and the frequency of microsatellite mutations.

A disproportionate number of cases of partial albinism occurred in the red facial plumage, which has a coloration based partly on carotenoids, particularly lutein, whereas the blue dorsal coloration rarely had white feathers. This result

suggests a physiological link between mutation and carotenoids. This suggestion is further supported by the fact that male barn swallows from Chernobyl have a pale red coloration compared to males from a control area, in particular in males with long outermost tail feathers (Camplani et al. 1999). In contrast, there was no difference in coloration between females from Chernobyl and females from control areas (Camplani et al. 1999). Long-tailed males are usually very attractive to females (Møller 1994), and they are also usually in prime health status as indicated by high plasma levels of carotenoids and low circulating levels of leukocytes and immunoglobulins (Saino et al. 1999). Because carotenoids have the dual function of immuno-enhancement and free-radical scavenging, long-tailed males from Chernobyl may face a different trade-off between use of carotenoids for signals and free-radical scavenging when compared to males from other populations (Ames 1983; Krinsky 1989, 1998; Edge et al. 1997; Rice-Evans et al. 1997; Bast et al. 1998; Rao and Agarwal 1999; for an extensive review, see Møller et al. 2000). Hill (1999) suggested that these patterns were not clear, but he based this conclusion on a very small fraction of the available evidence, which is fully reviewed in Møller et al. (2000). Camplani et al. (1999) hypothesized that male barn swallows in the Chernobyl region may use large amounts of carotenoids for free-radical scavenging, leaving little left for the production of plumage signals. Because long-tailed male barn swallows are particularly successful in terms of mating, elevated activity due to mating effort by such males may produce large amounts of free radicals that must be neutralized by carotenoids, that is, radioactive contamination may be an additional factor contributing to the use of carotenoids in the Chernobyl population.

We used partial albinism as a phenotypic marker of mutation in the present study. This approach is justified by the germline nature of partial albinism of barn swallows from the Chernobyl region (Ellegren et al. 1997). If carotenoids play an important role in free-radical scavenging in the barn swallow, we could hypothesize that the suboptimal development of phenotypic traits would correlate with the presence of partial albinism, assuming that the absence of red plumage coloration is indicative of disrupted carotenoid absorption, deposition, or utilization. We are currently investigating pat-

terns of carotenoid distribution in barn swallows from Chernobyl and uncontaminated populations.

Partially albinistic male barn swallows were characterized by reduced mean phenotypic values compared to barn swallows without signs of partial albinism (Tables 1, 2). This was the case both for skeletal characters that develop during early ontogeny in the nest, such as beak length, but also for feather characters that develop once a year in the African winter quarters (these include wing and tail length and wingspan). A potential mechanism accounting for this effect is that the efficiency of the antioxidant function of a given amount of carotenoids is limited in Chernobyl due to the effects of radiation. Growth, which is a process that generates free radicals, may be inhibited by the lack of available free-radical scavengers (i.e., carotenoids). However, although females were almost equally likely to show partial albinism (Table 2), there was no significant relationship between albinism and morphological trait values among females. While the mean difference between albinos and nonalbinos accounted for 64.6% of the variance in mean phenotypic values of males ( $r^2 = t^2/[t^2 + df]$ , where  $r$  is Pearson's product-moment correlation coefficient and  $t$  is the  $t$ -statistic; Rosenthal 1994), the comparable estimate for females was 25.0%, or less than half of that in males. At present we have no easily testable hypotheses to explain the observed sexual dimorphism in phenotypic correlations to albinism in this species. We hypothesize that male barn swallows may be more limited by carotenoid availability than females, because only males from Chernobyl had a reduction in the intensity of red plumage coloration (Camplani et al. 1999), perhaps due to the greater needs for carotenoids by males for coloration, immune function, and free-radical scavenging.

If there is natural selection against mutants, we might expect that the category of partially albinistic birds contained a smaller frequency of juveniles. This could bias some of the calculations due to age-dependent changes in phenotype. Whereas skeletal characters change very little or not at all in a cohort of birds, feather characters have been shown to change. For example, wing and tail length increase slightly from the first to the second and much less to the third year of life, with a subsequent decrease due to senescence (Møller 1991; Møller and de Lope 1999). These changes are very small, averaging about 2 mm for male tail length between year 1 and 2. Given that annual survival rate is about 30% (Møller 1994), birds older than one year comprise about 12% of the population. However, even in the unlikely event that the Chernobyl population consisted of two or three times as many old birds as other populations, this could not account for the mean difference of 5 mm in tail length between albinistic and nonalbinistic males (Table 2). Although we could not determine the age of the birds from Chernobyl, it seems unlikely that age-dependent mortality could account for the phenotypic differences between albinistic and nonalbinistic individuals.

Given that partially albinistic barn swallows generally had lower mean phenotypic values we should expect a priori that their variance in phenotype also should be smaller than that of nonalbinistic individuals. This prediction is based on the common observation that means and variances are positively correlated. Surprisingly, we found that partially albinistic

male barn swallows had significantly higher phenotypic variation than nonalbinistic individuals for five different tests, whereas the expected number of tests showing such a pattern was only 0.75. This pattern was not true for females: Only a single test showed a larger variance in albinos compared to nonalbinos, whereas the opposite pattern was observed for another character. The expected number of tests for females showing these results was 0.75, which is close to the observed number. Thus, partially albinistic male barn swallows seemed to have a wider range of phenotypes compared to nonalbinistic individuals, which was not the case in females. Again, it is not obvious why the effects of mutation should be greater in males than in females.

The fitness costs of mutations have been debated to a considerable extent during the past few decades (review in Lynch et al. 1999). The aberrant white plumage color of barn swallows from Chernobyl was associated with a generally small body size, and this may be costly for the following reasons. Because germline mutations arise every generation, we should expect a steady decrease in mean phenotypic values with time and an increase in partial albinism in barn swallows from the Chernobyl region, because an increasing fraction of the population would be comprised of mutants, unless small, albinistic mutant phenotypes were selected against. Six of the 10 morphological characters showed significant variation among samples from 1991, 1996, and 2000 (Table 3). Only two of these characters demonstrated a steady decrease in phenotype, whereas the others showed no consistent temporal pattern. Tarsus length decreased by 3.7 standard deviations during 1991–2000 in Chernobyl, and beak height decreased by 2.8 standard deviations during the same period. The same person measured all birds in a standardized way, thus the differences cannot be attributed to changes in measurement methods or definitions of characters. These changes are very large compared to responses to selection recorded in other birds, such as 0.50–0.75 standard deviations in Darwin's finches (Grant and Grant 1995) and a similar magnitude or even larger in cliff swallows (*Petrochelidon pyrrhonota*; Brown and Brown 1998; Price et al. 2000). The initial responses to selection in the studies by Grant and Grant (1995) and Brown and Brown (1998) were subsequently followed by evolutionary responses in the opposite direction.

Given that different morphological characters showed different temporal patterns of change, it might be more appropriate to investigate the overall measure of body size reflected by the first principal component. This overall measure of body size in barn swallows did not show significant variation among periods (Table 3). Similarly, there was no increase in the frequency of albinistic individuals during the study period (Fig. 2), which would be expected if germline mutations were continuously accumulating. Therefore, we can conclude that there was no overall decrease in mean body size, although some characters showed dramatic changes in size during the study period. The lack of change in overall body size is as expected if mutant genes did not increase in frequency in the population. This suggests that there is considerable natural selection against small and albinistic phenotypes. Ellegren et al. (1997) showed that partially albinistic barn swallows from a Danish population had a reduced probability of survival compared to other conspecifics. It seems unlikely that vi-

sually searching predators would be able to cue in on prey with such small amounts of white feathers. In humans, partial albinism has been associated with immune deficiency due to defective melanosomes, platelet granules, and lysosomes (Introne et al. 1999; Baumeister et al. 2000; Spritz 2000). We hypothesize that carotenoid-limitation of efficient immune function and/or free-radical scavenging is the mechanism generating reduced survival of partial albinos. The generally low frequency of partial albinos in free-living populations, despite the germline basis of this plumage aberration, suggests that there is selection against such coloration. This interpretation is also consistent with the frequency of partially albinistic birds in cities, smaller towns, and rural areas (Il'enko 1960). The higher frequency of such cases of albinism in cities may be associated with the presence of higher concentrations of mutagens in this environment, but an alternative (although not necessarily mutually exclusive) explanation is that cities generally have a low abundance of predators compared to rural areas. Thus, natural selection against partial albinism may be less severe in cities compared to rural areas.

Finally, a previous study of barn swallows in Chernobyl has also demonstrated an increased frequency of outermost tail feathers with aberrant morphology, and individuals with such disrupted phenotypes reproduced later than others (Møller 1993). This observation suggests that radioactive contamination may disrupt normal phenotypic development, but that sexual selection due to female choice or male-male competition may select against such deviant morphology.

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