Rapid decrease in asymmetry associated with increase in size of a secondary sexual character

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Fluctuating asymmetry in morphological characters arises as a consequence of the inability to control developmental processes. The level of individual asymmetry may reflect the adversity of environmental conditions during development and the ability to resist such insults. I investigated temporal trends in asymmetry of wing and tail length of barn swallows *Hirundo rustica* during the period 1989-2004, when environmental conditions during spring migration deteriorated, and when the length of a condition-dependent secondary sexual character in males increased by over 1.2 standard deviations. The degree of composite asymmetry based on asymmetry of the two characters decreased significantly during the study, and this decrease was significantly associated with the increase in tail length in males. The relationship between asymmetry and length of tails in males changed from significantly negative to no relationship during the course of the study. There was little evidence that selection against asymmetry changed during the study period. These findings are consistent with the hypothesis that tail length of male barn swallows is a reliable indicator of the ability of individuals to undergo stable development, since an increase in tail length of males has been associated with a decrease in asymmetry.

Key words: composite asymmetry; condition-dependence; developmental instability; phenotypic plasticity; selection.

INTRODUCTION

Developmental instability causes the imprecise expression of developmental design due to perturbations during the developmental period (Zakharov 1989; Parsons 1990; Møller and Swaddle 1997; Polak 2003). Such imprecision is generally assumed to arise from lack of perfect adaptation to the environment (due to mutation, host-parasite coevolution, sexually antagonistic evolution and other processes). Developmental stability is presumed to modulate the impact of perturbations during ontogeny of a trait by a number of different mechanisms, including feedback mechanisms that can stabilize growth processes. Measures of the degree of developmental instability include fluctuating asymmetry and the frequency of phenodeviants. The latter are major deviations from normal phenotype such as extra or missing digits, limbs, bristles or other morphological characteristics. Fluctuating asymmetry is characterized as randomly directed deviations from perfect symmetry between the two sides of a symmetry axis. The distribution of signed differences between the two sides is assumed to have a mean value of zero and a frequency distribution that deviates slightly from normal (Gangestad and Thornhill 1999). These deviations from normality reflected by the leptokurtotic distribution are directly proportional to heterogeneity in individual differences in developmental stability (Gangesatd and Thornhill 1999). Fluctuating asymmetry is caused by genetic factors (mutation, inbreeding, outbreeding, homozygosity, hybridization, co-adaptation) and the environment (radiation, ultraviolet light, electromagnetic radiation, chemicals, temperature, light, parasitism, competition, herbivory, predation).

The repeatability of fluctuating asymmetry is the proportion of phenotypic variance attributable to individual differences in developmental instability (Whitlock 1996; Van Dongen 1998), with the sample size weighted estimate across species and characters being 0.071 (Gangestad and Thornhill 2003). Such underlying individuals differences in the ability to resist developmental perturbations would imply small, but significant correlation between asymmetry and fitness. Meta-analyses of the available literature suggested that the sample size weighted correlation between asymmetry and growth, fecundity and survival, respectively, was – 0.15, -0.35 and –0.25 (Møller 1999; Møller and Manning 2003). This is within the range predicted by the standard model of developmental stability, when experimental studies are excluded (Gangestad and Thornhill 1999, 2003).

Does indicators of developmental instability such as fluctuating asymmetry reflect the ability of individuals to control developmental processes? Several pieces of evidence suggest that this is the case. (1) There is highly significant consistency in the degree of asymmetry across repeated developmental periods such as molts in the barn swallow *Hirundo rustica* (Møller 1994b). (2) Tail asymmetry of barn swallows when developed in two different environments (during normal molt in winter and during induced molt in summer) was strongly positively correlated (Møller 1996), indicating inherent individual differences in the ability to control development. (3) Individuals that are variable in asymmetry among years have lower fitness, while individuals that show a high degree of consistency in asymmetry have higher fitness (Shykoff and Møller 1999). In fact asymmetric individuals have lower fitness both before and after development of asymmetry. (4) Statistical control of individual asymmetry deviations removes the genetic relationship between developmental stability and mating performance (Polak and Stillabower 2004). (5) There are consistent correlations between individual asymmetry and underlying developmental control, as reflected by a meta-analysis of the literature (Gangestad and Thornhill 1999, 2003).

Here I use the opportunity that the size of a secondary sexual character in males has increased dramatically, by over 1.2 standard deviations, during the last 20 years (Møller and Szép 2004) to test the prediction that asymmetry decreased as the size of the secondary sexual character increased. Such as decrease would be predicted if tail length reliably reflected the ability of an individual to cope with adverse environmental conditions. The increase in tail length of male barn swallows during the period 1984-2004 is linked to a dramatic increase in male mortality during spring migration in Algeria (Møller and Szép 2004). There is little evidence that the increase in male tail length can be explained by a temporal change in phenotypic plasticity, since variability in tail length of the same individuals across molts decreased significantly during the study (Møller and Szép 2004). An increase in variability would be expected if plasticity accounted for the temporal change in phenotype. Furthermore, the observed change in tail length was very similar to that predicted from the breeders' equation using available estimates of heritability of tail length and field estimates of intensity of selection (Møller and Szép 2004). There is no evidence that environmental conditions during breeding, migration or in winter have ameliorated during the period 1984-2004 (Møller and Szép 2004). Since long outermost tail feathers of male barn swallows are costly in terms of viability, as revealed by a number of field experiments (Møller 1989; Møller and de Lope 1994), we might pose the alternative hypothesis that the amount of stress imposed by the secondary sexual character increased as its size increased. If that was the case, we would predict an increase in tail asymmetry as tail length increased across generations.

The specific objectives of this study were to test (1) whether composite asymmetry in morphology increased as a consequence of an increase in the adversity of environmental conditions, and (2) whether composite asymmetry decreased as a consequence of a micro-evolutionary increase in tail length of male barn swallows. More specifically, I first tested whether asymmetry of two morphological characters was significantly positively correlated. Second, I tested for sex, year and sex by year effects on composite asymmetry. Third, the change in asymmetry during the study period was related to year and mean tail length of male and female cohorts. Fourth, temporal change in degree of phenotypic plasticity for the same individuals when one year old and when two years old was assessed for male and female barn swallows, since any evidence of a temporal trend in phenotypic plasticity would suggest that the temporal changes in asymmetry were caused by changes in phenotypic plasticity. Finally, I investigated how the intensity of natural and sexual selection affected asymmetry by calculating directional and quadratic selection differentials on asymmetry. Since character asymmetry was related to character size, I also estimated selection gradients after accounting for indirect selection acting through selection on the size of the character. Selection differentials were estimated for each year separately, but also for the entire sample to estimate to which extent selection across the study period was overall directional, stabilizing or disruptive. These estimates were compared with estimates derived from the ‘breeders’ equation’ that predicts response to selection from intensity of selection and heritabilty (Falconer and Mackay 1996).

The barn swallow is a small, semi-colonial passerine bird that breeds in most of the Holarctic, while migrating to tropical regions in winter (Møller 1994a). Males attract females to their small breeding territories, where both construct a nest; females incubate and both sexes provision the one to three annual broods of offspring. Males and females are monomorphic in most characters with the exception of the outermost tail feathers that on average are considerably longer in males than in females. Females use the length of the outermost tail feathers as a cue to their mate choice, thereby affecting a number of different components of fitness of males (Møller 1988, 1990, 1993a, 1994a; de Lope and Møller 1993) and females (Møller 1990, 1995). The size of the male secondary sexual character is condition-dependent, being influenced by parasites, environmental conditions (and hence food abundance) in the winter quarters, and senescence (Møller 1990, 1991, 1994a; Møller and de Lope 1999). Tail length of males and females demonstrates fluctuating asymmetry by having a normal frequency distribution of signed left-minus-right character values not deviating from a normal distribution (Møller 1994b; Van Dongen et al. 1999). Increased tail asymmetry develops in response to haematophagous mites, poor foraging conditions in the African winter quarters, radiation and senescence (Møller 1992b, 1993, 1994; Møller and de Lope 1999). Both males and females are significantly repeatable in their tail asymmetry among years (Møller 1994b), with individuals when yearlings and older than four years having slightly, but significantly larger asymmetry than at intermediate ages (Møller and de Lope 1999). Females show a mate preference for males with symmetric tail feathers (Møller 1993a, 1993b, 1994b), and tail asymmetry is negatively correlated with laying date in females and survival prospects in males, even when controlling statistically for male tail length (Møller 1994b).

MATERIALS AND METHODS

Study area

Barn swallows were studied during 1989-2004 at Kraghede 57^{o}12’ N, 10^{o}00’E), Denmark, a site where barn swallows have been studied since 1971 (Møller 1994a). This study area consists of open farmland habitat with scattered mixed plantations, groves and bogs. Barn swallows breed at farms where the number of breeding pairs ranges from single pairs to more than 50.

Measurements

Barn swallows were captured shortly after arrival to the breeding grounds using mist nets with the same capture and measurement procedures being followed in 1989-2004, with all measurements being made by APM, thus avoiding inter-observer variability in measurements. A number of morphological measurements were made for the left and the right side of the body, including the length of the outermost tail feathers and the flattened wing, with a ruler with an accuracy of 1 mm. A small blood sample was collected before banding with numbered aluminum bands and color bands. Individuals were assigned to nests using individual identification.

Farms were visited and nest contents checked at least once per week during the breeding season, which allowed determination of mating status, start of laying of the first clutch, brood size at fledging, and whether a second brood was produced. If an individual disappeared between years, it was considered to be dead since the probability of recapture of surviving individuals exceeded 99% (as determined from the presence of color banded birds that were not recaptured)(Møller and Szép 2002, 2004), and since dispersal of breeding birds between seasons was very limited (Møller 1994a), with only 4 out of 3365 individuals ever changing breeding site.

Tail and wing length was defined as the mean length of the left and the right outermost tail feathers and the left and the right wing, while signed asymmetry was defined as the signed difference in the length of the left and the right character. Absolute asymmetry was defined as the unsigned difference in the length of the left and right characters. More detailed information on methods of data collection and measurements than given above can be found in Møller (1992b, 1994a, b).

Measurement errors may seriously confound estimates of fluctuating asymmetry, but also the size of morphological traits (e. g. Lundström 1960;

Greene 1984). Measurement errors have been estimated blindly for all morphological measurements of the barn swallow, because a number of individuals erroneously were measured twice, since they were unknown to have been captured previously the same season (Møller 1994b). The measurement errors in these tests were small relative to the level of asymmetry (repeatability of tail asymmetry based on two measurements: males: $R = 0.77$, $F = 7.56$, df = 16,32, $P < 0.001$; wing length: $R = 0.84$, $F = 11.90$, df = 16,32, $P < 0.001$; females: $R = 0.89$, $F = 16.63$, df = 16,32, $P < 0.001$; wing length: $R = 0.83$, $F = 10.67$, df = 16,32, $P < 0.001$ (Møller 1994b)). Niels Cadée, who worked in the same population of barn swallows 1996-1998 found a similarly high degree of repeatability of tail asymmetry ($R = 0.95$, $F = 8.00$, df = 36,72, $P < 0.0001$). Measurements of asymmetry in more than 200 pairs of tail feathers of barn swallows collected over the years by students and APM have shown a high degree of inter-observer reliability of asymmetry measurements. The repeatability of signed tail asymmetry measured by a student and APM was very high ($R = 0.95$, $F = 41.81$, df = 235,274, $P < 0.0001$). Similarly, repeated measurements of this set of feathers by APM on two different days revealed a high repeatability ($R = 0.95$, $F = 39.05$, df = 235,274, $P < 0.0001$). In addition, a comparison of asymmetry measured in the field with measurements based on removed tail feathers also provided evidence of a high degree of repeatability ($R = 0.96$, $F = 41.02$, df = 235,274, $P < 0.0001$). Finally, we also assessed the effect of measurement error by estimating unbiased estimates of tail asymmetry using REML analysis (Van Dongen et al. 1999) of a sample of adult barn swallows measured twice during the breeding season 1998. This analysis gave the following results: There was no evidence of directional asymmetry ($F = 1.84$, df = 34, $P = 0.18$). Fluctuating asymmetry was significantly greater than zero ($\chi^2 = 130.05$, df = 1, $P < 0.0001$). REML estimates of unsigned asymmetry were strongly positively correlated with uncorrected, unsigned asymmetry (Pearson product-moment correlation coefficient: $r = 0.94$, df = 33, $P < 0.0001$).

I deliberately excluded any individuals with broken or damaged feathers from the analyses. The tip of the outermost tail feathers of barn swallows is rounded, and since feathers are composed of small barbs, any broken barb immediately leaves an irregular shape of the feather that is readily visible to anyone familiar with birds. Since barn swallows molt their tail feathers in late winter just before returning to the breeding grounds, and since birds were captured shortly upon arrival, the number of birds with damaged tail feathers was less than 3% in any given year. This is unlikely to have seriously biased the samples of birds in any particular way.

All individuals involved in experiments with the exception of untreated controls were excluded from the data sets, but since treatments were assigned randomly, this cannot have caused any bias in the data. Experiments in any given year only involved a small fraction of individuals.

Composite asymmetry

Individual asymmetry of a single character is a poor estimate of underlying developmental instability (Whitlock 1996; Van Dongen 1998; Gangestad and Thornhill 1999, 2003; Leung et al. 2000), and composite asymmetry of two or more characters provides much more reliable estimates of the ability to control developmental processes than asymmetry of any single character (Leung et al. 2000). Therefore, I calculated composite asymmetry by standardizing unsigned asymmetry of wing length and tail length to a mean of zero and a variance of one. These two estimates for each individual were then summed to obtain an overall estimate of composite asymmetry, subsequently standardizing this sum to a standard normal deviate (with mean of zero and a variance of one). This procedure provides equal weight to asymmetry in two or more characters without biasing the composite estimate (Leung et al. 2000). Composite size was estimated as the sum of the standard normal deviate of tail length and the standard normal deviate of wing length, subsequently standardizing this sum to a standard normal deviate (with mean of zero and a variance of one).

Phenotypic plasticity in asymmetry

Phenotypic plasticity in asymmetry was estimated from composite asymmetry of an individual when one year old minus composite asymmetry of the same individual when two years old. This measure of change in asymmetry with age provides an estimate of within-individual plasticity in asymmetry between molts. Mean plasticity in asymmetry was then calculated for each year, and the regression of mean plasticity in asymmetry on year provided a statistical test of the null hypothesis that plasticity did not change during the study period.

Selection analyses

I analyzed selection using the approach developed by Lande and Arnold (1983) and Arnold and Wade (1984). Briefly, intensity of directional selection on phenotype can be estimated from linear regression models. The dependent variable is a standardized measure of fitness, where fitness during a given selection episode is divided by mean fitness to obtain a relative estimate of fitness. Phenotypic characters are standardized to a mean of zero and a variance of one before selection. Estimated regression coefficients for phenotype provide estimates of selection acting on the trait. The statistical significance of selection was derived from the significance of the linear regression models. While selection differentials estimate selection on the phenotype in question, selection gradients provide estimates of selection after controlling for selection on correlated characters (Lande and Arnold 1983). Since asymmetry is sometimes correlated with character size in the barn swallow (Møller 1994b), I entered both composite asymmetry and a measure of composite size, as described above, in multiple linear regression analyses. I used selection differentials and selection gradients as estimates of effect size simply because they have properties of standardization similar to Hedges' d in meta-analysis (e. g. Hedges and Olkin 1985; Rosenthal 1991, 1994). Even without statistical significance, such a standardized measure of effect size will provide the best estimate of the strength of a relationship.

The selection episodes and the fitness components considered here are the following: (1) Mating selection reflect the ability to acquire a mate (in males only since unmated females were never recorded). Mated individuals were assigned a fitness of one and unmated individuals a fitness of zero. (2) Fecundity selection was quantified as the total number of fledglings produced in a year. (3) Survival selection was whether an individual survived or not, as reflected by recapture or re-sighting of individuals. This procedure of using recaptures and re-sightings as a substitute for survival is justified by annual recapture rates of barn swallows in this study exceeding 98% (see Møller and Szép 2002, 2004).

The use of number of total number of fledglings per year as a fitness measure requires justification for male barn swallows, since approximately 30% of all nestlings are extra-pair offspring (Møller and Tegelström 1997; Saino et al. 1997). Hence, it seems likely that estimates of selection based on simple counts of number of offspring may be biased. However, Saino et al. (1997) have shown that there is a strong positive correlation between the number of offspring fathered in the own nest and the number of offspring fathered elsewhere. This demonstrates that male barn swallows losing paternity in their own nest do not simply gain paternity elsewhere, thereby compensating for the loss of paternity in their own nest. These arguments suggest that the overall magnitude of selection in males for these three fitness components may be biased, although extra-pair paternity only seems likely to increase the variance in fitness derived from the number of nestlings in the own nest.

Statistical analyses

Sample sizes per year varied between 28 and 134 in males, with a total of 1376 males, and between 35 and 148 in females, with a total of 1465 females. Since not all information was available for all individuals, sample sizes sometimes differ among tests.

I tested for sex, year and sex by year effects on composite asymmetry using a two-way factorial ANOVA. The relationship between annual mean and variance in composite asymmetry and year was explored using linear regression analysis. Likewise, the relationship between composite asymmetry and mean tail length was investigated using linear regression analysis.

I quantified the relationship between tail length and tail asymmetry and composite asymmetry, respectively, using the Pearson correlation coefficient as an estimate of effect size. Separate analyses for the two sexes were used to estimate the relationship between asymmetry and character size.

The table-wide significance levels were adjusted to 5% for the number of tests made using Bonferroni-correction (Holm 1979). Strict application of this method severely reduces the power of tests (Wright 1992), but such sacrificial loss of power can be avoided by choosing an experimentwise error rate higher than the usually accepted 5%. We used 10% as suggested by Wright (1992) and Chandler (1995).

Values reported are means (SE).

RESULTS

Character asymmetry and composite asymmetry

Summary statistics for asymmetry and composite asymmetry are reported in Table 1. First, I tested whether unsigned wing and tail asymmetry were positively correlated, as expected if they reflected the same underlying developmental control mechanisms. In males there was a weak but significant positive relationship (r = 0.06, t = 2.23, d.f. = 1375, P = 0.02), while the relationship did not reach significance in females (r = 0.03, t = 1.15, d.f. = 1465, P = 0.26). This implies that the two measures of asymmetry are virtually statistically independent, justifying their use in a composite asymmetry index.

TABLE 1 ABOUT HERE

Temporal change in asymmetry

A two-way ANOVA on composite asymmetry revealed a significant sex effect (Table 2; mean (SE) males: 0.04 (0.03), N = 1376, females; -0.04 (0.03), N = 1465), and a significant year effect, but no sex by year interaction (Table 1).

TABLE 2 ABOUT HERE

Composite asymmetry decreased significantly during the period 1989-2004 in both males and females (Fig. 1A). Likewise variance in composite asymmetry decreased significantly during the study period (Fig. 1B).

FIG. 1 ABOUT HERE

Estimates of phenotypic plasticity in asymmetry for individuals from age one year old to age two years was available for 382 individuals. An ANOVA revealed a marginally significant effect of sex, while the effects of year and sex by year were far from statistically significant (Table 3). There was no consistent trend in phenotypic plasticity during the study period (Fig. 2).

TABLE 3 AND FIG. 2 ABOUT HERE

Change in asymmetry with change in phenotype

Composite asymmetry decreased as tail length increased, although the relationship was only significant for males (Fig. 3). The regression for males explained 43% of the variance in mean asymmetry among years.

FIG. 3 ABOUT HERE

The relationship between tail length and absolute asymmetry changed during the study period from significantly negative to close to zero in males, while there was no significant change in females (regression analysis with year as the independent variable, based on z-transformed correlation coefficients: males: $F = 11.17$, d.f. = 1,14, $r^2 = 0.44$, $P = 0.005$, slope (SE) = 0.02 (0.01); females: $F = 1.48$, d.f. = 1,14, $r^2 = 0.10$, $P = 0.25$). The relationship between tail length and absolute asymmetry changed with increasing mean tail length of each cohort in males, but not in females (analysis based on z-transformed correlation coefficients: males: $F = 4.37$, d.f. = 1,14, $r^2 = 0.24$, $P = 0.04$, slope (SE) = 0.04 (0.02); females: $F = 2.16$, d.f. = 1,14, $r^2 = 0.13$, $P = 0.16$). The relationship between tail length and absolute asymmetry changed with decreasing composite asymmetry of each cohort of males, but not of females (Fig. 4). A polynomial regression for the data on females in Fig. 4 showed that only the quadratic term reached significance ($F = 8.67$, d.f. = 2,13, $r^2 = 0.57$, $P = 0.004$, slope (SE) for linear term: -0.07 (0.13), $t = 0.54$, $P = 0.60$, slope (SE) for quadratic term: -2.61 (0.63), $t = 4.12$, $P = 0.001$).

FIG. 4 ABOUT HERE

Selection on asymmetry and change in asymmetry

Based on the 16 years of data we found five (6%) significant directional selection differentials out of 78 possible (Table 4). After controlling for selection on size of the characters, only four (5%) selection gradients were statistically significant. After sequential Bonferroni correction for multiple tests only one selection differential remained significant: There was significant directional selection on tail asymmetry with respect to mating success in 1997. Mate choice tended to select against asymmetry since 14 of 16 selection differentials and selection gradients were negative, differing significantly from a random distribution (binomial test, $P = 0.004$), while that was not the case for the other selection episodes. Likewise, the mean selection differential for mating success differed significantly from zero (mean (SE) = -0.032 (0.008); one-sample t-test, $t = 3.92$, d.f. = 15, $P = 0.001$), while that was not the case for any of the other selection differentials. There was little evidence of changes in patterns of selection during the course of the study with no significant temporal trends, suggesting that temporal trends in selection cannot account for the temporal change in composite asymmetry that I documented.

TABLE 4 ABOUT HERE

Estimates of overall selection based the entire data set revealed statistically significant directional mating selection and fecundity selection in males, while none of the other components of selection were statistically significant (Table 5). Only the former remained significant after Bonferroni correction. Selection gradients were only significant for mating selection in males (Table 5), even after sequential Bonferroni correction for multiple tests. Therefore, this overall analysis revealed a conclusion similar to that based on analyses for individual years.

TABLE 5 ABOUT HERE

The change in composite asymmetry of males from 1989 to 2004 was 0.4, and since the variance in composite asymmetry in 1989 was 1.31, this implies that composite asymmetry decreased by 0.31 standard deviation units. In females the change was 0.37, and since the variance in composite asymmetry was 1.44, this implies that composite asymmetry changed by 0.26 SD. If we use the mean estimate of change in composite asymmetry in the two sexes, which is (0.31 + 0.26) / 2 = 0.285, we can test if the change in asymmetry is within the limits expected from available estimates of heritability of developmental instability from the literature and from estimates of the intensity of selection, using the breeders' equation $R = S \times h^2$, where R is response to selection, S is intensity of selection and h^2 is heritability (Falconer and Mackay 1996). I assume that the heritability of developmental instability is 0.35 to 0.55, with a mean of 0.45 (Gangestad and Thornhill 1999, 2003). I can estimate generation time as $T = A + P / (1 – P)$, where A is age at first reproduction (1 year (Møller 1994)) and P is annual survival rate (0.343 (Møller and Szép 2002)). This gives T = 1.522, and since the study period was 16 years, this equals 10.512 generations. With the change in phenotype being 0.285 SD, total net selection should have been 0.285 / 0.45 = 0.633 or an intensity of selection of 0.060 per generation. This intensity of selection is of a similar order of magnitude as the intensities of selection reported in Table 5.

DISCUSSION

The main findings of this study were that (1) two measures of developmental instability were very weakly positively correlated; (2) a measure of composite asymmetry differed significantly between sexes and years; (3) mean and variance in composite asymmetry decreased during 1989-2004; (4) phenotypic plasticity in asymmetry did not change during 1989-2004; (5) mean composite asymmetry decreased as tail length of males increased by 1.2 standard deviations during 1989-2004; (6) the relationship between tail asymmetry and tail length in males changed from significantly negative to flat during 1989-2004; and (7) directional selection differentials and gradients for mating, fecundity and viability selection were all weak, with only selection during mating consistently acting against asymmetric males.

There was a strong decrease in composite asymmetry during 1989-2004 in both sexes. This change in asymmetry was related to change in tail length since composite asymmetry of males decreased as male tails became longer, while that was not the case for females. Asymmetry was predicted in the Introduction to change in either of two ways as a response to change in a secondary sexual character. First, if the size of a secondary sexual character reliably reflects that ability of individuals to cope with perturbations during development (Møller 1990), we should expect that an increase in the size of a secondary sexual character to be associated with a decrease in overall asymmetry. Second, secondary sexual characters are exaggerated beyond the optimum under natural selection, with their costs of production and maintenance potentially affecting their degree of developmental instability (Møller and Pomiankowski 1993). If this hypothesis was correct, we should expect asymmetry to increase as the size of a secondary sexual character increased. The results presented here are consistent with the first hypothesis that the size of a secondary sexual character reliably reflects the ability of control development of feathers during molt, while the second hypothesis is refuted. The findings do not support the second hypothesis that as tail length increased, there was a decrease in developmental control. If the environment had ameliorated, we should also expect asymmetry to decrease over time. However, there is empirical evidence suggesting that environmental conditions have deteriorated over time (Møller and Szép 2004). Furthermore, annual adult survival rate of males has been reduced by a factor two since 2004 (Møller and Szép 2004), suggesting that it is unlikely that the environment has ameliorated. Thus, there is no empirical evidence suggesting that the decrease in asymmetry is due to an amelioration of environmental conditions.

The temporal change in composite asymmetry could be due to a temporal change in phenotypic plasticity in asymmetry, a temporal change in the intensity of selection against asymmetric individuals, or a micro-evolutionary change in the ability to control developmental processes that affect the development of small asymmetries. Phenotypic plasticity in composite asymmetry could be quantified in the present study because individuals undergo a molt each year, thereby reproducing the development of feathers annually. In fact, individual barn swallows are significantly repeatable in their ability to re-produce asymmetry, since degree of asymmetry is consistent among molts, even when individuals are forced to re-grow their feathers during summer by pulling out feathers (Møller 1994b 1996). Change in composite asymmetry between years showed evidence of phenotypic plasticity differing marginally between the sexes, while there was no evidence of temporal change in phenotypic plasticity of composite asymmetry. These analyses suggest that it is unlikely that the temporal decrease in composite asymmetry can be accounted for by a temporal decrease in phenotypic plasticity. I am only aware of two other studies investigating temporal trends in asymmetry. Manning and Chamberlain (1994) investigated temporal trends in dental asymmetry of teeth from museum specimens of the gorilla *Gorilla gorilla*, and they found evidence of a dramatic increase that was interpreted to reflect a deterioration in environmental conditions experienced by gorilla. Badyaev (1998) in a study of grizzly bears *Ursus arctos horribilis* from Yellowstone National Park showed that detail asymmetry increased dramatically following closure of garbage dumps. However, neither of these studies provided information on the relative importance of phenotypic plasticity or micro-evolutionary change. This leaves open two other possibilities: (1) Composite asymmetry has changed during 1989-2004 as a consequence of a change in the intensity of selection against asymmetric individuals, or (2) composite asymmetry has changed as a consequence of a change in the underlying developmental control mechanisms responsible for preventing the development of asymmetries. I will discuss these two possibilities.

The present case study is interesting because environmental conditions during spring migration have deteriorated, causing an increase in the intensity of viability selection in males and a dramatic increase in adult male mortality rate (Møller and Szép 2004). This increase in annual mortality rate could potentially cause more intense selection against asymmetry, thereby reducing the population level of asymmetry. However, patterns of survival selection were not related to composite asymmetry, and there was no clear temporal trend in directional viability selection against composite asymmetry in either males or females. There was little evidence of directional selection acting on composite asymmetry, with mate selection in males being the only clear exception. The importance of mate choice as a factor affecting composite asymmetry is consistent with previous descriptive studies (Møller 1990, 1994b) and experiments on the barn swallow (Møller 1992a, 1993b) that have consistently shown sexual selection to be related to tail asymmetry. The lack of evidence of selection significantly affecting composite asymmetry was not due to the confounding effects of indirect selection acting on a phenotypic character that was closely related to composite asymmetry. Patterns of directional selection on composite asymmetry based on selection differentials were very similar to patterns based on selection gradients controlling for selection on size of morphological characters, suggesting that size was not an important confounding variable in these estimates. I am unaware of any other long-term study of selection on asymmetry.

Could the change in composite asymmetry of the barn swallow be attributed to a micro-evolutionary change in developmental instability? Heritability of developmental instability was estimated by (Møller and Thornhill 1997; see all commentaries to this paper in the same issue) to be 0.19. Whitlock and Fowler (1997) provided a more conservative estimate of 0.025 after removing studies that could be biased due to maternal effects. Since then several additional studies have investigated this question empirically (e. g. Blanckenhorn et al. 1998; Woods et al. 1998; Cadée 2000; Polak and Starmer 2001; Polak et al. 2004; reviews in Gangestad and Thornhill 1999; Van Dongen 2000, Fuller and Houle 2003). Van Dongen (2000) obtained a mean Bayesian hierarchical estimate of 0.046 across studies. Fuller and Houle (2003) in the so far most extensive review of this literature obtained an estimate of 0.03. If the repeatability of fluctuating asymmetry is 0.072, as estimated by Gangestad and Thornhill (1999) in their extensive review of the literature, then the heritability of developmental instability would be 0.35 to 0.55 depending on the estimate of a single trait's fluctuating asymmetry. The change in mean composite asymmetry during the study was 0.31 SD in males and 0.26 in females. Using the mean of these two estimates, the estimates of heritability of developmental instability from the literature, and the number of generations elapsed, I estimated an intensity of selection per generation of 0.06. This value of the required intensity of net selection is of a similar magnitude as the intensities of selection reported in Table 5. This exercise suggests that observed and predicted values do not deviate considerably from each other. Obviously, these calculations are based on a literature estimate of the heritability of developmental instability that so far have not been estimated specifically for the barn swallow. The only conclusion that I can draw is that currently available estimates are consistent with a micro-evolutionary change in developmental instability.

The decrease in composite asymmetry during 1989-2004 was associated with an increase in mean tail length of males, but not significantly so in females. At the start of the study in 1989 males with long tails generally had the smallest degree of tail asymmetry, but also the smallest composite asymmetry, while males all had small degrees of asymmetry independent of tail length at the end of the study in 2004. Thus, asymmetry was independent of size of the secondary sexual character at the end of the study. This finding suggests that the temporal change in the relationship between asymmetry and size has occurred as a consequence of asymmetric phenotypes being eliminated from the population. If this is the case, we should expect the variance in asymmetry to decrease with increasing tail length, as was actually observed (Fig. 1B). Therefore, temporal change in composite asymmetry seems to be directly linked to a change in the size of a secondary sexual character, with selection on tail length having improved the ability of individuals to cope with stressful conditions that can upset control of developmental processes. I am only aware of one other study of showing a decrease in asymmetry due to the integration of an initially disruptive pesticide resistance allele into the genome of Australian blowflies *Lucilia cuprina* (Clarke and McKenzie 1987; Davies et al. 1996). If the micro-evolutionary interpretation is correct, we should expect a number of other measures of ability to cope with stress to have changed since 1989. In particular, we should expect a change in corticosterone concentration in response to a standard challenge. Furthermore, we should expect a temporal change in induction of heat shock proteins since 1989. I am currently testing the second possibility.

In conclusion, a long-term study of composite asymmetry in barn swallows revealed evidence of a strong temporal decrease in asymmetry during a period of only 16 years. This decrease was closely associated with an increase in the size of a secondary sexual character. There was no evidence suggesting that the temporal pattern of asymmetry could be accounted for by temporal changes in phenotypic plasticity or temporal changes in intensity of selection. This leaves the possibility that the observed change in composite asymmetry is a consequence of a micro-evolutionary change in the ability to cope with problems that disrupt stable development of the phenotype.

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Legends to figures

Fig. 1. Temporal change in (A) relative composite asymmetry and (B) variance in relative composite asymmetry in male and female barn swallows during the period 1989-2004. The lines are the linear regression lines (full line – males, hatched line – females). For mean male asymmetry $F = 49.86$, d.f. = 1,14, $r^2 = 0.78$, $P < 0.0001$, slope (SE) = -0.03 (0.004), and for mean female asymmetry $F = 12.02$, d.f. = 1,14, $r^2 = 0.46$, $P = 0.004$, slope (SE) = -0.03 (0.01). For variance in male asymmetry $F = 8.42$, d.f. = 1,14, $r^2 = 0.38$, $P = 0.01$, slope (SE) = -0.07 (0.03), and for variance in female asymmetry $F = 15.89$, d.f. = 1,14, $r^2 = 0.53$, $P = 0.001$, slope (SE) = -0.07 (0.02).

Fig. 2. Phenotypic plasticity in asymmetry of individuals when aged one year and two years expressed as the mean change in asymmetry (± SE). The linear regressions have the statistics: males: $F = 0.32$, d.f. = 1,13, $r^2 = 0.02$, $P = 0.58$; females: $F = 0.24$, d.f. = 1,13, $r^2 = 0.02$, $P = 0.63$.

Fig. 3. Relative composite asymmetry (± SE) of (A) male and (B) female barn swallows in relation to annual mean tail length of different cohorts. The lines are the linear regression lines. For mean male asymmetry $F = 10.37$, d.f. = 1,14, $r^2 = 0.43$, $P = 0.006$, slope (SE) = -0.05 (0.02), and for mean female asymmetry $F = 1.29$, d.f. = 1,14, $r^2 = 0.08$, $P = 0.27$.

Fig. 4. Relationship between asymmetry and length of tail feathers in male and female barn swallows in relation to mean composite asymmetry of different cohorts during the period 1989-2004. The line is the linear regression line for males, with the statistics, based on z-transformed correlation coefficients: $F = 8.30$, d.f. = 1,14, $r^2 = 0.37$, $P = 0.01$, slope

(SE) = -0.70 (0.24). The relationship for females was not significant (F = 0.21, d.f. = 1,14, r^2 = 0.02, P = 0.65).

Fig. 1

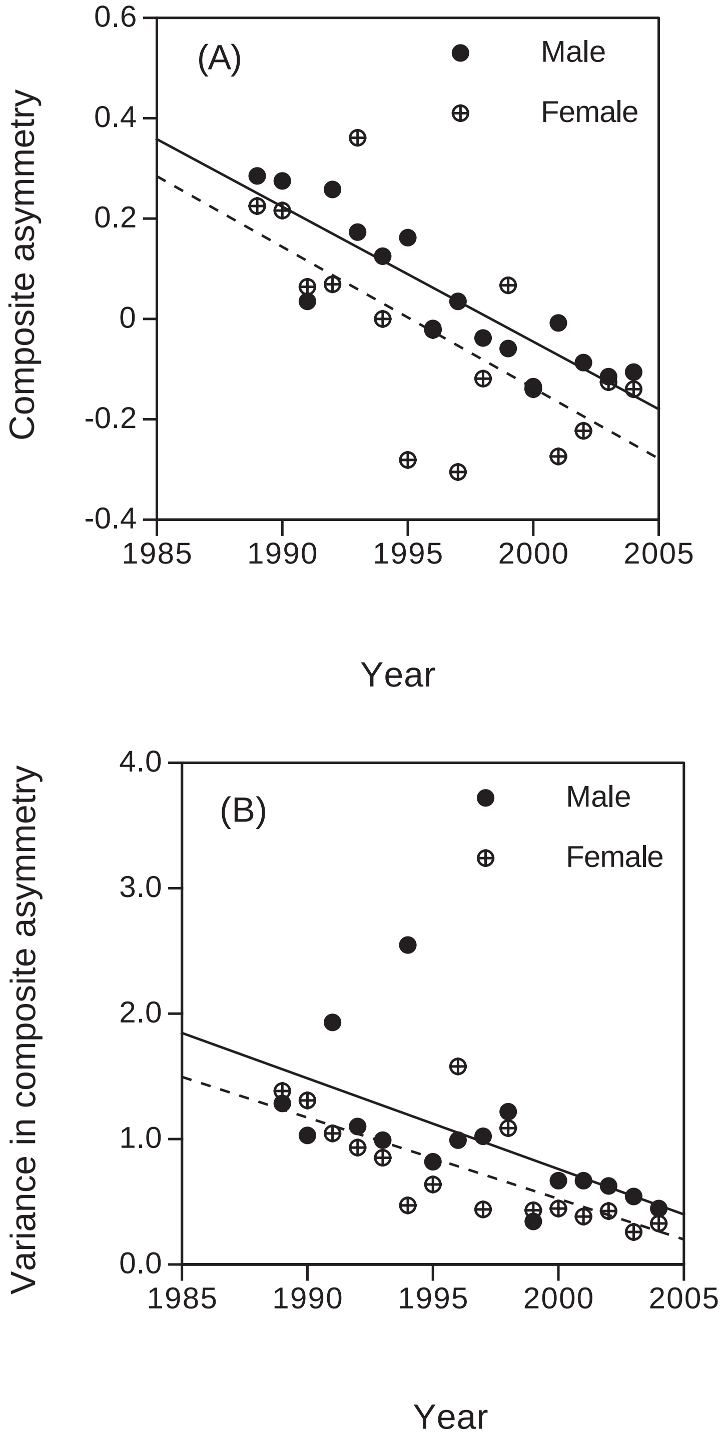
(A)
Male
Female
Composite asymmetry
0.6
0.4
0.2
0
-0.2
-0.4
1985
1990
1995
2000
2005
Year
(B)
Male
Female
Variance in composite asymmetry
4.0
3.0
2.0
1.0
0.0
1985
1990
1995
2000
2005
Year

Fig. 2

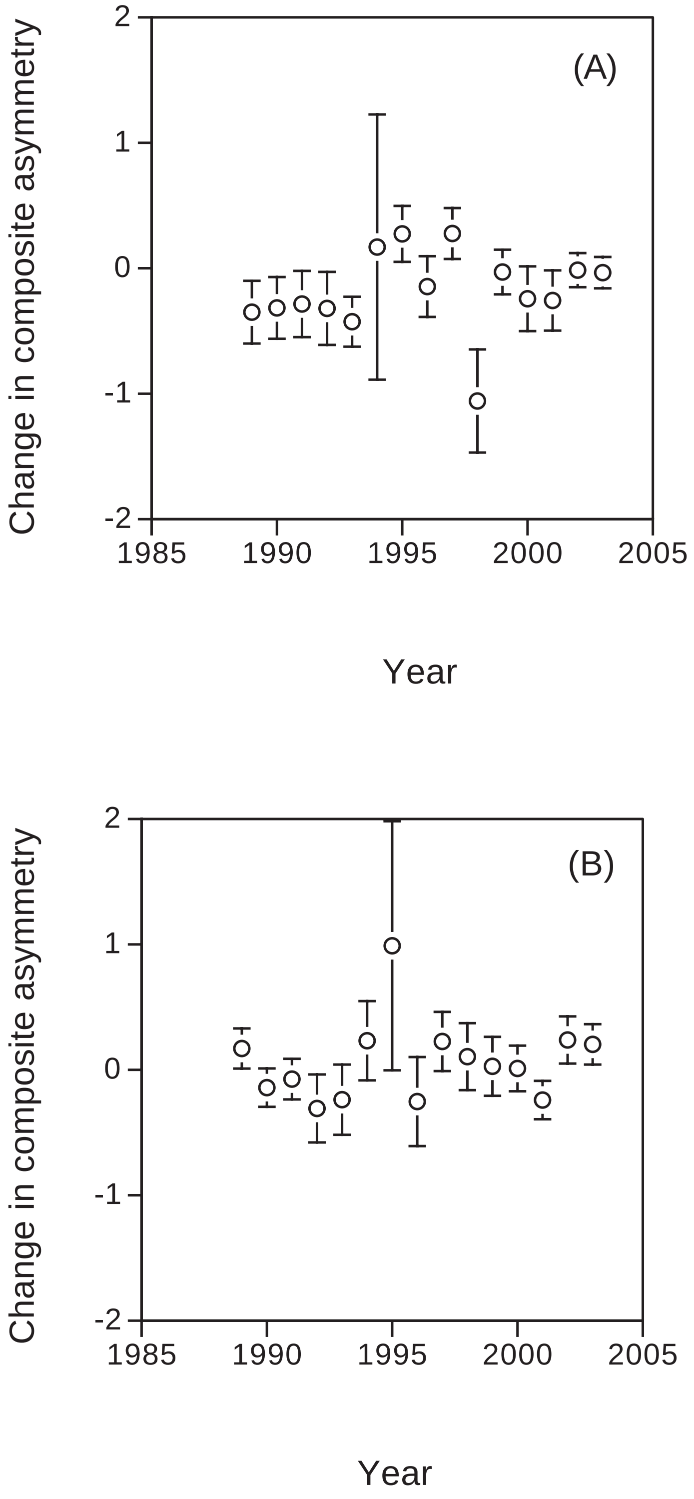
(A)
Change in composite asymmetry
2
1
0
-1
-2
1985
1990
1995
2000
2005
Year
(B)
Change in composite asymmetry
2
1
0
-1
-2
1985
1990
1995
2000
2005
Year

Fig. 3

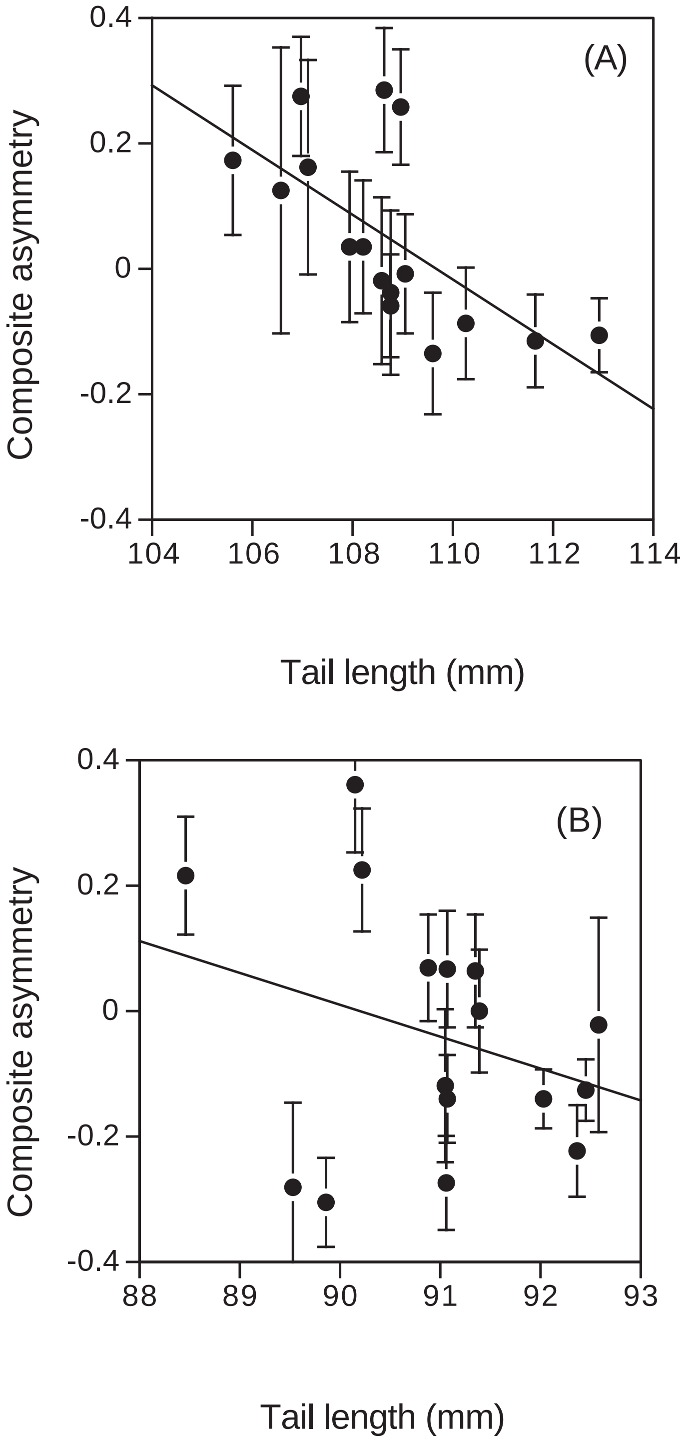
(A)
Composite asymmetry
0.4
0.2
0
-0.2
-0.4
104
106
108
110
112
114
Tail length (mm)
(B)
Composite asymmetry
0.4
0.2
0
-0.2
-0.4
88
89
90
91
92
93
Tail length (mm)

Fig. 4

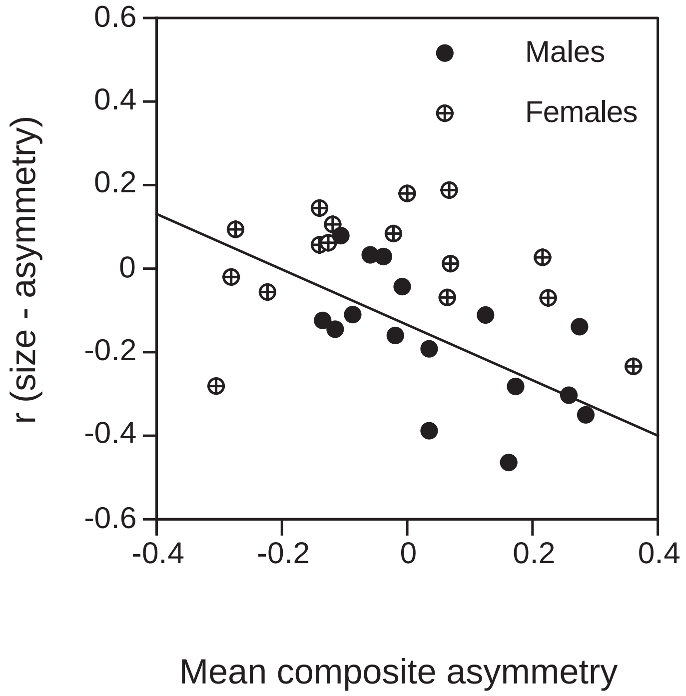
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.4
-0.2
0
0.2
0.4
● Males
⊕ Females
r (size - asymmetry)
Mean composite asymmetry

Table 1. Summary statistics for asymmetry of adult barn swallows for each of the years 1989-2004. Composite asymmetry is calculated as the standard score of the sum of the standard score for signed tail asymmetry and the standard score for signed wing asymmetry.

	Males					Females				
Year	Unsigned tail asymmetry (mm) (SE)	Unsigned wing asymmetry (mm) (SE)	*N*	Composite asymmetry (SE)	Variance in composite asymmetry	Unsigned tail asymmetry (mm) (SE)	Unsigned wing asymmetry (mm) (SE)	*N*	Composite asymmetry (SE)	Variance in composite asymmetry
1989	2.42 (0.30)	0.45 (0.05)	131	0.29 (0.10)	1.28	1.68 (0.20)	0.50 (0.05)	144	0.23 (0.10)	1.38
1990	2.16 (0.28)	0.58 (0.07)	114	0.28 (0.10)	1.03	1.95 (0.23)	0.65 (0.06)	147	0.22 (0.09)	1.31
1991	2.80 (0.54)	0.41 (0.05)	134	0.04 (0.12)	1.93	1.56 (0.24)	0.55 (0.05)	129	0.06 (0.09)	1.05
1992	1.85 (0.21)	0.59 (0.06)	130	0.26 (0.09)	1.10	1.97 (0.21)	0.44 (0.05)	129	0.07 (0.09)	0.93
1993	2.44 (0.42)	0.43 (0.07)	70	0.17 (0.12)	0.99	1.71 (0.28)	0.49 (0.08)	73	0.36 (0.11)	0.85
1994	4.00 (1.23)	0.26 (0.08)	49	0.13 (0.23)	2.55	1.49 (0.24)	0.33 (0.07)	49	0.00 (0.10)	0.47
1995	3.93 (2.61)	0.43 (0.11)	28	0.16 (0.17)	0.82	1.31 (0.41)	0.34 (0.09)	35	-0.28 (0.14)	0.64
1996	2.04 (0.33)	0.49 (0.08)	56	-0.02 (0.13)	0.99	1.80 (0.32)	0.74 (0.10)	54	-0.02 (0.17)	1.58
1997	1.91 (0.30)	0.47 (0.06)	91	0.04 (0.11)	1.02	2.35 (0.74)	0.55 (0.06)	87	-0.31 (0.07)	0.44
1998	2.48 (0.39)	0.73 (0.07)	71	-0.04 (0.13)	1.22	1.38 (0.15)	0.69 (0.07)	73	-0.12 (0.12)	1.09
1999	1.55 (0.26)	0.22 (0.06)	51	-0.06 (0.08)	0.34	1.58 (0.25)	0.31 (0.06)	50	0.07 (0.10)	0.43

2000	1.79 (0.36)	0.23 (0.05)	71	-0.14 (0.10)	0.67	1.26 (0.18)	0.24 (0.04)	91	-0.14 (0.07)	0.45
2001	1.65 (0.24)	0.26 (0.05)	74	-0.01 (0.10)	0.67	1.44 (0.24)	0.25 (0.05)	68	-0.27 (0.08)	0.38
2002	1.92 (0.27)	0.20 (0.04)	79	-0.09 (0.09)	0.63	1.61 (0.25)	0.19 (0.04)	80	-0.22 (0.07)	0.43
2003	1.65 (0.29)	0.15 (0.03)	99	-0.12 (0.07)	0.54	1.49 (0.19)	0.10 (0.03)	108	-0.13 (0.05)	0.26
2004	1.91 (0.20)	0.11 (0.03)	128	-0.11 (0.06)	0.45	1.12 (0.16)	0.14 (0.03)	148	-0.14 (0.05)	0.33

Table 2. Two-way analysis of variance with composite asymmetry as dependent variable and sex, year and sex by year interaction as factors.

Factor	MS	d.f.	*F*	*P*
Sex	4.34	1	4.34	0.037
Year	3.65	15	3.65	< 0.0001
Sex x Year	0.83	15	0.83	0.59
Residual	1.00	2809		

Table 3. Two-way analysis of variance with change in composite asymmetry between age one year and age two years as dependent variable and sex, year and sex by year interaction as factors.

Factor	MS	d.f.	*F*	*P*
Sex	3.88	1	3.81	0.052
Year	1.38	15	1.29	0.13
Sex x Year	0.83	15	0.37	0.97
Residual	1.02	350		

Table 4. Directional selection differentials (directional selection gradients in parentheses after controlling statistically for composite size) for composite asymmetry with mating success, fecundity and survival as fitness components for adult male and female barn swallows for each of the years 1989-2004. For further details, see Material and Methods.

	Males			Females	
Year	Mating	Fecundity	Survival	Fecundity	Survival
1989	-0.021 (-0.019)	-0.061 (-0.058)	-0.068 (-0.065)	-0.019 (0.017)	-0.068 (-0.066)
1990	-0.018 (-0.012)	-0.064 (-0.063)	-0.065 (-0.063)	0.016 (0.014)	-0.071 (-0.074)
1991	-0.042** (-0.040**)	-0.150** (-0.133*)	0.115 (0.148)	-0.072 (-0.072)	0.113 (0.111)
1992	-0.039 (-0.025)	0.031 (0.067)	0.259 (0.202)	-0.030 (-0.030)	0.198 (0.187)
1993	0.015 (0.030)	0.069 (0.073)	-0.009 (-0.127)	-0.027 (-0.007)	-0.141 (-0.139)
1994	-0.017 (-0.006)	-0.044 (-0.031)	0.132 (0.131)	0.068 (0.068)	-0.300 (-0.299)
1995	-0.053 (-0.045)	-0.030 (-0.021)	-0.181 (-0.172)	0.028 (0.042)	0.023 (0.011)
1996	-0.084 (-0.076)	-0.019 (-0.008)	0.354 (0.344)	-0.014 (-0.015)	0.018 (0.089)
1997	-0.109*** (-0.103***)	-0.135 (-0.132)	0.019 (0.020)	-0.039 (-0.039)	0.028 (0.032)
1998	-0.027 (-0.030)	-0.021 (-0.066)	0.017 (-0.010)	-0.024 (-0.024)	-0.604* (-0.624*)

1999	-0.004 (-0.002)	0.090 (0.087)	0.044 (0.036)	0.011 (-0.006)	-0.167 (-0.177)
2000	0.002 (0.007)	0.020 (0.043)	0.066 (0.064)	0.047 (0.067)	0.125 (0.144)
2001	-0.032 (-0.034)	-0.098 (-0.098)	0.158 (0.155)	0.083 (0.081)	0.245 (0.234)
2002	-0.013 (-0.002)	-0.002 (0.033)	-0.323 (-0.302)	0.090 (0.101)	-0.366 (-0.395)
2003	-0.059 (-0.054)	-0.136* (-0.119)	0.020 (0.017)	0.020 (0.024)	0.119 (0.120)
2004	-0.006 (-0.003)	0.043 (0.057)	-	0.063 (0.067)	-

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$

Table 5. Directional selection differentials and directional selection gradients for composite asymmetry (gradients after controlling statistically for composite size) based on all data for the period 1989-2004. For further details, see Material and Methods.

Selection episode	Directional selection differential	Directional selection gradient	*N*
Males			
Mating	-0.025**	-0.018**	1226
Fecundity	-0.042*	-0.028	1226
Survival	0.054	0.052	1076
Females			
Fecundity	-0.012	-0.011	1134
Survival	-0.050	-0.043	905

* $P < 0.05$, ** $P < 0.001$