

Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*)

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The causes and magnitude of inbreeding depression are of considerable importance for a wide range of issues in evolutionary and conservation biology, but we have only a limited understanding of inbreeding depression in natural populations. Here, we present a study of inbreeding in a large wild population of collared flycatchers (*Ficedula albicollis*). Inbreeding was rare, to the extent that we detected only 1.04% of 2139 matings over 18 years that resulted in offspring with a non-zero inbreeding coefficient, $f > 0$. When it did occur, inbreeding caused a significant reduction in the egg-hatching rate, in fledgling skeletal size and in post-fledging juvenile survival, with the number of offspring being recruited to the breeding population from a nest of $f = 0.25$ being reduced by 94% relative to a non-inbred nest. A maximum-likelihood estimate of the number of lethal equivalents per gamete was very high at $B = 7.47$, indicating a substantial genetic load in this population. There was also a non-significant tendency for inbreeding depression to increase with the strength of selection on a trait. The probability of mating between close relatives ($f = 0.25$) increased throughout the breeding season, possibly reflecting increased costs of inbreeding avoidance. Our results illustrate how severe inbreeding depression and considerable genetic load may exist in natural populations, but detecting them may require extensive long-term datasets.

Keywords: *Ficedula*; genetic load; inbreeding avoidance; lethal equivalents; dominance variance; mate choice

1. INTRODUCTION

Although inbreeding depression plays a central role in evolutionary theory, detailed analyses of its realized effects in natural populations are rare. Inbreeding depression, or the loss of fitness due to matings between relatives, affects numerous aspects of biological systems, such as the evolution of mating patterns and dispersal behaviour (Charlesworth & Charlesworth 1987; Pusey 1987), the genetic architecture of quantitative traits (de Rose & Roff 1999) and the maintenance of genetic variation (Charlesworth & Hughes 1999). It also has important consequences for the conservation of small isolated populations (Soulé 1986) and the captive breeding of endangered species (Ralls *et al.* 1988). An extensive literature exists documenting its effects in laboratory or zoo populations, in which the fitness consequences of an array of artificially constructed mate combinations can be assessed (e.g. see reviews by Soulé 1986; Hedrick & Kalinowski 2000). However, despite a general effect of a reduction in fitness due to inbreeding, patterns of inbreeding depression will vary as a function of a population's environment or genetic make-up, and of any interaction between the two (Hedrick & Kalinowski 2000). Observations from captive populations therefore have only lim-

ited relevance for our understanding of inbreeding depression in wild populations in natural environments.

Studies of inbreeding depression in natural environments are rare because several factors combine to make quantifying inbreeding in the wild difficult. Firstly, establishing the relatedness between individuals in a breeding pair requires either reliable pedigree knowledge or inferences from genetic information, in particular levels of heterozygosity (e.g. Slate *et al.* 2000; Amos *et al.* 2001). Secondly, complete life-history data are generally rare for free-living individuals, with the result that most studies have concentrated on juvenile traits (e.g. see review in Crnokrak & Roff 1999). Finally, if inbreeding occurs at very low frequencies—possibly due to the evolution of mechanisms preventing it—or if its effects are subtle, the sample sizes that are typical of studies of natural populations may not afford sufficient statistical power to detect any effect.

Long-term studies of bird populations, in which marked individuals are monitored through all breeding attempts, provide a valuable opportunity to quantify levels of both inbreeding and inbreeding depression under natural conditions. To date, the most extensive studies of inbreeding in birds have involved isolated populations on small islands with relatively high rates of inbreeding (e.g. van Noordwijk & Scharloo 1981; Gibbs & Grant 1989; Keller 1998; Grant *et al.* 2001; Keller *et al.* 2002). In this paper, we consider the occurrence and consequences of inbreeding in a large wild population of collared flycatchers

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(*Ficedula albicollis*) in one part of the island of Gotland, Sweden, in which, as we show, inbreeding is apparently rare. We quantified the effects of inbreeding on a range of traits related to fitness. Recent evidence indicates that inbreeding depression is greater for traits more closely tied to fitness than for those under weaker selection (de Rose & Roff 1999; Slate *et al.* 2002). We therefore tested for a relationship between the selection on a trait and the inbreeding depression it exhibited, using the framework for quantifying inbreeding depression proposed by Slate *et al.* (2002). Finally, we determined the genetic load through the number of lethal equivalents in the genome (Morton *et al.* 1956), estimated using a maximum-likelihood method (Kalinowski & Hedrick 1998), which we extended to test for temporal variation in survival rates and inbreeding depression.

2. MATERIAL AND METHODS

(a) *Study population*

The population of *F. albicollis* in the south of the island of Gotland has been the subject of intensive monitoring since 1980 (see Sheldon & Ellegren 1999; Merilä *et al.* (2001a) and references therein). Territory establishment and mate choice occur on the birds' return from winter migration, after which each breeding pair lays a single clutch of approximately six eggs (genuine second clutches, as opposed to replacement clutches due to, for example, predation, are unknown). Over the study period, 11% of nestlings fledging in the study area returned as breeding adults (Kruuk *et al.* 2001). When fledgling tarsus length had reached adult size, it was measured with digital callipers (to the nearest 0.1 mm), and nestlings were weighed using a Pesola spring balance (to the nearest 0.1 g) and marked with individually numbered aluminium rings. The total study area was divided into 15 smaller areas, corresponding to different wood lots that ranged in size from *ca.* 5 to 66 ha.

(b) *Inbreeding coefficients*

We constructed pedigrees for the population assuming that the adults attending a nest were the genetic parents of the chicks in the nest. Any extra-pair paternities (EPPs) will have introduced errors into the pedigree construction, but, as we outline in § 4, these should only make the tests for inbreeding depression more conservative. For broods involved in any of the cross-fostering experiments that have been conducted in the population (see Kruuk *et al.* 2001; Merilä *et al.* 2001a), parents were identified from the nest where the eggs were laid. For the chicks in each brood, an inbreeding coefficient f , or the probability of identity by the descent of an allele, was estimated using the software package PEDIGREE VIEWER (<http://www-personal.une.edu.au/~bkinghor/pedigree.htm>). Identifying inbreeding events requires a sufficient pedigree depth and so, following the methodology described by Marshall *et al.* (2002), we restricted analyses to cases where it was possible to detect an inbreeding event of at least $f = 0.125$. To avoid any bias caused by differences in pedigree length, we further restricted the analysis to 1983 onwards. Mating attempts that involved either pied flycatchers (*Ficedula hypoleuca*) or hybrid birds (Veen *et al.* 2001) were excluded.

(c) *Occurrence of inbreeding*

Lineages in the flycatcher pedigree extended up to 15 generations, with an average of 2.92 generations. Of 2107 pairings in

which some form of inbreeding would have been detectable, 22 were between relatives and thus produced offspring with $f > 0$. Sixteen of these pairings involved 'close' inbreeding, at the level of $f = 0.25$ (i.e. mother-son, father-daughter or brother-sister). This high frequency of close inbreeding events (73% of inbreeding events) may reflect the relatively short length of many of the pedigrees in the dataset: when pedigrees were restricted to broods for which all eight great-grandparents were known, it fell to 50%. However, this reduction was not statistically significant (Fisher's exact test of difference: $p = 0.26$), and the restriction of the dataset reduced the total number of inbreeding events to just 10. We therefore used the full dataset, with the proviso that as we were probably not detecting all occurrences of inbreeding between more distant relatives (as indicated by the low frequency of, for example, cousin matings) our tests for inbreeding depression may have had reduced power. In addition, if dispersal from the breeding or natal site is random in direction, and the number of potential breeding sites available per generation increases faster than the number of relatives in the population, one may expect an excess of close inbreeding events.

We tested for temporal and spatial variation in the probability of inbreeding using logistic regression with zero for an outbred mating ($f = 0$), unity for an inbred mating ($f > 0$). Independent variables in the model were: year and area (both as multilevel factors), breeding time (first egg date, relative to the annual mean and corrected for differences between first breeders and older birds) and female's age (in years: fitted as a quadratic equation).

(d) *Effects on fitness*

We used generalized linear models (GLMs) to examine the effect of inbreeding on components of the productivity of a nest. For each trait, a GLM was fitted with an appropriate error structure to account for the different trait distributions (McCullagh & Nelder 1989). We considered the following traits.

- (i) Clutch size—the number of eggs laid, analysed in a model with normal errors.
- (ii) Hatching success rate—the proportion of eggs that hatched, analysed in a binomial errors model with clutch size as the binomial denominator and a logit link function.
- (iii) Fledging success rate—the proportion of chicks that survived to fledging, analysed with binomial errors, with brood size (total number of chicks that hatched) as the binomial denominator and a logit link function.
- (iv) Fledgling tarsus size and
- (v) fledgling body condition—averaged for each brood and analysed with normal errors. Body condition was defined as the residual from a linear regression of body mass on tarsus length (see Merilä *et al.* 2001a for details). In both models we included brood size as an explanatory variable to account for competition.
- (vi) Juvenile survival rate—the proportion of fledglings that returned to the study area as breeding adults, analysed with binomial errors with the number of fledglings as the binomial denominator and a logit link function. Brood average tarsus length and body condition were also included as explanatory variables, so that survival was assessed independently of body size.
- (vii) Total recruitment—the total number of birds recruiting to the breeding population from each nest, analysed with negative binomial errors and a logarithmic link function.

We excluded 1998 and 1999 from the last two analyses in case birds from those years had not yet recruited to the population.

For all statistical analyses the breeding attempt, rather than the individual chick, was considered as an independent unit to avoid pseudoreplication. The inbreeding coefficient f was fitted after the following environmental terms, as described in the previous paragraph: year, area, breeding time and female's age (as a quadratic function). To test whether inbreeding depression was exaggerated under harsh environmental conditions, we ran further analyses in which the 'year' factor was replaced by a direct measure of population performance: the population average body condition (as described in the previous paragraph) of all fledglings in a given year (see Merilä *et al.* 2001a). The annual average condition and its interaction with the inbreeding coefficient were then included in the model as continuous variables.

Statistical significance was assessed by F -ratios for models with normal errors, or from the change in deviance (on respective degrees of freedom) when the term was dropped from a full model for non-normal errors (McCullagh & Nelder 1989). The model of the fledgling success rate showed overdispersion of the residual deviance, so we assessed significance using an F -ratio. The average differences between the inbred and non-inbred nests were calculated by comparing fitted values from the models for the different categories. Sample sizes varied because of gaps in the data.

(e) Multitrait comparison

We estimated the correlation between a trait's inbreeding depression and its association with fitness. Inbreeding depression was quantified using the metric ID_α of Slate *et al.* (2002), defined as the percentage of total deviance in a trait explained by the inbreeding coefficient f , where deviance was taken from the GLMs described above. ID_α therefore reflects both the distribution of inbreeding coefficients in the population and the magnitude of inbreeding depression (Slate *et al.* 2002). Selection on a trait was quantified by the squared correlation, r^2 , between the trait and the total number of recruits from each nest. Due to the fact that each of the traits was not independent of the others we considered partial correlations, calculating r^2 using the residuals from a linear regression of a given trait on other traits that were capable of affecting it—or, in other words, had preceded it in time. Thus, each of the traits listed above was corrected for the traits preceding it in the list. We tested the significance of the correlation between ID_α and r^2 by comparing the observed value with a distribution generated by randomization of the observed measures 10 000 times, defining the p -value as the proportion of the correlations from randomized values with absolute magnitude greater than the observed value (generating a two-tailed test).

(f) Lethal equivalents

The number of lethal equivalents gives a standardized measure of the potential for inbreeding depression in a population, where a lethal equivalent is defined as a unit of genetic variation that would cause death in an individual in a homozygous state (Morton *et al.* 1956). If the loci affecting survivorship have independent, deleterious and multiplicative effects, then the survival rate S is expected to decline approximately exponentially as a function of the inbreeding coefficient: $S = S_0 e^{-Bf}$, with S_0 as the survival rate of non-inbred individuals. The number of lethal equivalents in a gamete, B , is typically estimated as the slope of a weighted least-squares regression of $\ln(S_i)$ on f_i (Morton *et al.* 1956; Lynch & Walsh 1998), where S_i is the survival rate among

all individuals with inbreeding coefficients f_i , so $S_i = N_{\text{surv},i}/N_{\text{total},i}$, where $N_{\text{surv},i}$ is the number of survivors in class i , and $N_{\text{total},i}$ is the number of eggs laid in class i . This regression method runs into difficulties if there is a zero-survival rate among all individuals of a given inbreeding level, because the logarithm of zero is undefined. A possible solution to the zero-survival problem is to use a small sample correction (Templeton & Read 1984), replacing S_i with $S'_i = (N_{\text{surv},i} + 1)/(N_{\text{total},i} + 2)$. However, with data such as ours, the correction can generate highly misleading results. For example, we had a zero-survival rate in an $f = 0.125$ brood of six chicks, so $S_{0.125} = 0$. With the correction, $S'_{0.125} = 0.125$, which is higher than the survival rate of non-inbred offspring, and dramatically alters the estimates of B (see § 3).

As an alternative approach, Kalinowski & Hedrick (1998) recommend calculating a maximum-likelihood estimate of B , using a binomial expectation of survival probability. Maximum-likelihood estimates \hat{B} and \hat{S}_0 (and 95% confidence intervals) are estimated by maximizing the log-likelihood function

$$\ln L = \sum_i N_{\text{surv},i} \ln \hat{S}_i + (N_{\text{total},i} - N_{\text{surv},i}) \ln(1 - \hat{S}_i), \quad (2.1)$$

where $\hat{S}_i = \hat{S}_0 e^{-\hat{B}f_i}$ and the summation is over the different inbreeding classes i . We extended this method to test for the differences between years, firstly in the estimates of S_0 and then in the estimates of B , by replacing \hat{S}_i with a year-specific value $\hat{S}_{y,i}$. For the differences between estimates of S_0 , this required maximizing the function

$$\ln L = \sum_y \sum_i N_{\text{surv},i} \ln \hat{S}_{y,i} + (N_i - N_{\text{surv},i}) \ln(1 - \hat{S}_{y,i}), \quad (2.2)$$

where $\hat{S}_{y,i} = \hat{S}_{0,y} e^{-\hat{B}_y f_i}$ and $y = 1983, 1984 \dots 1997$. The difference in deviance (i.e. -2 times the difference in log-likelihood) between this model and that in equation (2.1) was tested against a χ^2 -distribution on 14 d.f. (corresponding to the 14 new parameter estimates $\hat{S}_{0,y}$ added to the model).

Adding the between-year differences in B gave $\hat{S}_{y,i} = \hat{S}_{0,y} e^{-\hat{B}_y f_i}$ where B_y is the estimate of lethal equivalents in year y . Here, the change in deviance from equation (2.2) was tested on 9 d.f., in five of the years considered there was no inbreeding, so none of these years require estimates of B_y . All maximization procedures were done in MICROSOFT EXCEL 2000, using the SOLVER tool (after Kalinowski & Hedrick 1998). Note that this procedure could be extended to test for heterogeneity in any aspect of inbreeding depression, for example, in individual age- or sex-related survival rates.

The maximum-likelihood estimates produce less biased estimates than the traditional regression approach (Kalinowski & Hedrick 1998). However, for comparison with estimates in the literature calculated using the latter, we also present the regression parameter, calculated initially using the small sample correction (Templeton & Read 1984) and later considering only the categories $f = 0$ and $f = 0.25$, the only ones with sample sizes greater than five (and thus excluding all zero-survival classes, using eqn (10.21b) from Lynch & Walsh (1998)).

3. RESULTS

(a) Occurrence of inbreeding

The total frequency of inbreeding events was 1.04%, generating an average inbreeding coefficient in the study population of 0.002 06. There was no evidence of significant differences between the years or between areas in the

frequency of inbreeding events ($\chi^2_{16} = 15.56$, $p = 0.48$ and $\chi^2_{14} = 15.88$, $p = 0.32$, respectively). Similarly, within a year, the probability of any inbreeding event ($f > 0$) did not change with breeding time ($\chi^2_1 = 2.619$, $p = 0.10$). However, the probability of a 'close' inbreeding event, at $f = 0.25$, increased significantly for later breeders ($\chi^2_1 = 4.60$, $p = 0.03$), with $f = 0.25$ pairs breeding an average of 2.36 (± 1.03 s.e.) days after outbred pairings.

(b) *Effects on fitness*

Inbreeding did not affect clutch size (table 1a(i)), but it did have a significant adverse effect on the hatching success rate (table 1a(ii)): the probability of an egg hatching in a clutch of $f = 0.25$ was reduced by 6.4%, relative to that of a non-inbred clutch (figure 1a). There was no evidence of any effect of inbreeding on the hatching to fledging survival rate (table 1a(iii)).

There was also a significant effect of the inbreeding coefficient on body size, tarsus length, so that the average tarsus length in a brood with $f = 0.25$ was 0.56 of a standard deviation less than that of a non-inbred brood (table 1a(iv); figure 1b). Inbreeding did not affect the fledging body condition (table 1a(v)).

The rates of juvenile survival from fledging to recruitment the following year were greatly reduced by inbreeding (table 1a(vi)). The average survival rate from an $f = 0.25$ nest—correcting for the other significant variables such as fledging body size and breeding time—was reduced by 93.4%, relative to that of a $f = 0$ nest (figure 1c).

(i) *Total number of recruits*

There was significant inbreeding depression in the total number of offspring recruiting to the breeding population from a nest (table 1a(vii)). The number of recruits from a nest with $f = 0.25$ was reduced by 94.2% of the number expected from a non-inbred nest (table 1a(vii); $94.2 = 100 \times (1 - e^{-11.38/4})$; CI of ± 2 s.e.: 80.7–98.3%); see figure 1d.

Only five individuals with $f > 0$ recruited to the breeding population, of which only one, a male, was closely inbred at $f = 0.25$. Sample sizes were therefore too small to analyse the effect of inbreeding on adult reproductive performance.

(c) *Environmental conditions*

The year and area had significant effects on all traits (table 1). Breeding time also had a significant effect: late broods performed less well, with the exception of hatching success that, surprisingly, increased in later clutches (table 1a(ii)). There were also significant effects of female's age on all traits except for hatching success and juvenile survival rates.

However, we found no evidence to indicate that the magnitude of inbreeding depression varied with environmental conditions. When we included the average population body condition in the models instead of the year, it explained a significant (or near significant) amount of the variance in several traits (data not shown), but its interaction with the inbreeding coefficient was never significant.

(d) *Comparison of inbreeding depression across traits*

The proportion of deviance explained by inbreeding, ID_{∞} , was always very low, with a maximum of 0.70% for recruitment (table 1). The correlation between the effect of inbreeding and the amount of selection (r^2) on a trait was 0.750 ($n = 7$, $p = 0.073$; figure 2). The values for ID_{∞} and r^2 for each trait are given in table 1.

(e) *Lethal equivalents*

The maximum-likelihood estimate was $B = 7.47$ (95% CI: 2.60–15.52). Allowing between-year variation in the overall survival rate gave a significantly better fit to the data ($\chi^2_{14} = 124.23$, $p < 0.001$). However, there was no effect of adding between-year variation in B ($\chi^2_6 = 10.63$, $p = 0.30$), and hence no evidence of temporal variation in inbreeding depression.

For comparison, the number of lethal equivalents per gamete calculated using the regression approach with the small sample correction was 1.358. Restricting the analysis to only cases where $f = 0$ or $f = 0.25$ (i.e. excluding all zero-survival classes) gave an estimate of $B = 7.462$.

4. DISCUSSION

We have shown that inbreeding was apparently rare in this collared flycatcher population, but that when it did occur it had severe consequences, particularly for characters closely related to fitness. Qualitatively, the patterns of inbreeding depression in the collared flycatcher are in agreement with those from other studies of wild bird populations (table 2). For example, the deleterious effects of inbreeding on hatching success have been reported in all but two cases, whereas the lack of differential mortality between hatching and fledging fits with findings in five other species. However, despite this qualitative agreement with other studies, to our knowledge, the number of lethal equivalents estimated here (7.47) is greater than any other presented in the bird literature (table 2). Keller *et al.* (2002) report estimates ranging from zero to 6.3 in a review of the literature on wild populations, containing results for five bird and two mammal species. The value of 7.47 is also in the top 10% of estimates of B based on juvenile survival in captive mammal populations (Ralls *et al.* 1988). This high value indicates a substantial genetic load, and hence indicates relatively little purging of deleterious alleles in the Gotland population.

Generalizing from the contrast between this population and others is clearly difficult, especially because a direct comparison of inbreeding frequency between populations will undoubtedly be confounded by differences between studies in pedigree length. Furthermore, conclusive evidence of purging in natural populations is rare (Hedrick & Kalinowski 2000). However, it is interesting to note that the high genetic load in this population corresponds to the largest population size and the lowest frequency of inbreeding among the published bird studies (table 2), which should theoretically have resulted in relatively lower purging levels. Furthermore, immigration into the study area and hybridization with the pied flycatcher (Veen *et al.* 2001) will both have maintained a steady flow of genetic variation and hence potentially deleterious mutations into the population. A population's initial genetic constitution,

Table 1. Effect of inbreeding on measures of nest productivity in the collared flycatcher.

((a) Significance of each term when dropped from a full model containing the inbreeding coefficient f . Δ deviance, change in deviance. The F -ratio and Δ deviance were calculated for each term when fitted last in a model; residual SS or dev., residual sums of squares or residual deviance; N , total number of nests in the analysis. (b) Parameter estimates from minimal model, which contains all terms that were significant in the full model plus the inbreeding coefficient f . The year and area were included in all models, but the parameter estimates are not given. Note that the parameter estimates are dependent on the link function used in the GLM. The values in bold represent the significant effects of inbreeding on trait value. ID_α is the proportion of deviance accounted for by the inbreeding coefficient in the minimal model (Slate *et al.* 2002). r^2 is the (squared) partial correlation between the trait and the number of recruits, and therefore gives an indication of the selection on a trait; see § 2.)

(a) full model		(i) clutch size	(ii) hatching success	(iii) fledging success	(iv) tarsus length	(v) body condition	(vi) juvenile survival rate	(vii) no. of recruits
term	d.f.	F -ratio	Δ deviance	Δ deviance ^a	F -ratio	F -ratio	Δ deviance	Δ deviance
year	16	6.65***	379.787***	615.418***	32.16***	18.00***	91.959***	66.981***
area	14	2.29**	23.737*	73.515**	3.95***	3.82***	57.311***	45.700***
female's age + age ²	2	36.40***	0.808	14.496*	5.17** ^b	3.691*	0.450	1.519
breeding time	1	111.76***	5.139*	19.075*	20.35***	97.32***	7.554**	21.754***
brood size	1	—	—	—	28.12***	27.52***	0.317	—
tarsus length	1	—	—	—	—	—	34.868***	—
body condition	1	—	—	—	—	—	34.126***	—
f	1	0.07	5.265*	2.942	6.48*	0.66	11.212***	13.222***
residual SS or dev.		880.17	1489.14	4735.64	407.23	2043.37	1938.36	1727.72
N		2088	2066	2065	1971	1966	1764	1764
(b) minimal model + inbreeding coefficient		estimate (s.e.)	estimate (s.e.)	estimate (s.e.)	estimate (s.e.)	estimate (s.e.)	estimate (s.e.)	estimate (s.e.)
female's age		0.570 (0.076)	—	0.417 (0.130)	0.028 (0.010)	—	—	—
female's age ²		-0.069 (0.010)	—	-0.062 (0.017)	— ^b	—	—	—
breeding time		-0.037 (0.004)	0.027 (0.011)	-0.028 (0.007)	-0.011 (0.003)	-0.057 (0.006)	-0.023 (0.008)	-0.035 (0.007)
brood size		—	—	—	-0.069 (0.013)	-0.149 (0.028)	—	—
tarsus length		—	—	—	—	—	0.476 (0.080)	—
body condition		—	—	—	—	—	0.216 (0.037)	—
f		-0.177 (0.656)	-3.545 (1.179)	-1.846 (1.042)	-1.188 (0.474)	-0.866 (1.064)	-10.890 (4.822)	-11.384 (4.797)
ID_α		0.003	0.282	0.053	0.241	0.014	0.528	0.697
r^2		0.0009	0.0034	0.0137	0.0279	0.0219	0.8281	1.0000

^a Significance assessed from F -ratio due to over-dispersion of residual deviance.

^b Only female age significant (not age²).

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

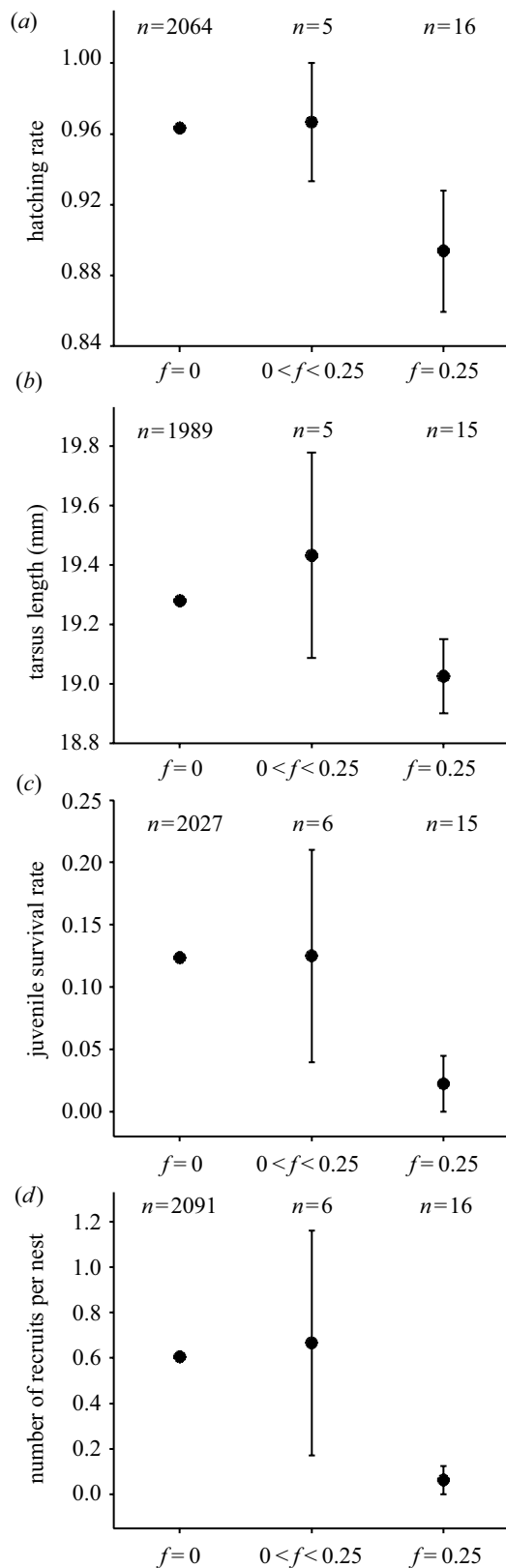


Figure 1. The effect of inbreeding on fitness. The mean and standard errors of (a) hatching rate, (b) brood average tarsus length, (c) juvenile survival rate from fledging to recruitment to breeding population, (d) total number of recruits to breeding population per nest. The figures show the observed values for non-inbred ($f=0$); moderately inbred ($0 < f < 0.25$) and closely inbred birds ($f=0.25$). n is the number of broods contributing to each category.

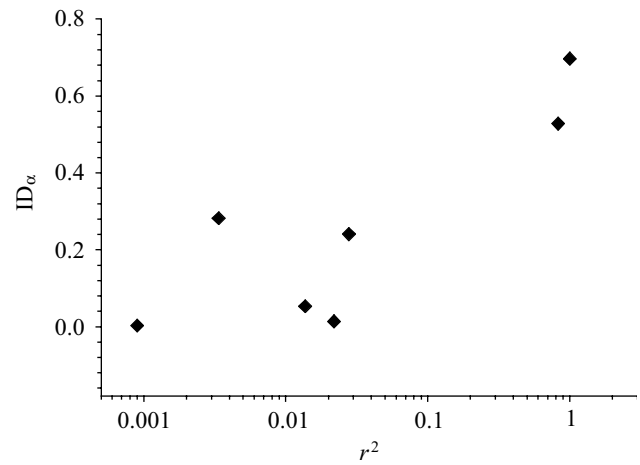


Figure 2. Relationship between the percentage of variance in a trait explained by inbreeding (ID_w) and the selection on that trait (r^2). Correlation = 0.750, $p = 0.073$.

as well as any stochastic demographic events, will also play a critical role in determining genetic load (Miller & Hedrick 2001), and it would be informative both to determine the history of the Gotland population and to compare the inbreeding depression here with that in other collared flycatcher populations.

Just as environmental conditions affect the components of fitness directly, so they also are likely to modify the action of inbreeding depression. For example, in a cactus finch (*Geospiza scandens*) population on the Galápagos islands, the severity of inbreeding depression increases under harsh environmental conditions (Keller *et al.* 2002), and a study of house mice (*Mus domesticus*) found significant inbreeding depression under semi-natural conditions but not in the laboratory (Meagher *et al.* 2000). Here, however, we did not find any evidence of variation in the magnitude of inbreeding depression in collared flycatchers: first, there was no indication that the effect of inbreeding varied according to the average condition of the population, and, second, replacing an overall estimate of the number of lethal equivalents with annual estimates did not provide a significantly better fit to the data. However, our statistical power to detect differences between years is unarguably low given the small sample sizes.

Severe inbreeding depression, such as that shown here, will generate selection for behaviour that reduces the chances of mating with a relative, either through sex-biased dispersal or through kin discrimination. There is a degree of sex-biased dispersal in this population (Pärt 1994), but, as in all populations, it may serve a variety of other evolutionary purposes (Pusey 1987). Studies that have considered inbreeding avoidance through kin discrimination in bird populations in detail have found little supporting evidence for its existence (Gibbs & Grant 1989; Pärt 1996; Keller & Arcese 1998), but defining a suitable null model is always difficult (Pärt 1996) and kin discrimination has been shown in studies of cooperatively breeding species (Russell & Hatchwell 2001). In this case, we believe the increased frequency of inbred pairings among late breeders to be indirect evidence of inbreeding avoidance through kin discrimination. This is because the evolution of inbreeding avoidance requires the potential costs of random mating to exceed the costs of actively

Table 2. Patterns of inbreeding depression in wild bird populations.

(Effects on: clutch size, by inbreeding coefficient of (1) female and (2) offspring; rate of hatching success by inbreeding coefficient of (3) female and (4) offspring; (5) survival I: from hatching to fledgings; (6) brood size, or number of fledglings in nest, incorporating clutch size, hatching success and nesting mortality; (7) fledging body size; (8) fledging body condition; (9) survival II: from fledging to one year; (10) total number of birds recruited to breeding population per nest; (11) survival III: annual survival after one year; (12) female and (13) male lifetime reproductive success (LRS), after accounting for differences in survival. All measures are with respect to inbreeding coefficient of offspring, unless stated otherwise. 'Method' indicates whether relatedness was determined by pedigree information ('ped.') or DNA similarity. '%f > 0' gives the percentage of nests with non-zero relatedness between the parent birds. 'Annual *n*' gives estimates of average annual population size, in numbers of breeding adults; *B*, number of lethal equivalents per gamete; blanks, data not given; n.s., test not significant; x, significant effect in the opposite direction; —, no test.)

species	moonhen ^a		red-cockaded woodpecker ^b		great tit ^c		great tit ^d		blue tit ^e		cactus finch ^f		medium ground finch ^g		medium ground finch ^h		large ground finch ⁱ		song sparrow ^j		Mexican jay ^k		great reed warbler ^k		great reed warbler ^l		collared flycatcher ^m		
	ped.	%f > 0	ped.	%f > 0	ped.	%f > 0	ped.	%f > 0	DNA	%f > 0	ped.	%f > 0	ped.	%f > 0	ped.	%f > 0	ped.	%f > 0	ped.	%f > 0	ped.	%f > 0	DNA	%f > 0	DNA	%f > 0	ped.	%f > 0	
method	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
%f > 0	5.6	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.
mean <i>f</i>	0.014	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
annual <i>n</i>	180	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(1) clutch size ⁿ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(2) clutch size ^o	—	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.
(3) hatch rate ⁿ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(4) hatch rate ^o	*	—	*	—	***	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(5) survival I	—	—	n.s.	—	—	—	—	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.
(6) brood size	—	—	—	—	—	—	—	*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(7) size	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(8) condition	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(9) survival II	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(10) recruitment	—	—	*	—	x	—	*	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.
(11) survival III	—	—	*	—	n.s.	—	—	—	—	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.	—	n.s.
(12) female LRS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(13) male LRS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>B</i>	—	—	—	—	0.77	—	1.16	—	—	—	4.27	—	—	0	—	3.026	—	—	—	—	—	—	—	—	—	—	—	—	7.47

^a McRae 1996; ^b Daniels & Walters 2000; ^c van Noordwijk & Scharloo 1981; ^d Bulmer (1973) and Greenwood *et al.* 1978; ^e Kempenaers *et al.* 1996; ^f Keller *et al.* 2002; ^g Gibbs & Grant 1989; ^h Grant *et al.* 2001; ⁱ Keller 1998; ^j Brown & Brown 1998; ^k Hansson *et al.* 2001; ^l Bensch *et al.* 1994; ^m this study; ⁿ in relation to mother's inbreeding coefficient; ^o in relation to chicks' inbreeding coefficient; ^p harmonic means.

* *p* < 0.05; ** *p* < 0.01; *** *p* < 0.001.

avoiding inbreeding, i.e. the costs of taking longer to find an unrelated mate. For collared flycatchers returning from migration, the availability of potential partners will decrease towards the end of the breeding season, and so the time required to find a suitable mate will increase. However, each day's delay in breeding leads to a 6% decrease in expected recruitment from a breeding attempt (Merilä *et al.* 2001b). As inbreeding avoidance is therefore more costly for birds returning late from migration, we would expect it to be more likely to break down later in the breeding season. In agreement with this prediction, late-breeding birds were more likely to pair with a close relative than early breeders. Our results therefore indicate the existence of costly mate-choice mechanisms through which pairing with close relatives is usually avoided, but which become increasingly expensive and thus fragile towards the end of the breeding season. Note that breeding time in males does not appear to be under genetic control, as it is in females (Sheldon *et al.* 2002), which argues against the observed pattern being driven by similarities between male and female relatives in the breeding time. In an interesting mirror of the situation, the probability that a collared flycatcher female in the study population pairs with a pied flycatcher male also increases throughout the breeding season—in this case because the cost of hybridization decreases with time (Veen *et al.* 2001).

We calculated inbreeding coefficients on the assumption that the adults recorded attending a nest were the true genetic parents of the offspring. This assumption will be valid for the female, as genetic analyses have never found any conflict between mother and offspring genotype (Sheldon & Ellegren 1999). However, rates of EPPs in this population have been estimated at 15% of chicks (Sheldon & Ellegren 1999), generating errors in the pedigrees from which f was calculated. If we assume that, on average, a female is no more related to her social partner than to her genetic partner, the existence of EPP can only result in a downward bias in the magnitude of inbreeding depression: if non-inbred individuals are mistakenly classified as inbred, and vice versa, the errors will reduce the slope of the decline in fitness with inbreeding coefficient. Simulation studies are required to calculate the potential magnitude of any such effect, but the results presented here may therefore be only conservative estimates of the true genetic load.

The comparison between traits provides useful insights into the genetic architecture of the different components of fitness. Inbreeding depression has been shown to be stronger for traits closely related to fitness, both in comparison across a range of taxa (de Rose & Roff 1999) and when considering a range of juvenile and adult traits within a single species (red deer, *Cervus elaphus*, Slate *et al.* 2002). Our results support the notion of substantial differences between traits in inbreeding depression, although the correlation with selection did not reach statistical significance (figure 2).

Inbreeding depression is generated by the existence of either deleterious recessives or overdominance (Charlesworth & Charlesworth 1987), both of which create dominance genetic variance. The extent of inbreeding depression for a trait may therefore reflect underlying levels of dominance variance, so our results indicate that

traits closely related to fitness may have relatively greater dominance variance. Crnokrak & Roff (1995) also present evidence showing increased levels of dominance variance in traits more closely associated with fitness than in weakly selected traits. Our results also allow indirect inferences about the amount of dominance variance underlying particular phenotypic traits. In this study, we found inbreeding depression in fledgling tarsus length, but not in body condition. In an earlier analysis of tarsus length, we reported that 15% of the total phenotypic variance was due to similarities between full-siblings, over and above that attributable either to additive genetic variance or to common environment effects experienced after two days of age (Kruuk *et al.* 2001). We suggested that this component was due largely to very early common environment effects, but we could not rule out the possibility that it was also due to dominance variance. In a parallel analysis of fledgling body condition, the corresponding component of variance was much smaller, accounting for only 2% of the total (Merilä *et al.* 2001a). The results presented here—significant inbreeding depression for tarsus length but not for body condition—indicate that at least some of the extra variance in tarsus length may be due to dominance genetic variance. The genetic architecture of different characteristics may therefore vary substantially even between morphological traits expressed at the same age.

Despite the magnitude of inbreeding depression, the inbreeding coefficient only ever explained an extremely low proportion of the variance in each of the fitness components. This is not surprising given the very low frequency of matings between relatives: even if no inbred individuals had survived at all, the proportion of variance explained would still have only been 1.21% (as compared with the 0.70% observed). Thus the statistical, but not the biological, significance of our results must depend on the significance of the unusually large sample sizes. The study therefore highlights the difficulties inherent in estimating inbreeding depression in large populations of wild animals in which inbreeding events are rare. The potential existence of substantial genetic load in such populations has important conservation implications should population numbers crash at some point in the future. We envisage that the ongoing accumulation of data from long-term, individual-based studies of natural populations such as the one presented here will, therefore, have an important role in improving our understanding of patterns of inbreeding depression in the wild.

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