THE EFFECTS OF ENVIRONMENTAL HETEROGENEITY ON MULTIVARIATE SELECTION ON REPRODUCTIVE TRAITS IN FEMALE GREAT TITS

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Describing natural selection on phenotypic traits under varying environmental conditions is essential for a quantitative assessment of the scale at which adaptation might occur and of the impact of environmental variability on evolution. Here we analyzed patterns of multivariate selection via fecundity and viability on three reproductive traits (laying date, clutch size, and egg weight) in a population of great tits (*Parus major*). We quantified selection under different environmental conditions using (1) local variation in breeding density and (2) distinct areas of the population's habitat. We found that selection gradients were generally stronger for fecundity than for viability selection. We also found correlational selection acting on the combination of laying date and clutch size; this is the first documented evidence of such selection acting on these two traits in a passerine bird. Our analyses showed that both local breeding density and habitat significantly influenced selection patterns, hence favoring different patterns of reproductive investment at a small-scale relative to typical dispersal distances in this species. Canonical rotation of the nonlinear selection matrices yielded similar conclusions as traditional nonlinear selection analyses, and also showed that the main axes of selection and fitness surfaces varied over space within the population. Our results emphasize the importance of quantifying different forms of selection, and of including variation in environmental conditions at small scales to gain a better understanding of potential evolutionary dynamics in wild populations. This study suggests that the fitness landscape for this species is relatively rugged at scales relevant to the life histories of individual birds and their close relatives.

KEY WORDS: Canonical rotation, clutch size, egg weight, great tit, laying date, natural selection, spatial scale.

The quantification of natural selection in the wild is central to the study of evolutionary ecology (see Endler 1986; Fairbairn and Reeve 2001), and the scale over which selection operates has long been recognized as a critical factor in population differenti-ation and adaptation (Slatkin 1973). Indeed, numerous empirical studies have confirmed the importance of natural selection in driv-ing the adaptive divergence of populations using different habitat (Endler 1986; Schluter 2000). There is evidence for variation in

selection pressures on a temporal scale, for example, due to climatic conditions (Etterson 2004; Nussey et al. 2005) or predation regimes (Reimchen and Nosil 2002), and there is also evidence for spatial variation in selection among populations (Smith et al. 1997). Studies of plant systems have suggested an important role of the environment in driving selective pressures at a small spatial scale (see Kalisz 1986; Huber et al. 2004), but much less is known about the impact of environmental heterogeneity on patterns of selection on phenotypic traits within animal populations. Such environment-specific estimates of selection are critical for a quantitative assessment of the spatial scale of adaptation and of the impact of environmental variability on evolution (see Svensson and Sinervo 2000, 2004; Blondel et al. 2006).

As studies of natural selection in the wild accumulate, it has become clear that the strength of selection will depend on the fitness component measured in estimates of both linear and non-linear selection, with fecundity selection gradients often being higher than viability (survival) selection gradients (see Kingsolver et al. 2001). However, rather few studies have looked at these two components simultaneously (although see Sheldon et al. 2003; Brommer et al. 2005; Wilson et al. 2005 for exceptions), and even fewer test for variation in selection via these fitness components with respect to spatial environmental variation. It thus remains unclear to what extent selection varies depending on the spatial scale at which it is measured, and whether this variation results in different trait optima with respect to spatial location, or even different combinations of traits yielding locally varying optima. Such variable selection could potentially result in the evolution of trade-offs among traits or fitness components (see Arnold and Wade 1984) and might also be an important mechanism underlying maintenance of genetic variation in wild populations (e.g., Felsenstein 1976; Postma and van Noordwijk 2005).

Studies of natural selection in the wild have typically focused on linear selection, with much less emphasis being devoted to investigations of nonlinear selection (reviewed in Kingsolver et al. 2001). This is surprising given the fact that natural selection is often expected to act in a nonlinear manner on a phenotypic trait (see Brodie 1992; Blows et al. 2003) and therefore that analyses of the general patterns of selection acting on traits, or combinations of traits, are misleading if quadratic and correlational components of selection are not considered (Philips and Arnold 1989). In particular, canonical analyses (see material and methods for details) of the matrix of nonlinear selection gradients may often reveal substantial levels of quadratic selection acting on key combinations of traits (see Blows and Brooks 2003; Blows 2007). These analyses are important because we are otherwise forced to consider only those traits that we ourselves define, rather than combinations of traits, which are perhaps more likely to be representative of the way that different aspects of the phenotype are interrelated. Characterizing nonlinear selection components also allows us to

investigate and visualize population fitness landscapes, a central concept in evolutionary biology that predicts the evolutionary trajectory of a given population (Simpson 1944; Arnold et al. 2001; see Brooks et al. 2005 for an example). However, small sample sizes and hence reduced statistical power in studies that assess patterns of selection may also render estimates of nonlinear and correlational selection unreliable or nonsignificant, even in cases where an effort is made to provide such estimates (Conner 2001).

In this study, we report an analysis of the form and strength of natural selection on three female reproductive traits in a population of great tits (*Parus major*) breeding in Wytham Woods, Oxford, U.K.: laying date, clutch size, and egg weight. This population shows variation in key habitat features at a small scale, the most striking of which is the variable density experienced by breeding individuals (see Minot and Perrins 1986; Garant et al. 2005; Wilkin et al. 2006). Population density affects population dynamics and reproductive processes of many territorial animals (Sinclair 1989), as competition for resources is more intense at high density. Clutch size (Perrins 1965), fledgling mass (Garant et al. 2004), and recruitment of offspring (Both and Visser 2000) have been shown to decline with increasing density in this species, not only generally, but also for this population in particular (Wilkin et al. 2006). On the other hand, effects of density on laying date and egg weight are less pronounced than for clutch size (Wilkin et al. 2006), and we therefore expect to find greater influence of density on selection on clutch size than on other reproductive traits. High density also has been shown to reduce the survival of territorial adults in this species (Both and Visser 2000).

Recent studies of this population have established that two different areas within the study population, henceforth referred to as North and East, show contrasting patterns of fledging mass differentiation and recruitment (Garant et al. 2005; Shapiro et al. 2006), which reflects variation in the mean habitat quality between these areas (see Garant et al. 2005 and Shapiro et al. 2006 for more details). Specifically, birds in the North lay larger clutches, produce heavier fledglings, have lower breeding failure rates, and have elevated recruitment rates, relative to the East. We thus used the continuous variation in breeding density and the contrast between these two parts of the population to analyze selection on reproductive traits under contrasting environmental conditions. The scale of the study site is relatively small relative to the natal dispersal potential of this species (mean distance of 3.7 km between potential breeding locations in the population, compared with mean natal dispersal distance of 0.7 km for males and 1.0 km for females [McCleery et al. 2004; see also Tufto et al. 2005 for estimates of dispersal parameters for other great tit populations]). Hence, all else being equal, genes in close relatives have the potential to experience the full range of the environmental conditions explored here.

Continuous monitoring of the population allowed us to estimate both fecundity selection (reproductive success) and viability selection (survival of breeders), and thus assess potential trade-offs among these fitness components. The present study is based on data collected over 36 years, which provides both the large sample size needed for selection analyses, but also allows us to assess general patterns of selection.

Materials and Methods
STUDY SPECIES AND DATA COLLECTION
We used data that were obtained during the long-term study of the great tit (*P. major*) population breeding in Wytham Woods, a about 390-ha woodland located in Oxfordshire, U.K. (see Perrins 1965, 1979 for more details). All breeding attempts in nest boxes are monitored from the date of egg laying until all nestlings had fledged, by regular visits to each nest box. All birds in this population are marked with individually numbered aluminium rings (see Perrins 1979; Perrins and McCleery 1989 for details). Here we specifically used data available for the phenotypic traits of (1) laying date (expressed as number of days since March 31, so that April 1 = 1); (2) clutch size (number of eggs); (3) mean egg weight (total egg mass in grams divided by the number of eggs in the clutch, for at least three eggs laid at the start of the clutch) for each female in each year from 1965 to 2000. Possible second clutches and repeat clutches laid after failure of the first clutch were removed from the data set (following Garant et al. 2004, 2005), as the processes causing these clutches to be laid are not representative of those affecting genuine first clutches. Sample sizes for each analysis are provided in Table 1; only about 85% of clutches were weighed, so a small proportion of nests and females could not be analyzed. Viability sample sizes were slightly larger than fecundity samples because (1) a few females every year were caught in the period just preceding the next breeding season but not during the breeding season (those females were still classified as survivors) and (2) nests that were experimentally manipulated were excluded from fecundity selection analyses but the females in such nests were identified as survivors and were included in viability analyses. There is no evidence that experi-

mental manipulations, such as clutch size modification, influence adult survival in this species (Verhulst et al. 1995; Tinbergen and Both 1999), and we thus kept these nests to maximize our power to detect differences in survival among females (see also Sheldon et al. 2003).

SELECTION ANALYSES
We estimated the fitness of individual females from (1) reproductive success, defined as the number of a female's offspring recruiting to the breeding population from each breeding attempt (fecundity selection) and (2) survival of a female to next year (viability selection). Estimates of fecundity and viability selection were based on recapture data under the assumption that nestlings and adults not returning to the study area in subsequent years had died (as in Garant et al. 2004, 2005). Although we know that this is not strictly accurate for nestlings, because a substantial fraction emigrates to other populations each year, adult birds are much less likely to emigrate between years. The use of recruitment as a measure of fecundity selection represents a reasonable measure of fitness relative to other broods in the same year as it is only those individuals who recruit that will contribute to any response to selection. Furthermore, we have no evidence that natal dispersal distance is related to the reproductive traits analyzed here, with the exception of laying date. Verhulst et al. (1997) showed that earlier-hatched nestlings (hatching date of individuals and laying date of clutches are strongly related) were more likely to emigrate from Wytham to breed in external woodlands. However, the effect is quite weak, and likely to be driven by spatial effects because of those birds breeding in the center of the study plot being at higher altitude and thus timing breeding to match later local phenology (Verhulst et al. 1997). Overall, this will lead to underestimates of the strength of selection on laying date in our data, although the effect is likely to be small.

In common with many other such studies, we therefore consider postnatal survival of chicks as a component of a mother's fitness (her reproductive success). Although theoretically this approach has potential drawbacks (see Wolf and Wade 2001), recent empirical evidence suggests that conclusions about patterns of

Table 1. Means with standard deviations (SD) of traits analyzed and number of observations (number of females in parentheses) available depending on the fitness component considered. Laying date is the number of days since March 31, clutch size is the number of eggs, and egg weight (g) is the egg mass divided by the number of eggs in the clutch. Note that the North and East area are two subdivisions that do not encompass the overall number of observations.

		North	East	Overall
Mean (SD)	Laying date	26.5 (8.0)	27.5 (8.4)	27.6 (8.3)
	Clutch size	9.00 (1.64)	8.61 (1.50)	8.76 (1.62)
	Egg weight	1.678 (0.128)	1.690 (0.131)	1.684 (0.130)
No. Observations (no. females)	Viability	2043 (1410)	1220 (825)	5061 (3478)
	Fecundity	1754 (1239)	1077 (749)	4389 (3083)

selection are unlikely to be dramatically changed by assignment of offspring survival to either maternal or offspring fitness (see Wilson et al. 2005), and the current approach allows us to quantify selection on maternal traits.

Fitness was converted to relative fitness by dividing each individual's observation by either the mean number of offspring recruited per breeding attempt in any given year, for fecundity selection, or by the proportion of surviving females in that year, in the case of viability selection. Each trait was standardized (zero mean, unit variance) within each year, to avoid confounding selection gradients with covariance of environmental conditions, trait values and fitness over time (see McAdam and Boutin 2003). Removing annual variation represents something of a compromise position between using raw data, and controlling for all possible environmental covariance (e.g., at the level of individual sites), as the latter process uses a very large number of degrees of freedom. Annual differences in these variables are the largest easily quantifiable environmental effects (R. A. Pettifor and B. C. Sheldon, unpubl. data).

We first estimated standardized linear (*i*) selection differentials (Endler 1986) using general linear models for both relative fecundity and viability for each standardized trait separately, and estimated nonlinear (*j*) selection differentials from a model that included both a linear and a quadratic term. Selection differentials provide a measure of both direct selection acting on a trait and indirect selection acting on correlated traits (Lande and Arnold 1983). We then used multivariate linear models that included all standardized reproductive traits in the same model to calculate selection gradients with respect to this set of traits (see Lande and Arnold 1983; Philips and Arnold 1989; Brodie et al. 1995). Selection gradients thus provide a measure of the direct selection on a trait, holding the effects of the other modeled traits constant. We estimated standardized linear selection gradients (β_i) for each phenotypic trait (z_i) from the model:

$$w = \alpha + \beta_{ld}x_{ld} + \beta_{cs}x_{cs} + \beta_{ew}x_{ew} + \varepsilon \quad (1)$$

where w is the relative fitness, α is the intercept, and ε is an error term, x_{ld} = laying date, x_{cs} = clutch size, and x_{ew} = egg weight. We also estimated nonlinear quadratic (γ_{ii}) and correlational (γ_{ij}) selection gradients from models of the form:

$$w = \alpha + \beta_{ld}x_{ld} + \beta_{cs}x_{cs} + \beta_{ew}x_{ew}$$
$$+ (\gamma_{ld}/2)x_{ld}^2 + (\gamma_{cs}/2)x_{cs}^2 + (\gamma_{ew}/2)x_{ew}^2$$
$$+ \gamma_{ldcs}x_{ld}x_{cs} + \gamma_{ldew}x_{ld}x_{ew} + \gamma_{csew}x_{cs}x_{ew} + \varepsilon \quad (2)$$

(as in Lande and Arnold 1983; Simms 1990). Quadratic partial regression coefficients (γ_{ii}) describe nonlinear selection that either reduces (convex or stabilizing selection, negative γ_{ii}) or increases (concave or disruptive selection, positive γ_{ii}) the variance on the trait (z_i). Correlational selection gradients describe selection that targets specific combinations of traits, and which favors either similar (positive γ_{ij}) or opposite combinations (negative γ_{ij}) (Brodie et al. 1995). Age (defined as the number of years since ringed as nestlings for females born in Wytham and as the number of years since first capture + 1 for immigrants that were first caught as adults—on average, 47% of females breeding in Wytham are born outside the wood) was included as a covariate in analyses of viability selection, because survival decreases with age (see McCleery et al. 1996).

Statistical significance of the selection differentials and gradients of either fecundity or viability were estimated with generalized linear-mixed models (GLMMs) with Poisson error structure for fecundity and binomial error structure for viability (Genstat ver. 8.2; VSN Intl. 2005). We included female identity as a random effect to control for repeated measurements of females (see Verbeke and Molenberghs 2000). The significance of each trait as a predictor of either the number of recruits or survival to breed in a later year was assessed from their Wald statistics, distributed as chi-squared on the appropriate degrees of freedom.

ENVIRONMENTAL DEPENDENCE OF SELECTION

Variation in selection patterns with respect to the environment, within this population, was assessed using two different approaches. First, we tested for variation in selection with respect to individual breeding density for the population as a whole. We defined the territory size of each female (available from 1965 to 1996, number of observations = 4283 and 3729, respectively, for viability and fecundity analyses) as a continuous measure of local breeding density. Territory size was calculated from Thiessen polygons that contained all points closer to a given nest box than to others, such that polygon boundaries are equidistant between occupied nest boxes. Territories that were larger than 2 ha were capped at 2 ha, as this represents the maximum effective territory size in the current population (see Wilkin et al. 2006 for a full methodological description, and for evidence that this measure is an appropriate way to describe relative access to space in this population). This measure is inversely proportional to the occupation of nest boxes in the area and thus to the number of breeders present at a local scale (see Garant et al. 2005; Wilkin et al. 2006).

Second, we have previously documented small-scale variation in evolutionary trajectories for nestling mass in this great tit population (Garant et al. 2005). Specifically, the area referred to as North and that referred to as the East show contrasting patterns of fledging mass differentiation over the period 1965–2000; the mean distance between the two locations is only 3.7 km. The spatial difference in fledging mass evolution is associated with differences in mean breeding density, and in recruitment, with birds breeding in the North having about 20% higher lifetime recruitment than those in the East. Every year, these subpopulations directly

exchange on average between 4% and 7% of their locally breeding birds, but are also linked by immigration between adjoining areas (see Garant et al. 2005 for details). We used these two areas to further test for potential differences in selective regimes on female reproductive traits at a small scale. We conducted selection analyses (as described in the previous section) where each trait was standardized to mean zero and unit variance for each year, within each area separately, and where relative fitness was also established within each area to assess the local selection gradients of fecundity and viability.

To compare the pattern and strength of linear and nonlinear selection acting on reproductive traits with respect to breeding density or area, we used a sequential model-building approach as outlined by Draper and John (1988; also explained in detail in appendix A in Chenoweth and Blows 2005). First, we fitted a model that contained the variable of interest (either breeding density or area) as a fixed effect and all three traits as linear covariates. We then compared this with a model in which we added variable of interest by linear covariate interactions to test whether there were differences in linear selection depending on either breeding density or area. We then tested whether quadratic selection differed between "treatments" (density or area) by comparing a model containing all linear and quadratic terms with a model to which we added variable of interest by quadratic covariate interactions. Finally, we produced a model containing all linear and correlational terms and compared this with a model in which we added correlational by either density or area interactions to test whether correlational selection differed between treatments. Because our measures of fitness followed a Poisson (fecundity) and binomial (viability) distribution, significance was established using the change in deviance between the reduced and complete models and tested against a chi-square distribution with appropriate change in degrees of freedom.

CANONICAL ROTATION AND CUBIC SPLINE VISUALIZATION

The importance of rotating the nonlinear selection matrices, to potentially reveal quadratic selection along some of the canonical axes, has been reemphasized recently (Blows and Brooks 2003; Blows 2007; see also Philips and Arnold 1989), where the canonical axes define major axes of selection, specifically those with maximum levels of quadratic selection (Kruuk and Garant 2007). However, there are relatively few cases where such analyses have been performed, which makes it hard to conclude much about the general utility of this method (Kruuk and Garant 2007). We thus performed canonical rotation of the γ matrix to analyze nonlinear relationships along the major axes of selection: first, at the population level, and second, in the two areas separately. The γ matrix is built from the nonlinear quadratic gradients γ_{xx} along its diagonal and the correlational interaction gradients γ_{xy} off the

diagonal. The canonical analysis (using SAS PROC RSREG, following Blows and Brooks 2003) then returns the eigenvectors (m_i) of γ that together form the **M** matrix, which describes the major axes of the fitness surface with the effect of the cross-product terms removed (Phillips and Arnold 1989; Blows and Brooks 2003). The corresponding eigenvalues (λ_i) of each eigenvector reflect the strength and pattern of nonlinear selection on the associated trait combination. Positive eigenvalues indicate concave selection, whereas negative eigenvalues indicate convex selection (Lande and Arnold 1983; Phillips and Arnold 1989; Blows et al. 2003). As the eigenvectors represent new combinations of the independent variables, we tested for significance of the eigenvalues by rerunning the quadratic analysis with the new independent variables, and thus obtaining the new linear coefficients θ, and the new quadratic coefficients corresponding to the eigenvalues of γ with their standard errors (Blows and Brooks 2003). We again used sequential model building (as above) to test whether there were interactions between breeding density or area (the environmental measures of interest) and our linear- and quadratic-rotated variables. The surfaces were visualized using a cubic spline fitting procedure, with the software STATISTICA (StatSoft 1999).

Results

Means with standard deviations of traits analyzed, as well as data on sample sizes for each fitness component, are presented in Table 1.

POPULATION-LEVEL ANALYSES
Linear selection
When we analyzed the population as a whole, we found significant linear fecundity selection differentials for laying date and for clutch size, but not for egg weight (Table 2). In contrast, the viability selection differential was weaker, but still significant, for laying date but not for clutch size, whereas it was significant for egg weight (Table 2). The magnitude of the linear selection differentials was rather similar to that of linear selection gradients (when including all three traits) for both laying date and egg weight (Table 2A, 2B). In general these results suggest that females that had laid eggs earlier had higher reproductive success and survival, and females that laid larger eggs had higher survival. On the other hand, the clutch size fecundity selection gradient was significantly smaller than the selection differential ($t_{8776} = 2.39$, $P = 0.017$), indicating that the latter was most likely inflated by the indirect effect of laying date (Table 2; also see Fig. 1). Still both estimates showed that females that had laid larger clutches enjoyed a higher reproductive success (Table 2).

Nonlinear selection
At the population level, we found a highly significant negative nonlinear fecundity selection differential for clutch size (Table 2A); we also found highly significant negative quadratic

Table 2. (A) Standardized linear (*i*) and nonlinear (*j*) selection differentials and (B) linear (β_i) and nonlinear quadratic (γ_{ii}) selection gradients for fecundity and viability selection on female reproductive traits in great tits. Values are provided with their standard error. Bold estimates are significant at *P* < 0.05, as assessed from GLMMs.

	Laying date		Clutch size		Egg weight	
(A)	*i*	*j*	*i*	*j*	*i*	*j*
Fecundity	**−0.240±0.018**	−0.002±0.012	**0.147±0.019**	**−0.040±0.013**	0.000±0.019	−0.014±0.012
Viability	**−0.055±0.020**	−0.003±0.013	−0.015±0.019	−0.002±0.013	**0.040±0.019**	0.009±0.012
(B)	β	γ	β	γ	β	γ
Fecundity	**−0.212±0.021**	**−0.027±0.014**	**0.081±0.020**	**−0.057±0.014**	0.019±0.019	−0.003± 0.012
Viability	**−0.065±0.021**	0.002±0.014	−0.029±0.020	−0.006±0.014	**0.040±0.019**	0.012±0.012
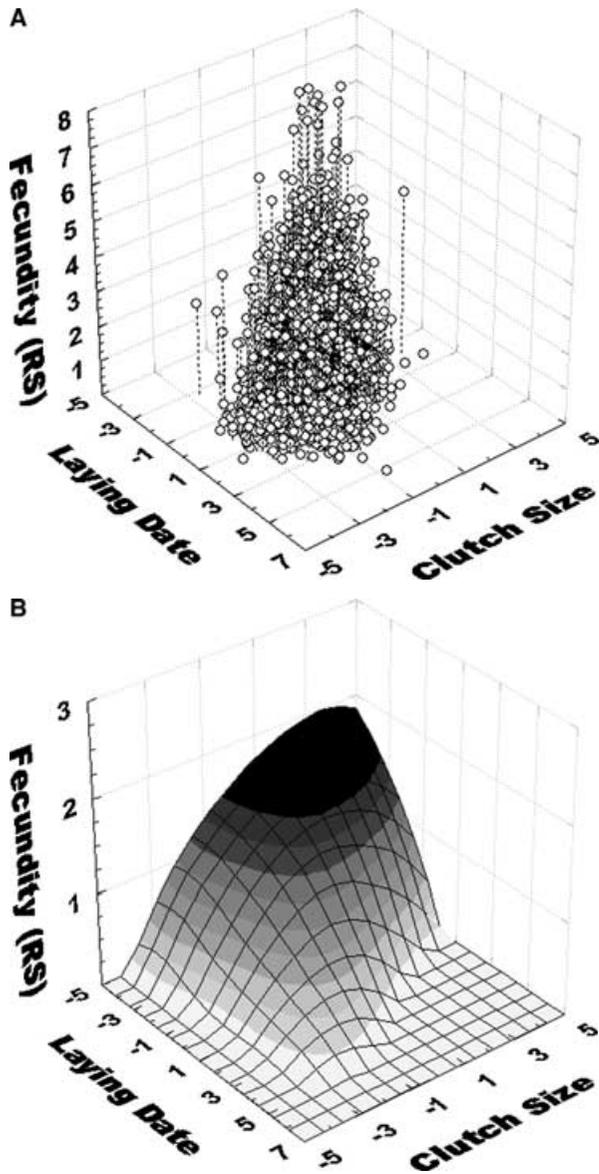
Figure 1. The relationship between fecundity (RS = number of recruits per breeding attempt) and the combination of laying date and clutch size for female great tits in Wytham Woods using (A) raw data from the 4389 observations and (B) a spline surface visualization. Traits are standardized values.

terms for both laying date and clutch size fecundity selection gradients (Table 2B), both of which were indicative of convex (i.e., stabilizing) selection acting upon these traits. In the same model, we also found a significant negative correlational selection gradient between laying date and clutch size (Table 3), in which individuals that laid larger clutches while laying early had the highest reproductive success (see Fig. 1). In contrast, we found no evidence for nonlinear components of viability selection (Tables 2 and 3).

NATURAL SELECTION WHEN LOCAL DENSITY VARIES
Fecundity selection
Partial changes in deviance obtained from the sequential model building showed that territory size did not influence selection on linear ($\chi^2_{(3)} = 2.43$, $P = 0.488$) or quadratic ($\chi^2_{(3)} = 2.65$, $P = 0.449$) terms (note that df = 3 to account for the addition of three interaction terms—see Methods). However, we found a significant effect of territory size on the correlational selection term ($\chi^2_{(3)} = 10.69$, $P = 0.014$). Further inspection of the interactions revealed that this effect was due to a significant increase in the clutch size–egg weight correlational selection term with increasing territory size (interaction term: $\chi^2_{(1)} = 8.18$, $P = 0.004$). An examination of Figure 2 suggests that it is especially beneficial to increase both clutch size and egg weight when territory sizes are at their largest (at 2 ha).

Table 3. Nonlinear correlational standardized selection gradients ($\gamma_{ij} \pm$ SE) for fecundity and viability selection on female reproductive traits in great tits. Estimates and their significance were not obtained using the same error structure (see text for details). Value in bold is significant at *P* < 0.05.

	Laying date	Clutch size
Fecundity		
Clutch size	**−0.057±0.022**	
Egg weight	0.011±0.020	0.002±0.020
Viability		
Clutch size	−0.014±0.023	
Egg weight	−0.033±0.020	−0.020±0.020
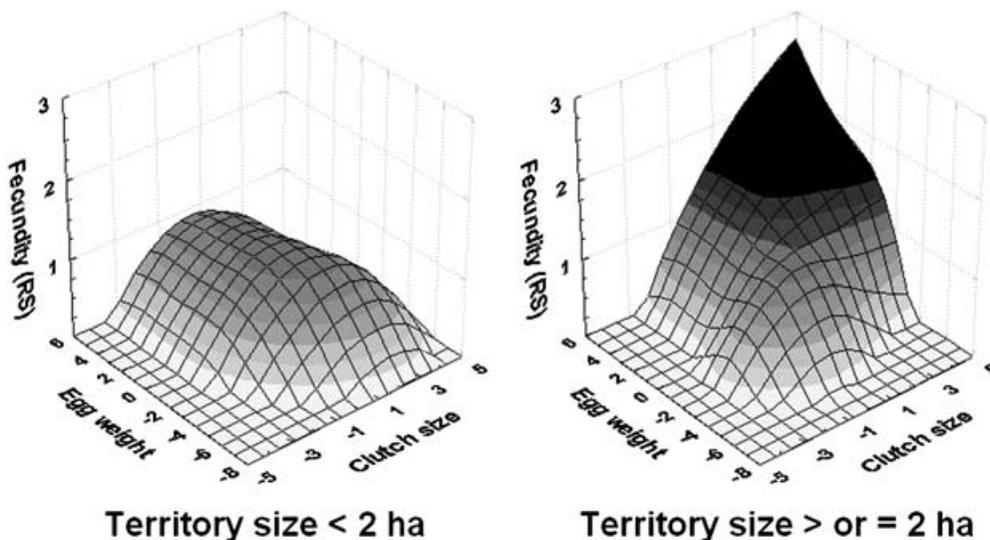
Figure 2. Fecundity fitness surfaces for the combination of egg weight and clutch size depending on the territory size for each female. For illustrative purposes, territories were separated into being either less than or greater than or equal to 2 ha, but in all analyses territory size was treated as a continuous variable.

Viability selection

Territory size tended to influence viability selection on the linear selection term ($\chi^2_{(3)} = 6.46, P = 0.091$). This trend was due principally to increasingly negative viability selection on clutch size with larger territory size ($\chi^2_{(1)} = 4.62, P = 0.032$). Territory size had no significant influence on quadratic ($\chi^2_{(3)} = 2.41, P = 0.492$) or correlational selection gradients ($\chi^2_{(3)} = 0.35, P = 0.950$).

NATURAL SELECTION IN DIFFERENT SUBPOPULATIONS

Fecundity selection

We found significant directional fecundity selection on laying date (negative) and clutch size (positive) in the North, and for laying date (negative) and egg weight (positive) in the East (Table 4). In both areas there was significant convex selection on clutch size (see Table 4), but the curvature was noticeably different between the two areas (Fig. 3). There was also a significant convex selection on laying date and egg weight in the North, but not in the East (Table 4; Fig. 3). Interestingly, we found that the significant negative correlational selection between laying date and clutch size that we had observed at the population level was only present in the North area, when the data were subdivided in this way (Table 4; Fig. 3).

Changes in deviance that were obtained from the sequential model building involving area revealed a marginally nonsignificant difference for linear selection terms depending on area ($\chi^2_{(3)} = 6.83, P = 0.078$), which was mainly due to the egg weight–area interaction ($\chi^2_{(1)} = 3.68, P = 0.055$) reflecting a stronger positive selection on this trait in the East than in the North (Table 3). We found no significant influence of area on either the quadratic terms ($\chi^2_{(3)} = 4.70, P = 0.195$) or the

Table 4. Summary of fecundity and viability selection gradients on female reproductive traits in great tits depending on area. (A) Fecundity and (B) viability. Note that effects and their significance were not obtained using the same error structure (see text for details). Values are provided with their standard error. Bold estimates were significant at $P < 0.05$.

	North	East
(A) Fecundity		
Linear *(β)*		
Clutch size	**0.074±0.031**	0.014±0.041
Laying date	**−0.195±0.032**	**−0.197±0.042**
Egg weight	0.003±0.029	**0.081±0.040**
Quadratic (γ_{ii})		
Clutch size	**−0.073±0.021**	**−0.055±0.031**
Laying date	**−0.039±0.022**	−0.008±0.030
Egg weight	**−0.029±0.018**	0.014±0.029
Correlational (γ_{ij})		
Laying date–clutch size	**−0.081±0.036**	0.006±0.043
Laying date–egg weight	0.037±0.031	0.028±0.043
Clutch size–egg weight	0.029±0.030	−0.011±0.043
(B) Viability		
Linear (β)		
Clutch size	0.020±0.035	**−0.104±0.044**
Laying date	−0.045±0.036	−0.033±0.045
Egg weight	0.014±0.033	**0.078±0.042**
Quadratic (γ_{ii})		
Clutch size	−0.019±0.024	−0.004±0.031
Laying date	0.004±0.025	−0.034±0.032
Egg weight	0.011±0.021	0.011±0.030
Correlational (γ_{ij})		
Laying date–clutch size	−0.001±0.041	0.020±0.046
Laying date–egg weight	0.024±0.035	−0.008±0.044
Clutch size–egg weight	0.006±0.034	−0.032±0.045
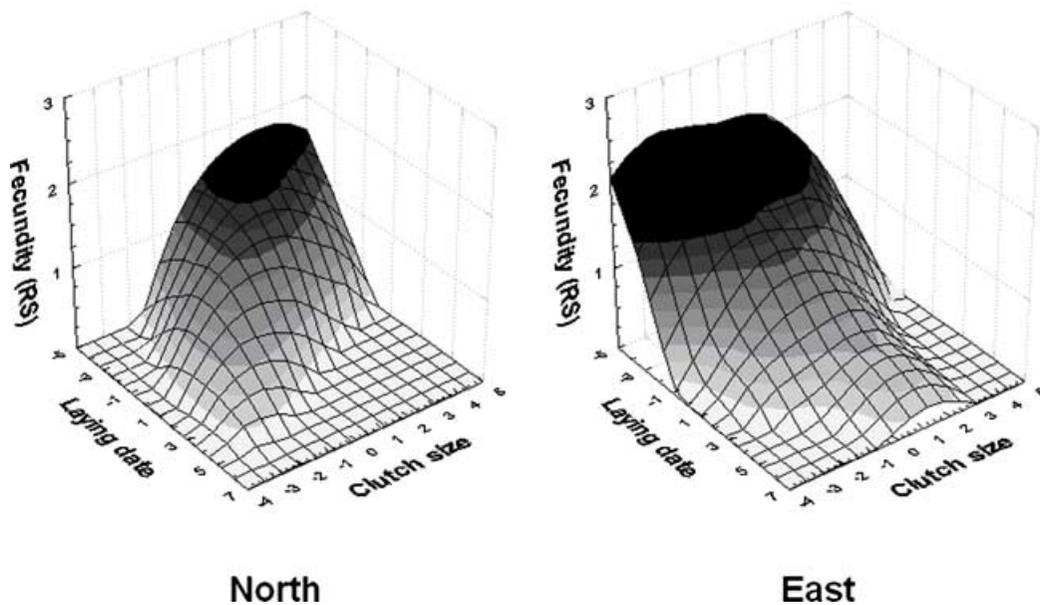
Figure 3. Fecundity fitness surfaces for combination of laying date and clutch size depending on the area of Wytham Woods.

correlational selection terms ($\chi^2_{(3)} = 3.63$, $P = 0.304$), despite the fact that the clutch-size lay–date interaction with area was marginally nonsignificant on its own ($\chi^2_{(1)} = 3.10$, $P = 0.078$), again reflecting stronger correlational selection in the North than in the East (Fig. 3).

Viability selection

We found evidence of linear selection in the East, but in opposite directions depending on the trait analyzed, with clutch size being under negative linear selection and egg weight being positively selected (Table 4). This suggests the presence of a trade-off between these traits in the East, where individuals producing larger eggs but smaller clutches had better survival. There were no other significant components of viability selection (Table 4).

Sequential model-building analyses revealed a highly significant interaction of linear selection gradients with area ($\chi^2_{(3)} = 17.69$, $P < 0.001$). This interaction in turn was explained by a significant interaction of clutch size gradient with area ($\chi^2_{(1)} = 10.70$, $P = 0.001$) reflecting the negative selection on this trait in the East only. The significant linear selection–area interaction was also partly explained by a marginally nonsignificant egg weight–area interaction ($\chi^2_{(1)} = 3.54$, $P = 0.060$) again reflecting the positive selection on egg weight in the East only. The quadratic term–area ($\chi^2_{(3)} = 5.63$, $P = 0.131$) and correlational term–area ($\chi^2_{(3)} = 1.12, P = 0.772$) interactions were not significant.

CANONICAL ROTATION

Fecundity matrix

At the population level, we found that two of the canonical axes m_2 and m_3 had negative eigenvalues (convex selection), which were highly significant and represented largely combinations of clutch size and laying date (Table 5), thus supporting the results obtained using traditional methods (see above). We also found that the stationary point of the population was a maximum (peak), as all three eigenvalues were negative (see Table 5A). This means that given constant fitness, the population mean should evolve toward the steepest uphill slope to reach a peak. There was no significant effect of territory size on linear ($\chi^2_{(3)} = 2.43$, $P = 0.488$) or quadratic ($\chi^2_{(3)} = 1.38$, $P = 0.710$) selection along the eigenvectors of the surface obtained from canonical rotation at the population level.

Analyses performed contrasting the two areas revealed a highly significant interaction of area with the linear selection terms ($\chi^2_{(3)} = 73.54$, $P < 0.001$), in which each main axis significantly interacted with area (m_1: $\chi^2_{(1)} = 21.61$; m_2: $\chi^2_{(1)} = 44.82$; m_3: $\chi^2_{(1)} = 4.74$; all $P < 0.05$). There was also an almost significant interaction of area with the quadratic term ($\chi^2_{(3)} = 7.63$, $P = 0.054$), which was due to a significant interaction between axis m_1 and area ($\chi^2_{(1)} = 4.52, P = 0.033$) together with a contribution from the nonsignificant interaction of axis m_3 and area ($\chi^2_{(1)} = 2.70$, $P = 0.100$; see Fig. 4).

The fitness surfaces in Figure 4 show how the eigenvector with the strongest nonlinear selection gradient in both areas (m_3) combines with the axis that is significantly different among area (m_1). The surface defined by m_1 and m_3 (Fig. 4A) shows a peak in the North that is due to the relatively strong convex selection on clutch size and laying date that both load quite heavily on the axis m_3 (Table 5). The peak shape is also driven by the strong linear selection on m_1 that largely illustrates the significant linear selection on laying date, which loads heavily on this axis (Table 5).

Table 5. The M matrix of eigenvectors (m_i) obtained from the canonical rotation analysis of the matrix γ of (A) fecundity and (B) viability selection. The eigenvalues (λ_i) give the strength and shape of nonlinear selection and θ provides the strength of linear selection. (i) entire population, (ii) North area, and (iii) East area. Values in bold are significant at $P < 0.05$.

	Clutch size	Laying date	Egg weight	θ	λ_i
(A) Fecundity					
(i) Overall					
m_1	−0.189	0.400	0.897	**−0.087**	−0.001
m_2	0.480	−0.759	0.440	**0.202**	**−0.012**
m_3	0.857	0.514	−0.049	−0.044	**−0.074**
(ii) North					
m_1	−0.407	0.805	0.431	**−0.185**	−0.008
m_2	0.461	−0.226	0.858	**0.065**	−0.026
m_3	0.788	0.548	0.279	−0.043	**−0.106**
(iii) East					
m_1	−0.050	0.429	0.902	−0.020	0.021
m_2	0.123	0.899	0.420	**−0.214**	−0.014
m_3	0.991	−0.090	0.097	0.040	**−0.056**
(B) Viability					
(i) Overall					
m_1	−0.144	−0.545	0.826	**0.074**	0.025
m_2	−0.747	0.607	0.271	0.000	0.003
m_3	0.649	0.578	0.494	−0.035	−0.020
(ii) North					
m_1	0.057	0.587	0.808	−0.017	0.020
m_2	−0.171	0.803	−0.571	−0.049	−0.005
m_3	0.984	0.106	−0.146	0.012	−0.019
(iii) East					
m_1	−0.561	−0.166	0.811	**0.133**	0.024
m_2	0.764	0.275	0.584	−0.038	−0.014
m_3	−0.320	0.947	−0.027	0.003	−0.038
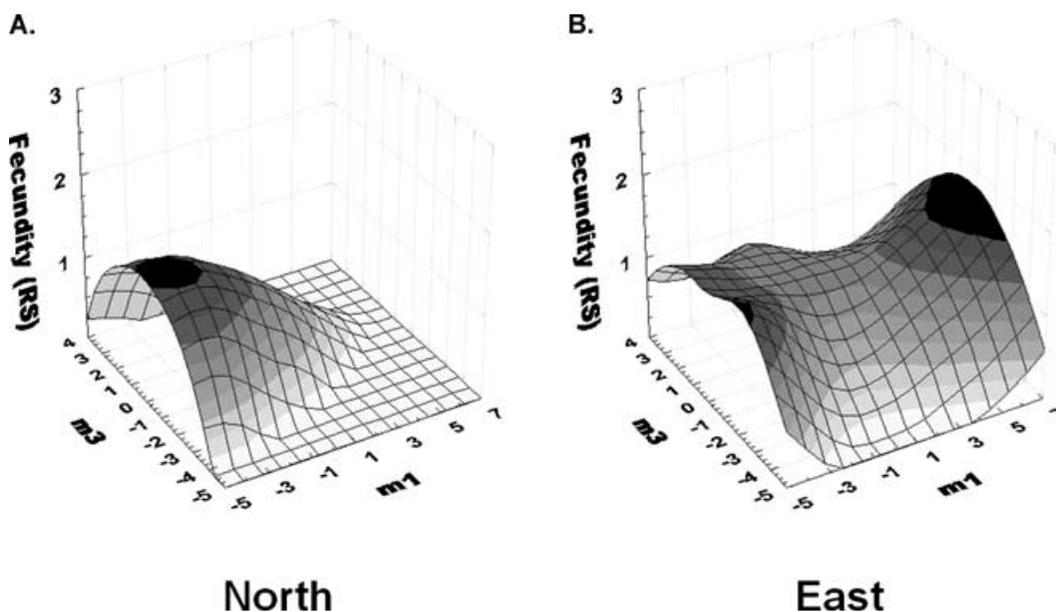
Figure 4. Fecundity selection surfaces visualized with cubic splines along the m_1 and m_3 main axes of selection.

This again suggests that high negative values of laying date are important determinants of female reproductive success.

The saddle-shaped fitness surface in the East (Fig. 4B) represents the concave, but nonsignificant, selection acting on m_1, and the convex selection acting on m_3. The combination of selection on m_1 and m_3 tended to produce two peaks, one for negative values of m_1 and one for positive values of m_1. Although the former is difficult to interpret, the positive peak most likely represents selection for larger egg weight. Both are located at intermediate values of m_3 because of the convex selection on clutch size, which loads very heavily on this axis (Table 5).

Viability matrix
No significant eigenvalues were found after rotating the viability nonlinear matrices, either at the population level or within area (Table 5). Interactions with territory size were also not significant (not shown). Interaction among areas with the linear terms of the rotated surfaces was significant, and only due to the difference among the m_1 axes that reflected positive selection for egg weight and negative selection for clutch size in the East but not in the North (see Tables 4B and 5B). No significant interaction was detected among areas for the quadratic term.

Discussion
Our analysis of a large sample of reproductive traits from a population of great tits showed that female reproductive traits are under natural selection (quite strong at times), and that the form of natural selection on these traits varied both with respect to the character and on the component of fitness being examined. More important, we found that patterns of selection were variable within the population studied, and that the way selection acted on individual traits and their combination depended on local breeding density and on a contrast between two parts of the study population.

Many studies aimed at detecting natural selection have suffered from low sample size and thus limited statistical power (Kingsolver et al. 2001; Hersch and Phillips 2004). This lack of power was shown to be even a greater problem when dealing with binomial distributions (Hersch and Phillips 2004) suggesting potential bias in conclusions on the relative strength of fecundity and viability selection (Kingsolver et al. 2001). Here we confirmed, using large samples from a long-term study, that fecundity selection is indeed stronger than viability selection in this system (see also Svensson et al. 2006). It is also possible that the difference in magnitude of the two kinds of selection we observed could arise because the strength of viability selection is suggested to decrease with the duration of the selection episode (see Hoekstra et al. 2001). However, a recent study by Svensson et al. (2006) also showed that fecundity was more important than viability selection irrespectively of the time period over which it was assessed. It must

also be pointed out that the traits analyzed here are directly associated with reproduction, and close links with fecundity might then be expected.

POPULATION-LEVEL ANALYSES
At the population level, we found that linear fecundity selection favored an earlier laying date. Thus, our results are in agreement with previous findings for this species, and for other small songbirds in temperate regions, where early timing of breeding has been shown to be important for recruitment of offspring to the breeding population (Verhulst et al. 1995; Verboven and Visser 1998; Sheldon et al. 2003). Our results also indicated linear selection (although weaker) for viability favoring early breeders. Previous studies have suggested that the difference in survival for early versus late breeders is mainly due to a difference in individual quality where early breeding females are able to breed early in all years and thus produce more young, but still survive better than late breeders who produce fewer offspring (Verhulst et al. 1995). It should also be noted that the relationships between both fitness components and laying date is if anything conservative and not a result of differential dispersal outside of Wytham by late breeders or their offspring, as previous studies in this population (Verhulst et al. 1997) have shown that dispersal outside the study area is slightly higher in earlier hatched birds.

We found positive fecundity selection for clutch size, which supports the general assumption that larger clutches than average are the most productive in terms of offspring produced (Lack 1966; Boyce and Perrins 1987; but see also Price and Liou 1989). Our findings, however, contrast with another large-scale analysis of selection on clutch size that failed to find any consistent direct selection for this trait in the collared flycatcher *Ficedula albicollis* (Sheldon et al. 2003). However, in that species clutch size is relatively less variable than in the great tit, which may reduce the scope for selection. We also found strong evidence for convex fecundity selection on clutch size, and to a lesser extent, on laying date, together with correlational selection on the combination of traits (with the combination of early laying dates and large clutches resulting in a greater reproductive success; see Price et al. 1988; Fig. 1). To our knowledge, this is the first documented evidence of correlational selection acting on these two traits in a passerine bird (but see also Perrins and McCleery 1989 for an early illustration of the presence of such process in the current population), although very few studies have estimated correlational selection on these traits (Charmantier et al. 2006). Negative correlational selection between laying date and clutch size has also been recently documented in a population of mute swans *Cygnus olor* (Charmantier et al. 2006), even though this species has a quite different breeding ecology from the great tit. This form of selection might be common among these reproductive traits in birds; a decline in clutch size with breeding date is commonly observed in wild bird

populations. Correlational selection has been argued to be an important determinant of the genetic covariance of traits (Sinervo and Svensson 2002; see McGlothlin et al. 2005 for an example). We might thus expect to find a negative genetic correlation between clutch size and laying date that would have developed as a result of a persistent negative correlational selection pressure acting on these traits, and preliminary investigations of the genetic architecture of these traits suggest that the genetic covariance is indeed negative (D. Garant, G. D. Hadfield, L. E. B. Kruuk, and B. C. Sheldon, unpubl. data).

Analyses performed at the population level for egg weight did not suggest any fecundity selection on this character. There is, in general, little evidence of a positive relationship between egg size and fitness in birds (reviewed in Williams 1994), which constitutes something of a puzzle, as the phenotypic correlation between egg size and clutch size is generally negative, consistent with a trade-off between egg size and number. Our understanding of the proximate causes of egg-size variation and the evolutionary significance of this variation thus remains unclear. Although variation could be due to differences in optimum egg size between individuals, the aspects of a female phenotype that would determine such optimum are unknown (Christians 2002). Nevertheless, in this study, egg weight exhibited a positive linear viability selection component. This relationship was presumably indicative of variation in female quality where individuals able to produce larger eggs enjoy elevated survival (see also Hõrak et al. 1997).

SELECTION AND BREEDING DENSITY

Our analyses of selection depending on local breeding density suggested that variation in this environmental effect generated different selection pressures. Specifically, larger territories (low density) seemed to favor female reproductive success from a combination of larger clutch size and egg mass (Fig. 2). This finding is broadly consistent with previous evidence that birds adjust their clutch size upwards in response to lowered density (Both 1998; Wilkin et al. 2006). In the current population, previous analyses of reproductive traits also showed that higher density of breeders negatively affected annual mean values of clutch size and egg weight (Perrins 1965; Perrins and McCleery 1989, 1994).

SELECTION IN SUBPOPULATIONS

Our analyses of natural selection made separately in two contrasting areas of Wytham Wood show that different patterns of combination of these reproductive traits are favored over quite small spatial scales. First, we found differences in linear fecundity selection patterns that depended on the area, with larger clutches only favored in the North and larger eggs only favored in the East. In addition, we found that viability decreased with a larger clutch size, but increased with larger egg weight in the East. There was no evidence of significant viability selection on these traits in the North. The patterns of selection that we documented correspond with the trait means for these characters in the two areas (Table 1; Clutch size: East $= 8.61 \pm 0.039$ eggs, North $= 9.00 \pm 0.033$ eggs, $t_{3996} = 7.75$, $P < 0.001$; Egg weight: East $= 1.690 \pm 0.004$ g, North $= 1.678 \pm 0.003$ g, $t_{3486} = 2.49$, $P = 0.013$). These observations suggest the possibility that female great tits show adaptive phenotypic plasticity to local conditions (Schlichting and Pigliucci 1998) adjusting the values of these traits with respect to cues of the form of selection on the traits. The observation of larger eggs in the East area also agrees with the suggestion that production of large eggs should be more beneficial under harsher conditions (Parker and Begon 1986; see Smith et al. 1995). We have shown previously that chick mortality is higher (Shapiro et al. 2006) and lifetime reproductive success lower (Garant et al. 2005) for birds breeding in the East compared to individuals from the North, effects that are indicative of a lower-quality habitat (but see also Postma and van Noordwijk 2005).

In both areas, earlier laying increased the number of surviving offspring. The observation that early breeding results in higher reproductive success, independent of habitat characteristics, is consistent with the suggestion that breeding early is physiologically challenging across all habitats, as it requires females to devote large quantities of resources to maturing eggs at a time (early spring) when food is relatively scarce (Perrins 1979). Still convex selection on laying date and correlational selection between laying date and clutch size, which we observed at the population level, were only detected in the North, indicating differences in selective pressures among areas on this trait. Convex selection on clutch size was present in both areas, but a closer examination of the fitness surface suggests that true stabilizing selection, in which an intermediate optimum within the range of phenotypes observed (Mitchell-Olds and Shaw 1987), was only present in the East (Fig. 3).

The choice of these two subpopulations for analysis was based on observations of differences in many reproductive traits (including recruitment success of offspring), and we must be cautious about drawing general conclusions from a potentially biased selection of populations. Nevertheless, the main point of choosing these two areas to compare the form of selection is that as we know the birds experience different environmental conditions, they provide a test of whether two different parts of a population can experience different forms of natural selection. The causes of the difference in form of selection between the two areas is not known: any two sites will differ along many axes that potentially contribute to fitness variation, but the demonstration of differences suggests that experimental tests of candidate mechanisms (e.g., by manipulating food availability, or the density of competitors) might be useful.

PREDICTING RESPONSES TO SELECTION

Our selection estimates suggest that reproductive traits in great tits could evolve differentially depending on density and habitat (and given heritable genetic variation). However, although selection analyses on phenotypes alone tell us directly about the way that the environment favors different trait combinations, predicting potential response to selection using phenotypic values is not straightforward. Indeed, in some instances, fitness could be associated with only the environmentally determined components of the phenotypes, in which cases there will be no evolutionary responses to selection (see Kruuk et al. 2003). Thus, if the main aim of a study (which was not the case here) is to use estimates of selection to predict evolutionary change between generations, a potential solution would be to assess selection acting directly on estimated breeding values (EBVs) of the traits of interest (see Rausher 1992). However, it should be noted that EBVs also come with their potential drawbacks. In particular, in cases where EBVs are obtained for sex-limited adult traits (as in our study), estimates are going to be less accurate than where there are more observations available for families (e.g., nestling characters; see Postma 2006 for a detailed discussion).

CANONICAL AXES OF SELECTION

Results of the canonical rotation analyses of the fecundity matrix revealed a single main axis of nonlinear selection in the North (m_3) and also in the East (m_3). Yet the underlying components of these axes were different depending on the area, as only clutch size loaded on this axis for the East, whereas both clutch size and laying date were represented in the North. Also we found that eigenvalues differed in sign in the East but not in the North. Consequently, the resulting fitness surfaces were of different shapes, having a peak in the former and a saddle in the latter (see Fig. 4). If fitness remains constant, the population mean is expected to evolve in the direction of the steepest uphill slope to reach a local peak. Alternatively, a saddle point is expected to be stable only in cases where selection changes when the population is reaching the peak and forces the population back to the saddle point (Lande 1976; Brodie 1992). The appearance of a saddle-shaped surface in the East must be interpreted cautiously, as concave selection on m_1 was not significant. Finally, canonical analyses did not provide any new insights with respect to the potential presence of nonlinear viability selection matrices. Rotation yielded the same patterns as the traditional nonlinear selection analyses, and thus reorientation of the axes had little impact on the general qualitative impression of the selection patterns in the current system (also see Kruuk and Garant 2007).

Conclusions

Our aim in this study was twofold: to conduct a thorough analysis of multivariate selection on female reproductive traits via differ-

ent fitness components, and to determine whether the form of natural selection depends on some ecologically relevant environmental parameters. Despite much attention to natural variation in breeding time and clutch size in wild bird populations, these traits have rarely been subject to multivariate selection analysis (Sheldon et al. 2003; Charmantier et al. 2006). Our analyses show that selection is substantial, interestingly nonlinear, and in the case of clutch size, that much of the directional selection differential on this trait is due to the indirect effect of selection on breeding time. Our results also suggest that consideration of ecological or spatial variation when estimating natural selection can lead to different conclusions about the way selection acts in the wild. Our analysis, and that of Svensson and Sinervo (2004), strongly suggests that reports of global population-level selection gradients should be complemented by analyses made on a local scale to gain a better understanding of evolutionary dynamics in wild populations. Finally, our results also underline the value of long-term data sets for studies of natural selection in the wild, and, especially, for assessing nonlinear selection components that are usually hindered by low sample size and statistical power.

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