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Natural selection on individual clutch size–laying date trends in the Ural owl

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ABSTRACT

Clutch size declines with advancing laying date in many birds. This relationship is thought to represent a reaction norm of individual optimization in response to local environmental conditions. This implies that: (1) individuals should vary in the properties of their clutch size–laying date trends; (2) these differences should be reflected in their fitness; and (3) parts of this variation should be heritable. Here, we show that 44 Ural owl females of known lifespan, with a statistically sufficient number (≥ 5) of clutch size–laying date observations each, differed individually both in the slope and the elevation of their clutch size–laying date relationships. As an estimate of individual fitness, we used the lifetime production of fledglings, which is a known correlate of recruitment in this population. Females with a higher elevation had a higher lifetime reproductive success. However, plasticity – that is, the slope of the clutch size–laying date relationship – did not have apparent advantages in terms of a female's fitness.

Keywords: clutch size–laying date trend, life history, lifetime reproductive success, phenotypic plasticity, *Strix uralensis*, Ural owl.

INTRODUCTION

Natural selection has moulded animals to respond conditionally to different environmental conditions – that is, they are phenotypically plastic. A trait is said to be phenotypically plastic when one genotype can produce different phenotypes in different environments (Bradshaw, 1965; Stearns, 1992; Roff, 1997). Usually, the traits considered have been morphological ones, but life-history traits are also plastic; for example, the decline in the optimal size at maturity with increasing developmental time depends on conditions during growth (e.g. Kawecki and Stearns, 1993). An adaptive reaction norm describes optimal phenotypic responses over a range of environments (Houston and McNamara, 1992; McNamara and Houston, 1996; Kisdi *et al.*, 1998). The reaction norm concept can also be applied to repeated reproduction of long-lived organisms (Rowe *et al.*, 1994). If environmental conditions vary during the lifetime of an individual, organisms should adjust their reproductive effort accordingly (Hirschfield and Tinkle, 1975); that is, they produce many

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offspring when the food supply is good, but produce fewer offspring or even refrain from breeding when food is in short supply.

Traditionally, quantitative genetic theory has considered selection on laying date (Price *et al.*, 1988) and clutch size (Price and Liou, 1989) separately. Nevertheless, in many bird species, clutch size and laying date are strongly coupled in that clutch size declines with advancing laying date (Klomp, 1970; Murphy and Haukioja, 1986). This seasonal decline has been addressed explicitly using optimization theoretical arguments. The clutch size–laying date relationship is an outcome of individual optimization of (1) the advantages of early breeding, which increase offspring survival, and (2) the delay of laying, which gives parents more time to gather resources (Daan *et al.*, 1990; Rowe *et al.*, 1994; Daan and Tinbergen, 1997). In fact, the decision of which clutch size to produce at a certain laying date is probably a female trait. Female kestrels (*Falco tinnunculus* L.) are thought to possess an endogenous physiological program, which adjusts first laying date and then clutch size to the nutritional conditions of the female (Meijer *et al.*, 1990). Variation in food supply is thus converted into variation in reproduction, both between individuals within a year and within individuals between years. As in birds of prey in general (Newton, 1979), Ural owl (*Strix uralensis* Pall.) females cease to hunt when they start forming eggs, and continue to be fed by the male until the late nestling phase (Lundberg, 1981). By depending on the male while forming eggs and caring for eggs and young, a female converts any variation in the environment into variation in reproduction. From the female's point of view, environmental variation includes general food supply, male quality as a hunter and variation in food supply between territories.

In this study, we extend the quantitative genetic framework for the clutch size–laying date relationship (Price and Liou, 1989) to study selection in progress at the reaction norm level (*sensu* Weis and Gorman, 1990), using 23 years of reproductive data. We consider the seasonal decline of Ural owl clutch size (Pietiäinen, 1989) as a female-determined trait, an individual policy that specifies how many eggs to lay at which laying date. This policy has two properties: first, the average clutch size produced over the range of laying dates, which we here term 'elevation', and, second, the deviations from this average due to plasticity, which are described by the slope of the individual's seasonal decline in clutch size (de Jong, 1990; Roff, 1997). Thus, the relationship between individual i's clutch c_i and laying date d is dictated by

$$c_i = e_i + s_i d + \varepsilon \qquad (1)$$

where e_i and s_i are the individual properties of elevation and slope, respectively, and ε is the error (environmental noise). Note, again, that this formulation assumes that laying date is environmentally determined, but that the decision which clutch size to produce at a certain date is determined by individual properties. These properties can be targets of natural selection (McNamara and Houston, 1996). For selection to operate, however, one should demonstrate first that individuals differ in the properties of their clutch size–laying date relationship. To our knowledge, this has not been studied previously in wild birds, although this link is an essential element in the evolution of the clutch size–laying date reaction norm.

MATERIALS AND METHODS

Since 1977, we have been studying a population of Ural owls with 80–100 pairs annually, although these do not necessarily breed every year (Brommer *et al.*, 2002). All pairs breed in nest boxes at a distance of 3–4 km from each other in a study area of about 1500 km^2 in southern Finland. All breeding females were identified annually by capturing them on their nest. Handling them did not markedly disturb them. Males were caught with a swingdoor trap attached to the nest box. Laying dates were determined either by a visit to an incomplete nest or by backdating from the wing-length of young. At the time of laying, the Ural owl mainly depends on microtine voles for prey. In southern Finland, these voles show large fluctuations in their population numbers, following a regular 3 year cycle of low, intermediate and high densities (Hanski *et al.*, 1991; Norrdahl, 1995). In our study area, the density of voles may vary by up to 50-fold (Brommer *et al.*, 1998). This environmental variation is reflected in the reproduction (clutch size, laying date) of the owls (Pietiäinen, 1988, 1989). Both sexes of the Ural owl are strictly territorial and highly site-tenacious all year round (Lundberg, 1979; Saurola, 1987). A breeding attempt was defined as the laying of a full clutch. Females that repeatedly produced clutches that never hatched were discarded from the analyses. Laying date was standardized with the long-term median (31 March), so that, for example, 1 is 1 April and −10 is 21 March.

Because we were interested in relating individual properties of the clutch size–laying date relationship to measures of lifetime fitness, we only used data for females that initiated and ended their breeding career in our boxes. Lifetime reproductive success was considered as the total production of fledglings during a female's lifetime. A female was considered dead if she had not bred for at least two consecutive years (see also Brommer *et al.*, 1998). We have shown previously that a female's lifetime production of fledglings correlates with her lifetime production of recruits – that is, offspring that recruit into the breeding population ($r = 0.48$, $n = 126$; Brommer *et al.*, 1998).

A mixed-model analysis of variance (ANOVA; Zar, 1999) was used to test for individual differences in the clutch size–laying date relationship. Individual was considered as a random effect and laying date as a fixed effect; a significant individual term indicates that individuals differ in their elevation (least square means) and a significant interaction term indicates that the slopes of the clutch size–laying date relationship differ (Zar, 1999). A female's least squares mean clutch size – that is, her expected clutch at the average laying date – was used as a measure of elevation.

We then calculated the female's slope, which we defined as the rate of decrease of her expected clutch size with advancing laying date. A female's slope was calculated as the regression coefficient (b) of the linear regression of clutch size against laying date for each individual separately. The accuracy of the coefficient b in a linear regression analysis is highly dependent on the sample size (n) used, because the power (β) of this test is given by

$$Z_\beta = (z - z_\alpha)\sqrt{n-3}$$

where Z_β is the normal deviate of the power β, z and z_α are the z-transforms of the sample's r and the critical r at the α significance level, respectively (Zar, 1999). For the coefficient b to have any reliability, n thus needs to be larger than 3 and the term $z - z_\alpha$ needs to be positive. In the Ural ow, $r_{\text{clutch size–laying date}} = 0.63$ (Pietiäinen, 1989) and thus $z = 0.74$. The critical z values for a one-tailed α of 0.05 are 0.81, 0.73 and 0.66 for sample sizes of 4, 5 and 6, respectively, when using Fisher's (1958) z corrected for small sample sizes (Zar, 1999). A minimal sample size of 5 is thus needed for $(z - z_\alpha)$ to be positive. We therefore only included females that bred at least five times in the analyses. The females in this sample form a selected subset of longer-lived individuals, because female Ural owls breed on average 2.7

times during their lifetime (Brommer *et al.*, 1998). For the mixed-model ANOVA described above, the result did not change when females which bred less than five times were also included. For consistency, however, we use the same data set throughout.

To analyse selection, we followed Lande and Arnold (1983), where the strength of directional selection, indicated by the coefficients β, on elevation and slope are the regression coefficients of linear regression on lifetime reproductive success. Stabilizing or disruptive selection coefficients γ are the regression coefficients for the quadratic terms in a model, which also includes the linear terms and the interaction 'elevation × slope'. For both models, the constant was included.

RESULTS

There were 44 females of known lifetime reproductive success, with at least five observations of laying date and clutch size per female. Although these females represented only 14% (44/313) of all females breeding during the study period, their reproductive output formed a significant contribution to the population's dynamics, as they produced 32% (286/907) of all clutches with known laying dates and 44% (46/104) of all recruits during 1977–98. Overall, the clutch size of these females declined with advancing laying date (constant: 3.4 ± 0.05, $t = 69.1$, $P < 0.001$; b: -0.064 ± 0.005, $t = 13.7$, $P < 0.001$; $n = 286$; $F_{1,284} = 187.2$, $P < 0.001$; $r^2 = 40\%$). There was a strong individual effect, which explained an additional 30% of the variance (laying date: sum of squares = 72.3, $F_{1,43} = 91.0$, $P < 0.001$; female: sum of squares = 65.1, $F_{43,198} = 3.0$, $P < 0.001$; female × date: sum of squares = 34.2, $F_{43,198} = 1.6$, $P = 0.02$; error: sum of squares = 99.4; $n = 286$, $r^2 = 70\%$). Females thus had different slopes and different elevations in their clutch size–laying date trends (Fig. 1). The difference between the smallest (2.36) and largest (4.65) elevation was almost two-fold.

Most females showed a trend for a decrease in clutch size with advancing laying date. The estimates for slope varied from a decrease of −0.168 eggs per day to an increase of 0.006 eggs per day and, on average, showed a decrease of -0.067 ± 0.006 eggs per day. Elevation and slope were not correlated ($r = -0.17$, $n = 44$, $P = 0.29$). Because an earlier study (Pietiäinen, 1989) as well as our subset of 44 females showed a linear decrease in clutch size, we tested each individual one-tailed for a significant seasonal decrease in clutch size (H_1: $b < 0$). One half (22/44) of the females had a statistically significant seasonal decrease in their clutch size (Fig. 1). It should be noted that the realized power for this test is higher than the minimal power for a sample size of 5 (see above), because for 64% (28/44) of the females more than five combinations of their clutch size and laying date were known (average 6.5 times, range 5–13).

Using multiple regression (Lande and Arnold, 1983), we evaluated the effect of elevation and slope of the females' clutch size–laying date relationship on the number of offspring produced during a female's lifetime. Because a female's lifetime is a major component of lifetime reproductive success (Brommer *et al.*, 1998; Fig. 2a), we included this factor in the analysis. The number of times a female bred was neither correlated with elevation ($r = -0.131$, $n = 44$, $P = 0.39$) nor with slope ($r = 0.017$, $n = 44$, $P = 0.91$). Females with a higher elevation in their clutch size–laying date trend produced more offspring during their lifetime (Table 1; Fig. 2b). In terms of lifetime reproductive success, the slope of the female's clutch size–laying date trend was unimportant, both in terms of directional and stabilizing selection (Table 1; Fig. 2c).

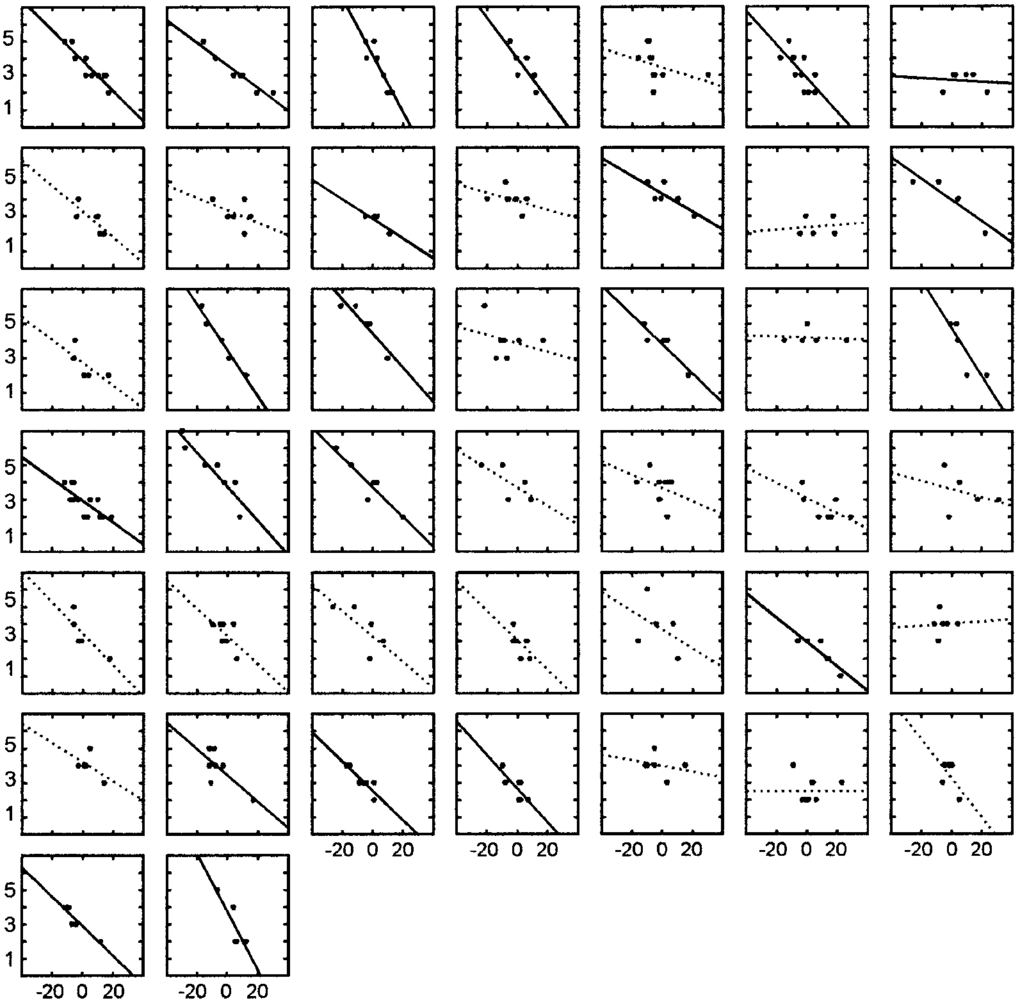

Fig. 1. The relationship between laying date and clutch size for 44 Ural owl females of known lifetime reproductive successs that bred at least five times. Laying date 0 indicates 31 March. The slope for each female is shown as a regression line. This line is dashed for females that showed a non-significant (one-tailed) decrease in clutch size with advancing laying date. The average laying date was 0.2 ($n = 286$); the expected clutch size at this date, as given by the regression line, is the elevation of the female's clutch size–laying date trend.

DISCUSSION

Our analyses are based on the perspective, motivated by quantitative genetic arguments (de Jong, 1990; Weis and Gorman, 1990), that the evolution and maintenance of a seasonal decline in clutch size can be attributed to selective differences for heritable, individual components that dictate the relationship between clutch size and laying date (equation 1). Instead of viewing clutch size and laying date as separate traits (e.g. Price *et al.*, 1988; Price and Liou, 1989), we consider these traits to be individually coupled (Daan *et al.*, 1990;

Table 1. Selection coefficients on lifetime reproductive success for elevation (least squares means) and slope of the linear trends in Fig. 1

Coefficient (property)	Estimate (± standard error)	*P*
Times bred	1.96 ± 0.26	< 0.001
β (elevation)	1.97 ± 0.96	0.04
β (slope)	−0.63 ± 12.6	0.96
γ (elevation^2)	3.16 ± 1.80	0.09
γ (slope^2)	−130 ± 251	0.61
γ (elevation × slope)	22.3 ± 24.1	0.36

Note: The number of times a female bred was included to correct for differences in this property across females (Fig. 2a). The strength of directional selection is indicated by the coefficients β. The coefficients γ indicate stabilizing (negative γ) or disruptive selection (positive γ). See text and Fig. 2 for further details.

Meijer *et al.*, 1990). Hence: (1) individuals could show variation in properties describing how clutch size and laying date are coupled; (2) this variation should be related to a measure of fitness; and (3) some part of this variation should be heritable (de Jong, 1990; Weis and Gorman, 1990; Roff, 1997; Schlichting and Pigliucci, 1998).

Our results relate to points (1) and (2) above. Our main finding is that females do indeed have individual differences in both the elevation and the slope of their clutch size–laying date trends. A seasonal decline in clutch size is the predominant trend observed in our study population and is attributable to an individual seasonal decline in clutch size, which about half of the females show. No female showed a significant seasonal increase in clutch size. This result thus supports the notion that natural selection on the clutch size–laying date relationship can operate through differential reproduction of females possessing individual reaction norms. We tested this notion in the Ural owl by analysing selection at the reaction-norm level (Weis and Gorman, 1990) and testing whether individual properties of the clutch size–laying date trend are linked to a measure of individual fitness. We considered lifetime reproductive success, a known correlate of the number of recruits in this population (Brommer *et al.*, 1998), as an estimate of individual fitness. For females that bred the same number of times, a higher elevation of the clutch size–laying date trend led to more offspring being produced over her lifetime. This result agrees well with previous findings, because a female's breeding performance at the average laying date (i.e. her elevation) correlates well with her average clutch size produced ($r = 0.78$ in our case). Average clutch size is an important component for variation in lifetime reproductive success (in general: Clutton-Brock, 1988; Newton, 1989; in the Ural owl: Saurola, 1989; Brommer *et al.*, 1998).

Our analysis further suggests that non-plastic females – that is, females that produce similar clutch sizes at every laying date – are equally successful as plastic females, who adjust their clutch size. However, we may not have adequate sample sizes to detect selection on slope, because this may be weak. In the only other study to quantify fitness at the reaction norm level, selection on slope was much weaker than selection on elevation (Weis and Gorman, 1990). Putative weak selection on slope is further aggravated by the larger statistical error inherent in measuring slope than in measuring elevation (Zar, 1999). Furthermore, lifetime reproductive success has been criticized as a measure of individual fitness (e.g. Grafen, 1988; Murray, 1992) and could, therefore, be unable to detect selection adequately. For example, lifetime reproductive success is insensitive to differences in the

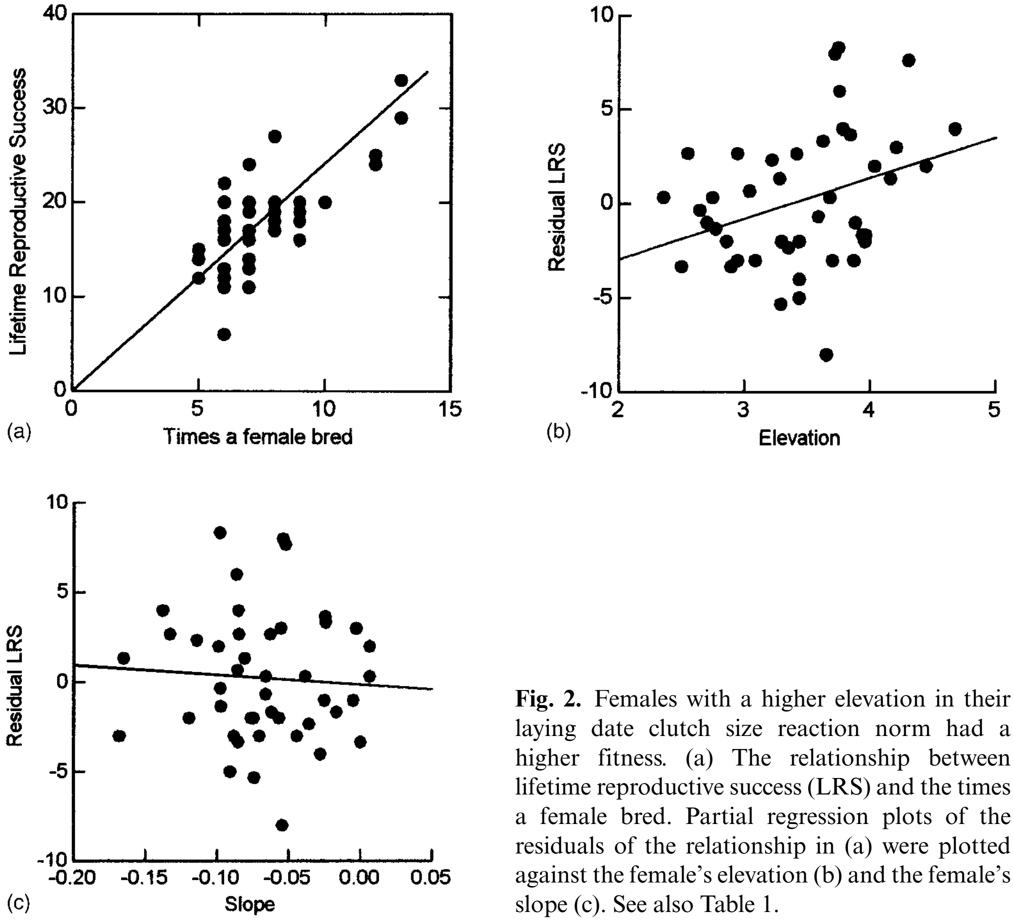

Fig. 2. Females with a higher elevation in their laying date clutch size reaction norm had a higher fitness. (a) The relationship between lifetime reproductive success (LRS) and the times a female bred. Partial regression plots of the residuals of the relationship in (a) were plotted against the female's elevation (b) and the female's slope (c). See also Table 1.

timing of first reproduction, which may form an important component of fitness (e.g. McGraw and Caswell, 1996).

An alternative view of the relationship between clutch size and laying date, which is based on optimality theory instead of quantitative genetic arguments, states that clutch size and laying date are individually optimized traits in response to local conditions (Daan *et al.*, 1990; Rowe *et al.*, 1994; Daan and Tinbergen, 1997). In these optimality models, local conditions conform to an environmentally determined correlate of laying date and clutch size – termed 'condition' – in the quantitative genetic models of Price *et al.* (1988) and Price and Liou (1989). The combination of omniscient optimization and the unique sequence of (territorial) conditions that an individual experiences during its life, will then determine the elevation and the slope of its clutch size–laying date relationship, irrespective of any individual or genetic components. From this 'environmental effect' perspective, there is no *a priori* reason to expect any selective differences in the individual slopes of the clutch size–laying date relationship *per se*, because this covariation is environmentally determined.

In understanding how individual properties govern the clutch size–laying date relationship of a wild bird population, estimating the heritability of the individual clutch size–

laying date relationship will be the critical next step. Heritability of the individual properties of elevation and slope will show what part of the individual variation we described here can be attributed to additive genetic effects rather than environmental effects (such as territory quality and noise). Unfortunately, because we have only one mother–daughter pair with sufficient observations, we cannot currently determine whether elevation and slope indeed are heritable properties. Intuitively, however, the effects of local conditions, individual-specific components and their interaction, which specify how environmental conditions are translated into reproductive decisions, may play a role in determining the clutch size–laying date relationship. The demonstration that clutch size and laying date are genetically correlated in the collared flycatcher *Ficedula albicollis* is one important step in considering the clutch size–laying date relationship as coupled traits (Merilä *et al.*, 2001). We feel that increased emphasis on individuals, which form the correct level of evolutionary and ecological explanation, will increase our understanding of the seasonal decrease in clutch size as a general phenomenon.

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