Selection on personality in a songbird affects maternal hormone levels tuned to its effect on timing of reproduction

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The increase or decrease in yolk androgens over the laying sequence of a clutch in birds may mitigate or enhance, respectively, the disadvantage of the last-hatched chicks, providing a potentially adaptive tool to adjust brood size to food conditions. This variation may involve a genetic component on which Darwinian selection can act. We found that two lines of a wild bird species selected for bold and shy personalities show, respectively, increased and decreased androgen concentrations over the laying sequence. The line showing the increase laid earlier in the season, when food conditions are normally sufficient to raise the whole brood. The line showing the decrease laid later, when food is normally scarce, which may facilitate brood reduction. The results indicate a correlated response in maternal hormone transfer to genetic selection on personality, which relates to ecological conditions.

Keywords: maternal effects; testosterone; personality; laying date; hatching asynchrony

1. INTRODUCTION

Maternal effects influence offspring development with potentially adaptive consequences (Mousseau & Fox 1998). One example are avian yolk androgens of maternal origin, which affect hatching time, stimulate begging, growth and thereby survival in the offspring (Groothuis *et al.* 2005). Androgen concentrations vary between species, and among and within clutches of the same species (Groothuis *et al.* 2005; Gil *et al.* 2007; Martin & Schwabl 2008).

One adaptive hypothesis explaining such variation relates to within-clutch differences. In many species, yolk androgens increase over the laying sequence, but decrease in others, which may allow mothers to adjust brood size to food availability (Schwabl *et al.* 1997). Parents often start to incubate before clutch completion, first eggs receiving incubation before the last are laid, inducing earlier eggs to hatch sooner than later eggs (hatching asynchrony, HA). Consequently, last-hatched chicks experience a handicap in competition with older siblings. Thus, high androgen concentrations in later-laid eggs may enhance chick competitive ability, mitigating the disadvantages of HA when food is plentiful, while the opposite pattern exacerbates disadvantages of later hatching, facilitating brood reduction under poor conditions. These different patterns were revealed by between-species comparisons. Although theory would also predict systematical and heritable variations within species, this has been overlooked. One study on starlings (Eising *et al.* 2008) showed that androgen levels are characteristic of individual females, while another found an effect of selection on behavioural traits on yolk androgens (Gil & Faure 2007), suggesting such heritable variation.

We sampled great tits selected for distinct personalities (Drent *et al.* 2003) and differing in laying date. In the field, this would confront them with different food conditions. In natural populations, first clutches show less HA than late and second clutches, in which brood reduction is common (Barba *et al.* 1995). We hypothesized that birds from the early laying line would show increased androgens over the laying sequence. Birds of the late-laying line would follow the brood reduction strategy, by exhibiting the opposite pattern. The predominance of either of these strategies depending on laying date is suggested by a study in a similar songbird (Tobler *et al.* 2007).

Yolk androgens exert long-term effects on the behaviour of the offspring (Strasser & Schwabl 2004; Eising *et al.* 2006). Thus, intra-clutch variation could influence lifelong individual differences. Individual great tits differ consistently in exploration, aggression and responses to stressors referred to as syndromes, coping styles or personalities with fitness consequences (Dingemanse & Réale 2005; Groothuis & Carere 2005). Selection on exploration resulted in two lines of bold or shy explorers (Drent *et al.* 2003). Selection on risk taking resulted in lines similar to the original ones (van Oers *et al.* 2004a). A quantitative genetic analysis indicates a significant maternal effect (van Oers *et al.* 2004b). Although the artificial selection minimized it by standard procedures (cross fostering, hand rearing), an effect mediated by egg hormones could still have shaped the personalities. Indeed, testosterone injection in quail produced a phenotype resembling the bold personalities (Daisley *et al.* 2005, but see Tobler & Sandell 2007). Furthermore, a difference in yolk testosterone between lines of quail selected for social reinstatement has been reported (Gil & Faure 2007). Our lines may validate these findings in a functional context in a species in which the personalities and their ecological relevance are largely documented.

To study the effect of personalities on yolk hormones over the laying sequence and laying date, we collected eggs of both lines under identical conditions and analysed the concentration of testosterone (T), androstenedione (A_4), 5α-dihydrotestosterone (DHT), 17β-oestradiol (E_2) and corticosterone (B).
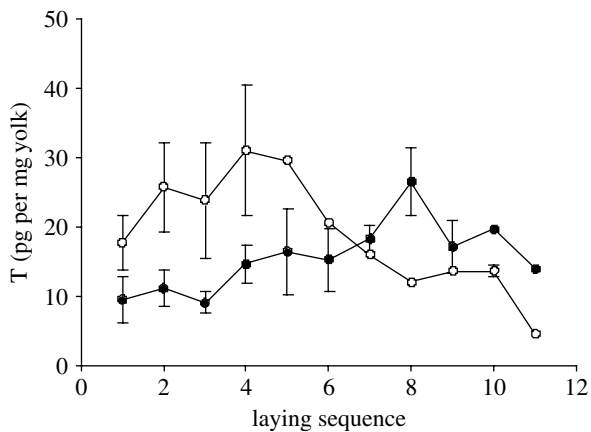
Figure 1. T yolk concentration in eggs across laying sequence of shy and bold great tits. Means $\pm$ s.e.m. are shown. n shy eggs: 5, 4, 3, 3, 1, 1, 1, 1, 1, 2, 1; n bold eggs: 7, 5, 4, 6, 4, 4, 4, 3, 4, 1, 1. Filled circles, bold line; open circles, shy line.

2. MATERIAL AND METHODS

Details are reported in the electronic supplementary material. We collected 66 fresh laid eggs from first clutches of six shy and nine bold pairs, (Drent *et al.* 2003), for which we recorded: (i) onset of nest building, (ii) laying date (first egg), (iii) onset of incubation, (iv) clutch size, and (v) degree of HA. The exploration tests used for selection are described elsewhere (Drent *et al.* 2003).

The procedures for extraction and radioimmunoassays of the hormones were as in Schwabl (1993). Data were analysed in MLwiN v. 1.10.0006 by hierarchical linear models (Rasbah *et al.* 2004).

3. RESULTS

The analyses of T and its precursor A_4 yielded similar results. Therefore, we report only T data. No significant results were found for DHT, E_2 or B (see the electronic supplementary material for levels and intercorrelations).

The lines differed in T concentrations across laying sequence (figure 1). In the bold line, T increased, while in the shy line, it decreased (line $\times$ sequence: $\chi_1^2 = 24.08$, $n = 66$, $p < 0.0001$). The increase in T in the bold line was also significant ($\chi_1^2 = 19.182$, $n = 45$, $p < 0.0001$). In the shy line, the best fit was for the model egg sequence $+$ (egg sequence)^2 ($\chi_1^2 = 13.821$, $n = 21$, $p < 0.0001$), indicating that the levels first increased, then decreased with sequence.

To test whether differences over the sequence relate to laying date, we analysed the line effect in onset of laying and clutch size. Bold females laid earlier than shy females ($F_{1,13} = 4.72$, $p = 0.049$, table 1; no effect of year or its interaction with line). A difference in laying date occurred in the same years in a larger sample of females (bold, $n = 19$; shy, $n = 21$, $p < 0.001$). To test whether laying date was an important predictor of T, we ran the previous model with T as dependent variable and line, sequence, and their interaction as predictors, but now adding laying date and its interaction with line. The model did not improve ($\chi_2^2 = 1.799$, $n = 66$, $p = 0.417$), while the interaction line $\times$ sequence was still significant ($\chi_1^2 = 8.812$, $p = 0.0029$), supporting line as the main explanatory factor. No line effect on egg mass, clutch size or its interaction with sequence was found.

We did not find an overall line effect, because in the model including line $\times$ sequence it disappeared

Table 1. Breeding parameters (mean $\pm$ s.e.m.) in the selection lines.

	bold	shy	n	p
clutch size	8.3 ± 0.8	7.0 ± 1.6	(9) (6)	n.s.
onset of nest building[a]	19.9 ± 6.4	37.2 ± 3.2	(8) (9)	0.036
laying date[a]	27.0 ± 5.5	42.7 ± 2.9	(9) (6)	0.049
onset of incubation[a]	36.4 ± 6.7	51.4 ± 1.9	(7) (5)	n.s.
hatching asynchrony[b]	0.43 ± 0.3	1.8 ± 1.1	(7) (5)	n.s.

[a] day 1, 1 April
[b] days

when the interaction effect was removed ($\chi_1^2 = 2.245$, $p = 0.134$). This might be due to the different patterns in eggs 8–11. Average clutch size in the lines is 7–8. The analysis of T for the first eight eggs yielded an overall effect of line ($\chi_1^2 = 5.185$, $p = 0.0227$), with higher concentrations in the shy birds.

4. DISCUSSION

The data support the hypothesis explaining variation in yolk androgens over the laying sequence: an increase from first- to later-laid eggs (bold line) would mitigate effects of HA under good food conditions, enabling parents to rear the full brood, while a decrease (shy line) would enhance them (brood reduction) under poor conditions (Schwabl *et al.* 1997). Until recently (Tobler *et al.* 2007), these strategies were described at the level of the species. We show that they occur within a species, in identical environmental conditions, have a genetic basis, and relate to laying date. In free-living great tits, late clutches show a larger degree of HA, face reduced food abundance and their late-hatching chicks have low survival (Barba *et al.* 1995). Indeed, the degree of HA tended to be larger in the shy than in the bold line. The results are consistent with the bold and shy phenotypes, being, respectively, risk taker and risk aversive in a life-history context (Wolf *et al.* 2007) the bold line taking the risk of rearing the full brood and the shy line avoiding rearing too many chicks.

The correlated response to selection in androgen levels suggests a genetic component for this trait. Consequently, Darwinian selection could act on hormone levels, supporting evolutionary explanations of hormone-mediated maternal effects. Although genetic variation in yolk hormones has previously been found in lines of domesticated species (Hayward *et al.* 2005; Gil & Faure 2007), this is the first report showing variation of within-clutch concentrations in association with laying date and personality.

The possibility that differences in attractiveness between males of the lines affected the results (Gil *et al.* 1999) is unlikely, since mate preference tests did not give an indication of females preferring males in relation to line (Groothuis & Carere 2005). Further, it is unlikely that attractiveness explains differences within clutch patterns.

Maternal androgens can affect various behaviours into adulthood, including those that form part of the tits' personality (see §1). Given that the majority of

the birds for selection came from the first eight eggs, the typical clutch size in the lines, we analysed T for only these first eight eggs. An overall line difference emerged, suggesting that the bold offspring would develop under lower androgen exposure than the shy offspring, contrary to expectations from effects of T injections (Groothuis *et al.* 2005, but see Tobler & Sandell 2007). However, this is consistent with findings in lines of mice similar to those of the great tit (Groothuis & Carere 2005). SAL mice (proactive, bold) experience lower embryonic T exposure than LAL (reactive, shy) mice (de Ruiter *et al.* 1992).

A potential problem is that the selection lines are not replicated. However, data from unselected birds collected from the field and data from another selection experiment provide validation (van Oers *et al.* 2004*a*,*b*; Groothuis & Carere 2005; see the electronic supplementary material).

In conclusion, we found a correlated response between selection on personality and yolk androgen concentrations over the laying sequence, in conjunction with co-selection for laying date, implying that the mechanisms underlying maternal effects could be heritable and may allow rapid responses to natural selection on reproductive strategies. Different personalities could result from differential exposure to hormones rather than the selection for behaviour traits and the selection may have acted on a maternal effect. Experimental evidence is necessary to confirm these interpretations.

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