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Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities

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Personalities are general properties of humans and other animals. Different personality traits are phenotypically correlated, and heritabilities of personality traits have been reported in humans and various animals. In great tits, consistent heritable differences have been found in relation to exploration, which is correlated with various other personality traits. In this paper, we investigate whether or not risk-taking behaviour is part of these avian personalities. We found that (i) risk-taking behaviour is repeatable and correlated with exploratory behaviour in wild-caught hand-reared birds, (ii) in a bi-directional selection experiment on 'fast' and 'slow' early exploratory behaviour, bird lines tend to differ in risk-taking behaviour, and (iii) within-nest variation of risk-taking behaviour is smaller than between-nest variation. To show that risk-taking behaviour has a genetic component in a natural bird population, we bred great tits in the laboratory and artificially selected 'high' and 'low' risk-taking behaviour for two generations. Here, we report a realized heritability of 19.3 ± 3.3% (s.e.m.) for risk-taking behaviour. With these results we show in several ways that risk-taking behaviour is linked to exploratory behaviour, and we therefore have evidence for the existence of avian personalities. Moreover, we prove that there is heritable variation in more than one correlated personality trait in a natural population, which demonstrates the potential for correlated evolution.

Keywords: selection response; risk-taking behaviour; *Parus major*; approach–avoidance; temperament; personality

1. INTRODUCTION

Consistent individual differences in behaviour have been found in many animal species (Wilson *et al.* 1994; Gosling & John 1999). These differences in a range of correlated behavioural traits have been labelled as temperament, coping strategies, styles or syndromes (Wechsler 1995; Boissy 1995; Koolhaas *et al.* 1999), comparable with human personalities (Eysenck & Eysenck 1985; Zuckerman 1991). Evidence is accumulating that these personalities exist not only in humans, but also in other animals (Wilson *et al.* 1994; Clarke & Boinski 1995; Gosling 2001; Gosling & Vazire 2002). Two conditions have to be fulfilled for separate behavioural traits to represent a behavioural syndrome or personality. First, the behavioural traits must be repeatable and heritable. Second, the behavioural traits have to be correlated with each other, within a single context.

In describing these personalities, several domains are distinguished. Two broad personality dimensions are approach and avoidance motivations (Budaev & Zhuikov 1998; Elliot & Thrash 2002). Approach motivation is defined as behaviour that is directed by a positive event, while in avoidance motivation the behaviour is directed by negative events (Elliot & Covington 2001). An important field of study in behavioural ecology is that concerning the trade-off between approach and avoidance in the form of a cost–benefit trade-off between foraging and avoiding the risk of predation (Lima & Dill 1990; Lima 1998).

Foraging activity may lead to an increase in predation risk (Godin & Smith 1988), but postponed foraging may have effects on the nutritional state of an animal (Van der Veen & Sivars 2000) and could thereby increase the probability of starvation (Sih 1997). Therefore, hungry animals are willing to take more risks, simply because the costs of hiding and the benefits of risk-taking increase with increasing hunger levels (Damsgard & Dill 1998). On the other hand, the absolute (Martin & Lopez 1999) and relative predation risks, and the predictability of predation risk (Sih 1992), may alter the balance between foraging and risk avoidance. Other factors influencing the trade-off between predation risk and feeding are food availability (Dill & Fraser 1997; Martin *et al.* 2003) and food properties (Cooper 2000), the quality of hiding places (Martin & Lopez 2000) and the distance to a possible hiding place or shelter (Walther & Gosler 2001). Culshaw & Broom (1980) showed that, when chicks were startled while foraging, the type of behaviour and the duration of the behavioural bout prior to the startle influenced the response to the startle. Apart from the environmental factors mentioned above, individual characteristics such as age, size, sex, reproductive state, parasite prevalence and dominance status (Koivula *et al.* 1994; Candolin 1998; Abrahams & Cartar 2000; Kavaliers & Choleris 2001; Lange & Leimar 2001) can be responsible for differences in risk-taking behaviour. Predation risk itself, on the other hand, affects various life-history decisions (Kavaliers & Choleris 2001). In addition, the phenotypic and genetic relationships with other personality traits could directly or indirectly influence the trade-off between risk-taking and foraging. Consistent individual differences in risk-taking have already been reported in guppies (Godin & Dugatkin

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1996) and pumpkinseed sunfishes (Coleman & Wilson 1998), for example. In humans it is suggested that approach–avoidance motivation even may be the foundation of several personality dimensions (Elliot & Thrash 2002). Covariation between risk-taking behaviour and individual differences in boldness and aggression has been found in several species, such as mice (Blaszczyk *et al.* 2000) and cichlid fishes (Brick & Jakobsson 2002), but the genetic basis of risk-taking behaviour and its relationship to other personality traits is unknown in wild animal populations.

In great tits, differences in exploration are phenotypically correlated with those in boldness (Verbeek *et al.* 1994), aggression (Verbeek *et al.* 1996; Drent & Marchetti 1999), feeding behaviour (Drent & Marchetti 1999; Marchetti & Drent 2000) and the reaction to physiological stress (Carere *et al.* 2001). In a four-generation bi-directional selection experiment on the combination of exploration and boldness (further referred to as early exploratory behaviour), Drent *et al.* (2003) showed that early exploratory behaviour has a genetic basis. In a study of wild great tits (Dingemanse *et al.* 2002) that were tested for exploration behaviour in the laboratory, individuals differed consistently in exploratory behaviour and using parent–offspring regression, heritabilities were found comparable with those of the study of Drent *et al.* (2003). These consistent, heritable and co-varying reactions towards novel challenges fit into the idea of the existence of avian personalities that are comparable to human personalities (Gosling & Vazire 2002). To study personalities with a non-human animal as a model species, one would preferably take a multidimensional personality approach (Budaev 1997), especially when studying personalities from an adaptive point of view. Natural selection influences different characteristics at the same time, and phenotypic correlations between personality traits have been shown in many studies. Hence, there is a need to study multiple behavioural traits using an integrative approach. In the present study we attempt to incorporate ecological reality and find evolutionary explanations for the underlying genetic structure of personalities.

To investigate whether and how risk-taking behaviour falls into the concept of avian personality, we tested whether risk-taking behaviour is repeatable and whether exploration and risk-taking behaviour are correlated. On the one hand, we investigated whether this correlation exists in hand-reared great tits collected from a natural population, and, on the other hand, whether lines bi-directionally selected for 'fast' and 'slow' exploration differ in risk-taking behaviour. Furthermore, we assessed two estimations of heritability: we tested whether similarity is greater within broods than across broods, which gives a rough heritability estimate; we also assessed the realized heritability of risk-taking behaviour by selecting for 'low' and 'high' risk-taking behaviour for two generations.

2. MATERIAL AND METHODS

(a) *Animals*

We collected 94 great tit (*Parus major*) nestlings from 15 nests in two wild populations, at Westerheide and Oosterhout (for details see Dingemanse *et al.* 2002), in 1998. Their biological parents raised these birds until 10 days after hatching. At this point we took the birds from their nests, brought them to the laboratory and hand-reared them under standard conditions in the laboratory until independence (for details see Drent *et al.* 2003). After this period the juveniles were housed individually, the tarsus was measured and they were tested in regard to exploration as described in § 2b. At the age of 10 weeks, a blood sample was taken for sex determination. Birds were sexed according to the method of Griffiths *et al.* (1998). Furthermore, we used 73 birds from the fourth generations of lines bi-directionally selected for 'fast' (FE; $n = 38$) and 'slow' (SE; $n = 35$) early exploratory behaviour (Drent *et al.* 2003) to investigate the relationship between risk taking and exploration.

(b) *Behavioural tests*

To measure the early exploratory behaviour we performed two types of behavioural tests: a novel-environment test (analogous to an open-field test; Walsh & Cummins 1976) was followed by two tests of reaction to different novel objects. The combination of the results of the novel-environment test (further referred to as exploration) and the novel-object test (further referred to as boldness) is referred to as early exploratory behaviour. Early exploratory behaviour was used as the selection criterion in the bi-directional selection experiment carried out by Drent *et al.* (2003). The exploration test was carried out between 30 and 35 days after hatching; the boldness tests were carried out 10 and 12 days later (for more details on the tests see Verbeek *et al.* (1994) and Drent *et al.* (2003)). Boldness tests were conducted in the home cages. The exploration and startle tests took place in an observation room of 2.4 m × 4 m × 2.3 m with 16 individual cages connected to the room via sliding doors of 20 cm × 20 cm (Drent *et al.* 2003). Birds were let into the observation room without handling, by manipulating the lighting conditions in the observation room and the adjacent cages.

(i) *Startle latency test*

Risk-taking behaviour was evaluated in terms of the time taken (latency) to return after a mild startle in a food context. This startle latency test took place 6–8 weeks after the boldness tests. For this test, we placed three artificial trees and a feeding table in the observation room (figure 1). The feeding table was equipped with a spring-loaded hinged steel plate of 7 cm × 7 cm, which was attached to the back of the tabletop. On the centre of the table we placed a bowl (diameter of 15 cm), the bottom of which was covered with mealworms. A cord attached to the plate, which was controlled from outside the observation room, made it possible to startle a bird: releasing the pressure on the cord at once caused the plate to spring up in front of the bird. After pulling the cord the plate returned to its initial position, invisible to the bird. The test involved three phases. After entering the room, birds landed on a tree after flying around for a short time (1–10 s). We measured the time from the moment each bird landed on a tree until the moment it took the first mealworm from the feeding bowl (first-worm latency). In all cases the birds ate the mealworm in an artificial tree. Since the experimental set-up in combination with the observation room was new to the birds, we expect the first latency to reflect a novelty effect. This first phase of the experiment reduces this effect and familiarizes the birds with the situation. In the second phase, we measured the time from the moment each bird had eaten the mealworm until it returned to the feeding table again and attempted to take a second one. Before the bird was able actually to take a mealworm, just as it landed on the feeding bowl, we startled the bird. We refer to this as 'second-worm

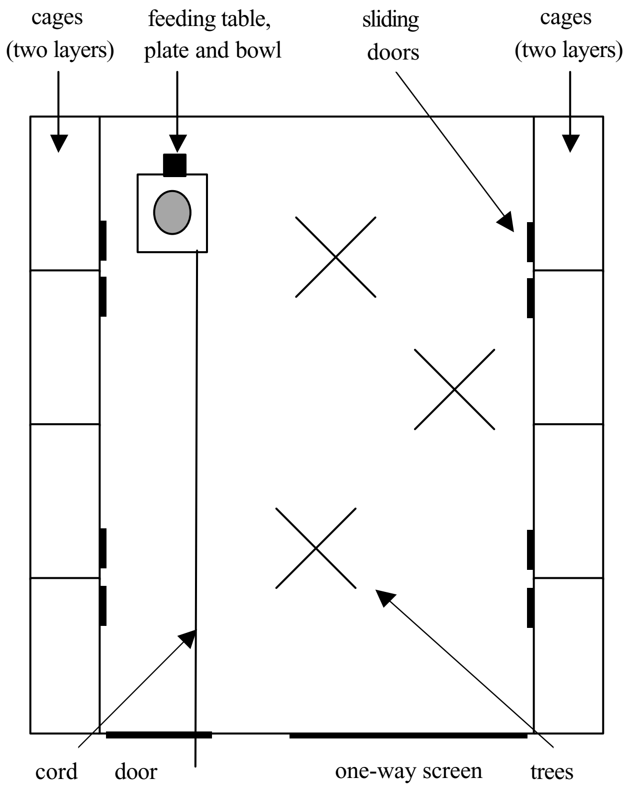

Figure 1. Plan of the observation room in which we tested risk-taking behaviour. Along each side wall are eight sliding doors (in two rows of four, one above the other), which connect the cages to the room. The front wall had a 0.9 m × 2.0 m door at the left side and a 1.1 m × 0.16 m one-way screen for observation. The room contained three artificial trees and a feeding table (1.30 m high; platter 30 cm × 30 cm). The trees were made of wood with a trunk of 4 cm × 4 cm and a height of 1.5 m. Each tree had four cylindrical branches of 20 cm in length. The upper two branches (5 cm below the top) were placed on opposite sides of the trunk, at right angles to the lower branches (25 cm below the top). Birds entered the room through one of the sliding doors.

latency'. In the third phase, after the startle, we measured the time it took the bird to return to the table and actually take the second mealworm, the startle latency (also referred to as risk-taking behaviour). If a bird did not return to the table to take a mealworm within 20 min of the startle, we stopped the test (only one case). This case was assigned a time of 1201 s. The time each bird took to manipulate and eat a mealworm was not included in any of the measurements.

(c) *Housing*

After hand-rearing, birds were kept individually in cages of 0.9 m × 0.4 m × 0.5 m with solid bottom, top, side and rear walls, a wire-mesh front and three perches. After the exploration and boldness tests the birds were housed in semi-open outdoor aviaries (2.0 m × 4.0 m × 2.5 m) in unisex flocks of six to eight individuals, for a period of six to eight weeks. Next, they were replaced in their home cages for the startle test. At all stages, we provided the birds with water *ad libitum*, commercial seed mixture and calcium. This was supplemented daily with mealworms and a mixture containing sour milk, ground beef heart, a multivitamin and calcium solution and commercial egg mixture. The birds were kept under natural light conditions, with visual and vocal contact with other birds. They had no access to food for 2 h prior to the tests, and were deprived of mealworms for 2 days before testing, to increase their tendency to take mealworms during the test.

(d) *Bi-directional artificial selection for risk-taking behaviour*

For the parental generation, we selected those juveniles that had the longest and the shortest startle latencies. However, to obtain labelling consistent with but distinguishable from that in former work, we used 'high risk-taking' for birds with a short latency and 'low risk-taking' for birds with a long latency. Both 'high risk' and 'low risk' lines were started with nine pairs. For the second generations we formed pairs from the first-generation offspring by selecting individuals with the shortest startle latency for the 'high risk' line and those with the longest startle latency for the 'low risk' line, avoiding full-sib and first-cousin mating. The second-generation individuals of the 'high risk' and 'low risk' lines were based on eight and five pairs, respectively. The pairs were kept in aviaries of 2.0 m × 4.0 m × 2.5 m from December onwards. All aviaries contained four nest-boxes, so the birds were able to choose nest-boxes in which to breed or roost. Our aviary pairs lay eggs synchronously with birds from natural populations. In the spring, aviaries were checked weekly when no nesting activity was observed (no material in nest-boxes or on the feeding table). This frequency was increased to once a day when the birds started nesting. Eggs were collected and replaced with dummy eggs on the day of laying. Clutches of eight eggs from a single pair were incubated by foster females. Nestlings were collected at the age of 10 days and then hand-reared in the laboratory (for details on hand-rearing see Drent *et al.* (2003)).

The parental generation was taken from two field populations in 1998. Because of sample problems, we needed 3 years (1999, 2000 and 2001) to obtain enough first-generation individuals to be able to produce enough second-generation pairs. The second generation was born in 2002.

(e) *Statistical analyses*

The untransformed data were used in Wilcoxon signed-ranks tests to compare within-individual latencies and in Spearman's rank correlations to compare the correlations between latencies. Because the variance in startle-latency time increased with the mean value, this variable was log-transformed (Zar 1999) for all analyses where normal distributions are assumed. Part of the phenotypic change from one generation to the next might result from environmental variation between successive years or generations. To control for this between-year variation in the selection experiment, we used 250 birds raised in the same years as these generations for a control population (see table 1). The mean logarithmically transformed first-worm, second-worm and startle latencies for these birds per year (table 1) were subtracted from the individual values of the animals involved in the selection experiment (Walsh & Lynch 2000). This same procedure was used for repeatability analyses, to separate sequence from year effects.

We calculated the repeatability of risk-taking behaviour for all individuals for which we obtained multiple measurements (52 individuals, twice; 40 individuals, three times; nine individuals, four times). Repeatability, the proportion of phenotypic variance explained by the individual (Falconer & Mackay 1996), was calculated following Lessells & Boag (1987) and its standard errors were calculated following Becker (1984). To test whether

Table 1. Control population means for log-transformed first-worm, second-worm and startle latencies with their standard errors. (Abbreviations: year, year of birth and first measurement; *n*, number of birds.)

year	*n*	first-worm	second-worm	startle
1998	109	1.90 ± 0.04	1.69 ± 0.03	1.82 ± 0.05
1999	43	1.60 ± 0.08	1.70 ± 0.03	1.91 ± 0.06
2000	37	1.30 ± 0.09	1.71 ± 0.06	1.85 ± 0.07
2001	39	1.15 ± 0.07	1.71 ± 0.04	1.65 ± 0.05
2002	22	1.15 ± 0.04	1.75 ± 0.03	1.62 ± 0.06

variation in risk-taking behaviour is related to sex, time of day, age or size, we used a general linear model (GLM) and type III sums of squares. We constructed a model with all explanatory variables and all two-way interactions, for all first tests of an individual. We calculated Pearson correlation coefficients to investigate relationships between early exploratory behaviour, exploration and boldness, and first-worm latency, second-worm latency and risk-taking behaviour. We used *t*-tests, assuming equal variance, to test whether lines bi-directionally selected for ‘fast’ and ‘slow’ early exploratory behaviour differed in their time to take the first worm, second-worm latency and risk-taking behaviour.

One method to measure the heritability (h^2) of a trait is to estimate the within-nest variance in relation to the between-nest variance by using one-way ANOVA with nest as a grouping variable. Heritability was calculated as twice the intra-class correlation coefficient (Falconer & Mackay 1996). The intra-class correlation coefficient (repeatability) is calculated as the between-nest variance divided by the sum of the between-nest and the within-nest variances. This is, however, a rough estimate of h^2, and sets an upper limit to it (but see Dohm 2002), since it is probably inflated by common environmental and genetic-dominance effects (Falconer & Mackay 1996).

Realized heritabilities, for each generation separately, were calculated by dividing the cumulative selection response by the cumulative selection intensity (Falconer & Mackay 1996; Lynch & Walsh 1998). Narrow-sense heritability (h^2) measures the proportion of the total variance that is attributed to the effect of genes. This is defined as the ratio of the additive genetic variance (V_A) to total phenotypic variance (V_P), i.e. $h^2 = V_A/V_P$ (Falconer & Mackay 1996). Realized heritability in the selection experiment was measured as the unweighted linear regression of the cumulative selection differential and the cumulative response to selection (Walsh & Lynch 2000). All statistical tests were two-tailed, and *p*-values less than 0.05 were considered to be significant. We used SPSS version 10.1 for Windows for all analyses.

3. RESULTS

(a) *Test results*

In our startle test we measured the lengths of the latencies in three phases. In each of the latencies there was considerable individual variation. Since part of the phenotypic variation in risk-taking behaviour may be a result of factors with non-permanent effects, we tested whether this variation (phase 3) was related to several control variables (time of day, sex, age, size). Risk-taking behaviour was not related to any of these variables (GLM: time of day: $F_{1,257} = 2.40$, $p = 0.12$; sex: $F_{1,328} = 2.22$, $p = 0.14$; age (juvenile or older): $F_{1,333} = 0.74$, $p = 0.39$; size (tarsus): $F_{1,209} = 0.29$, $p = 0.59$), nor to any of the interactions (GLM; all $p > 0.20$).

The time taken to return to the feeding table after eating the first mealworm (second-worm latency, mean ± s.e.m. 69.28 ± 10.16 s) was significantly shorter (Wilcoxon signed-ranks test: $n = 94$, $z = -6.05$, $p < 0.0001$) than that taken to obtain the first worm (first-worm latency, mean ± s.e.m. 142.31 ± 18.13 s); birds almost immediately returned to the feeding table to try to take another mealworm. The mean startle latency (mean ± s.e.m. 144.3 ± 19.0 s) was again significantly greater than the second-worm latency (Wilcoxon signed-ranks test: $n = 94$, $z = -4.59$, $p < 0.0001$), which shows that the startle had an effect on the behaviour of the birds. The first-worm latency (novelty effect) was positively correlated with second-worm latency ($r_p = 0.58$, $n = 15$, $p = 0.02$) and there was a tendency for a correlation with the startle latency ($r_p = 0.51$, $n = 15$, $p = 0.05$). The second-worm latency was not correlated with startle latency ($r_p = 0.20$, $n = 15$, $p = 0.49$).

(b) *Repeatability*

Owing to learning effects, repeatability is difficult to measure over a relatively short period (Dingemanse *et al.* 2002). When we measured repeatability of risk-taking behaviour (phase 3) with a between-test interval of 1 year, we found a repeatability of 0.26 ± 0.07. Neither the first-worm latency ($r = 0.06 \pm 0.07$) nor the second-worm latency ($r = 0.11 \pm 0.07$) were repeatable between tests, and none of the control variables (time of day, sex, age and size) or interactions between them were good predictors of the phenotypic variation found for first-worm latency (all $p > 0.34$) or second-worm latency (all $p > 0.35$).

To see whether a learning effect existed in the different phases of the test, we compared only the first two test results for each individual. We found a sequence effect for first-worm latency (GLM: $F_{1,201} = 8.36$, $p = 0.005$), but not for second-worm latency (GLM: $F_{1,201} = 0.71$, $p = 0.40$) or for risk-taking behaviour (GLM: $F_{1,201} = 0.25$, $p = 0.62$).

These measurements are based on individual test scores. Since siblings are expected to be more alike than non-relatives, these results contain a certain amount of pseudo-replication. This implies that *F*-values may be overestimated in the tested hypotheses, but this does not influence our finding of no relationships.

(c) *Risk-taking behaviour and early exploratory behaviour in hand-reared nestlings*

In the sample collected as nestlings from the wild, no significant correlation was detected between early exploratory behaviour and either first-worm latency ($r_p = 0.42$, $n = 15$, $p = 0.12$) or second-worm latency ($r_p = 0.14$, $n = 15$, $p = 0.62$). In figure 2, risk-taking behaviour is plot-

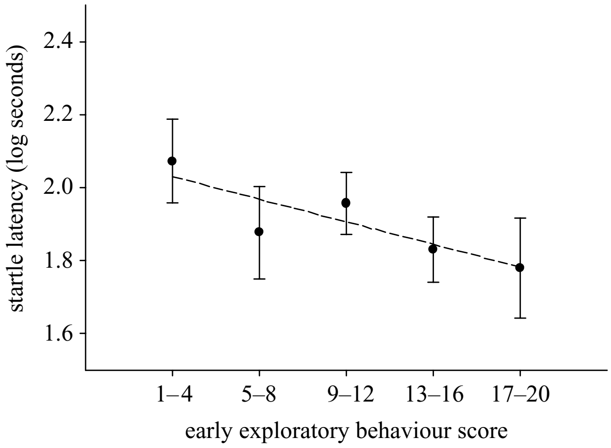

Figure 2. Mean startle latency with s.e.m. Individuals used were unselected birds collected from natural populations as juveniles. For reasons of clarity, early exploratory scores are presented in five groups (1–4, 5–8, 9–12, 13–16, 17–20). Statistical testing was carried out on the original values. These were used because all tests were carried out within a single year.

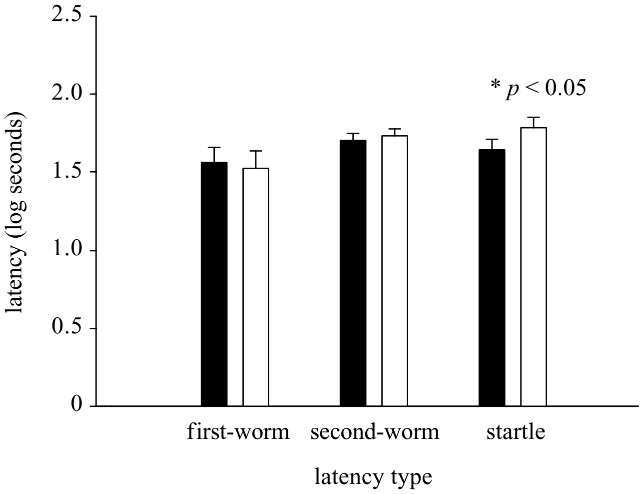

Figure 3. Time to take the first mealworm, the latency in returning to the feeding table after having eaten the first mealworm (second-worm latency), and the startle latency, plotted for the lines selected for 'fast' (filled bars) and 'slow' (open bars) early exploratory behaviour. Data are means and s.e.m.

ted against early exploratory behaviour. Although no significant relationship was found ($r_p = 0.45$, $n = 15$, $p = 0.09$), there was a tendency for fast explorers to come back to the feeding table after a startle sooner than slow explorers.

When looking at the separate components of early exploratory behaviour (exploration and boldness) we found that first-worm latency was correlated with exploration ($r_p = 0.84$, $n = 15$, $p < 0.0001$) but not with boldness ($r_p = 0.21$, $n = 15$, $p = 0.46$), which also holds for second-worm latency (exploration: $r_p = 0.62$, $n = 15$, $p = 0.02$; boldness: $r_p = 0.31$, $n = 15$, $p = 0.26$) and risk-taking behaviour (exploration: $r_p = 0.37$, $n = 15$, $p = 0.02$; boldness: $r_p = 0.17$, $n = 15$, $p = 0.55$). Although exploration and boldness are converted to inverted scores, which would have caused phenotypic correlations to be negative, all phenotypic correlations are calculated as being positive, since risk-taking behaviour, exploration and boldness were all originally measured in seconds.

(d) *Risk-taking behaviour in lines selected for early exploratory behaviour*

The results among the hand-reared juveniles were confirmed when looking at the lines bi-directionally selected for 'fast' and 'slow' early exploratory behaviour (figure 3). The lines differed neither in first-worm latency (t-test: $t_{71} = -0.91$, $p = 0.37$) nor in second-worm latency (t-test: $t_{71} = -1.08$, $p = 0.29$). In contrast to the first two phases, the time taken to return to the feeding table after the startle differed between the two lines: fast explorers came back sooner than slow explorers (t-test: $t_{71} = -2.15$, $p = 0.04$).

(e) *Heritability of risk-taking behaviour*

The mean brood size in the nests was 6.27 ± 0.87 (range of 3–10). The within-nest variance in risk-taking behaviour was smaller than the between-nest variance (GLM: $F_{14,79} = 2.21$, $p = 0.014$). This demonstrates that young from the same brood showed more resemblance to each other than to offspring of other broods. The heritability derived from this full-sib analysis was 0.32 ± 0.20, but the sample size was too small to make this significantly different from zero (t-test: $t_{14} = 1.88$, $p = 0.13$).

In table 2 the population measures of risk-taking behaviour are given for the parental generation, and first and second generations in the bi-directional selection study on 'high' and 'low' risk-taking behaviour. Although the response to selection fluctuated, we found a significant difference between the two lines (t-test: $t_{48} = -1.982$, $p = 0.05$) after two generations of selection (figure 4*a*), with birds of the 'high risk' line returning 73 s earlier to the feeding table than birds of the 'low risk' line. In figure 4*b* the cumulative response to selection (response compared with that of the starting population) has been plotted against the cumulative selection differential (the deviation of the mean of the individuals used as parents from the mean value in their generation). The realized heritability in the base population is the total of the observed phenotypic variance that can be attributed to genetic factors, which in this case is calculated from the regression coefficient of the cumulative response to selection over the cumulative selection differential, forced through the origin: 0.19 ± 0.03 (linear regression: $r^2 = 0.93$, $F_{1,4} = 37.85$, $p < 0.01$). The heritabilities calculated refer to log latency, which implies that changes are proportional rather than additive.

4. DISCUSSION

We showed that individual great tits collected from two populations and hand-reared in the laboratory consistently differed in risk-taking behaviour, independent of sex, size or time of day at which the test was carried out. There is a tendency for risk-taking behaviour to be correlated with early exploratory behaviour in these birds. Lines selected for 'fast' and 'slow' exploration differ in risk-taking behaviour. Fast explorers responded less to a startle, and thereby returned sooner to a feeding table than slow explorers. We also demonstrate that within-brood variation in risk-taking

Table 2. Population measures of risk-taking behaviour in the juvenile populations of the parental (P), first and second generations in the selection study, corrected for between-year differences.
(Abbreviations: S_{cum}, cumulative selection differential; n, total number of offspring tested; m, mean; s.e.m., standard error of mean; h^2, heritability; V_{P}, phenotypic variance; V_{A}, additive genetic variance.)

type	generation	S_{cum}	n	m	s.e.m.	h^2	V_{P}	V_{A}
	P		94	0.060	0.047		0.209	
high risk-taking	1	−0.338	10	0.030	0.104	0.09	0.108	0.010
	2	−0.476	28	−0.054	0.070	0.24	0.137	0.033
low risk-taking	1	0.249	23	0.136	0.113	0.31	0.295	0.091
	2	0.644	22	0.176	0.096	0.18	0.202	0.036

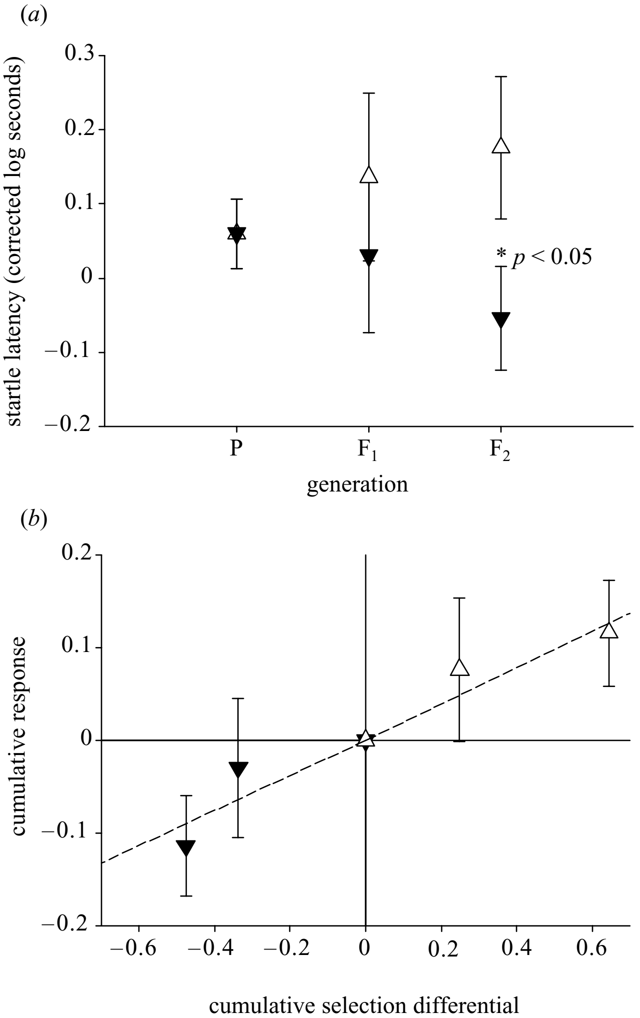

Figure 4. Response to artificial selection (*a*) per generation and (*b*) relative to the cumulative selection differential for both low risk-taking (open triangles) and high risk-taking (inverted filled triangles) behaviours, with s.e.m. Dashed lines are regression lines for low and high risk-taking. The slopes for low and high risk-taking behaviours separately are 0.19 (linear regression: $r^2 = 0.96$, $F_{1,2} = 21.14$, $p = 0.136$) and 0.20 (linear regression: $r^2 = 0.88$, $F_{1,2} = 6.97$, $p = 0.230$), respectively.

behaviour is smaller than between-brood variation. In a full-sib analysis, we found a heritability of 32%. Moreover, the results of the bi-directional selection study on 'high' and 'low' risk-taking behaviour proved that variation in risk-taking behaviour in a wild bird population is heritable. We found a realized heritability of 19%, based on selection over two generations.

Risk-taking behaviour as measured in our test in the laboratory can be seen as a standardized measure of the individual outcome of the trade-off between finding food and avoiding the risk of predation. For practical reasons no live predator could be used, and great tits do not react to video playback of predators (P. J. Drent, unpublished data). When attacked by a predator, birds fly away from the place where they are foraging, fly around and land in a tree or seek shelter (see, for example, Ficken & Witkin 1977). Although no predator was present in the vicinity of the birds, this test still reflects situations occurring in the wild, where birds react to sudden disturbances by fleeing, without always knowing the exact cause of the disturbance. The behaviour of birds startled by predators is similar to that seen when they are disturbed by a cause unknown to the observer (Van der Veen 2000). We recognize that the time taken to restart foraging also depends on social interaction with flock-mates, and a next step would therefore be to see whether the presence of other birds, and their behaviour, influence these decisions.

The time it took birds to take the first mealworm was correlated with exploration, but not with boldness and early exploratory behaviour. Hence, as expected, novelty effects were found the moment the birds entered the room, but they experienced only the room and the experimental set-up, and not the attributes themselves, as novel. This can be explained by the experience the birds gained before testing. The observation room was not new, since they had experienced it in the exploration test, six to eight weeks earlier. After the tests for boldness and exploration, the birds were submitted to group living in aviaries, where a feeding table and artificial trees were present. Therefore, the attributes were not novel to them at the time of testing, and no fear of novelty was present. The particular set-up of the trees and table in the room was new when the test for risk-taking behaviour was conducted for the first time, but not when this test was conducted for the second time, a year later. The decrease in latency between the years is therefore comparable with the difference between first-worm latency and second-worm latency within a test. This learning effect is also present in the exploration test itself, as has been shown in wild great tits (Dingemanse *et al.* 2002). The shorter the time between the separate tests, the faster the birds explore the observation room. In addition, the correlation between first-worm latency and startle latency suggests that both are measures of a common motivational state, elucidated by different challenges:

a novel experimental set-up at the beginning of the test, and an unexpected startle in a food context later on.

The fact that first-worm latency was correlated with second-worm latency indicates that, on the one hand, the novelty effects present in first-worm latency have not completely vanished in second-worm latency. This is confirmed by the correlations with exploration, which are present for both latencies, but the correlation between exploration and second-worm latency was smaller than that between exploration and first-worm latency, indicating an eroding effect. On the other hand, if second-worm latency resembles a hunger state, this is also likely to be present in both measurements. Risk-taking behaviour tends to be correlated with first-worm latency, and this points to a common motivational background for these two latencies. Since, however, it is not correlated with second-worm latency, the hunger state seems relatively less important.

The heritability found for risk-taking behaviour in this study is lower than the heritability found for early exploratory behaviour in the four-generation bi-directional selection experiment on 'fast' and 'slow' exploration ($h^2 = 54\%$; Drent *et al.* 2003). This confirms comparable findings in human personalities when using a three-dimensional personality questionnaire (TPQ). Here, heritabilities for harm avoidance (i.e. risk-taking behaviour) are typically lower than those for novelty seeking, for example (i.e. exploration and novelty; Ebstein *et al.* 2000). There are two possible reasons for this difference. First, the test of risk-taking behaviour was taken later in life than the exploration and boldness test. This leaves more scope for learning effects. Second, when the difference is a more adaptive one, it could be that there is more selection as regards risk-taking behaviour in the natural populations from which we derived the birds. Owing to stronger selection, additive genetic variation could decrease (e.g. Jones 1987; but see, for example, Frank & Slatkin 1992).

Laboratory estimates of heritability may not be good predictors of heritability in natural populations, owing to the reduction in environmental variability in the laboratory (Riska *et al.* 1989; but see Dingemanse *et al.* 2002; Drent *et al.* 2003). The results of several comparative studies, however, have shown that laboratory estimates are somewhat higher than, but not different from, values in natural populations (Weigensberg & Roff 1996; Bryant & Meffert 1998; Blanckenhorn 2002).

We cannot completely exclude environmental maternal effects from the estimation of the heritability of risk-taking behaviour. Females can alter the concentrations of maternal hormones deposited in the eggs (Schwabl 1993). Individual differences in female behaviour are known to cause between-nest differences in egg hormone concentrations (Whittingham & Schwabl 2002). The female is thereby able to influence indirectly the behaviour of her young (Schwabl 1993; Eising *et al.* 2001), despite the fact that in the present study the young were raised by foster parents until 10 days after hatching, and hand-reared from day 10 until independence. Maternal effects may also influence heritability (McAdam *et al.* 2002). In earlier work on the influence of maternal effects on phenotypic variation in exploration, however, we found that, although maternal effects are present, they are relatively small compared with additive and dominant genetic effects (Van Oers *et al.* 2004). Hormones, however, are known to play a role in risk-taking behaviour (Boissy 1995; Koolhaas *et al.* 1999; King 2002).

Our selection results were clear after correction for year effects. The correction of these kinds of effects through the use of a control population is a standard technique in selection experiments (Walsh & Lynch 2000). At the same time, between-year variation in risk-taking behaviour cannot be explained by variation in age, sex or size in our study. This indicates that environmental factors, in the form of experiences early in life, are important in the expression of risk-taking behaviour. These effects, however, seem to shift the distribution between years, rather than influencing the distribution within years.

We found that risk-taking behaviour is correlated with other aspects of avian personality. Novelty, exploration and risk-taking behaviours seem to be traits of the personality concept, which is in line with the results of other studies on personalities (Mather & Anderson 1993; Budaev & Zhuikov 1998; Weiss *et al.* 2000) and coping styles (Benus *et al.* 1991) in domesticated animals. Iguchi *et al.* (2001) showed, in a study of two groups of cloned siblings of red-spotted cherry salmon, that there were heritable consistent between-clone differences in three principal components derived from several behavioural measurements. They labelled these components boldness, activity and carefulness (comparable to boldness, novelty and risk-taking in our study), and showed integration of these genetically governed components. Whether these aspects of behaviour are real and independent is still unclear, and studies on the functional architecture of personality traits in natural populations are needed. Moreover, whether these seemingly independent traits are expressions of the same (genetic) system in other contexts, or arose by correlated evolution, needs further study.

Risk-taking behaviour is known to influence life-history decisions (Grand 1999), and evidence is also accumulating that other personality traits affect reproduction, survival and dispersal (Armitage 1986; Eaves *et al.* 1990; Réale *et al.* 2000; Fraser *et al.* 2001; Dingemanse *et al.* 2003). Our study shows that personality traits are correlated and have a substantial amount of additive genetic variance, and this therefore gives scope for co-selection of different traits or dimensions. This implies that natural selection of a trait in one context could have consequences for the evolution of another trait (Price & Langen 1992). To study the coexistence of adaptive individual strategies in natural populations, these genetic correlations between different personality traits need more study.

Budaev (1997), in his study on guppies, has already shown the importance of the use of more than one dimension in animal personality studies. With the results of two artificial selection experiments, we now have evidence for the genetic basis of at least two personality dimensions in great tits. These traits are correlated and consistent in the present context. With this we have a powerful tool to investigate the interactions between multiple personality dimensions, and, moreover, we will be able to get a better grip on the genetic architecture of personalities in animals from and within wild populations.

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