Original Article

Quantitative genetics and fitness consequences of neophilia in zebra finches

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Consistent between-individual differences in context-general behavioral traits (often called personality traits) are particularly interesting for behavioral ecologists because they might show unexpected cross-context correlations and explain maladaptive behavior. In order to understand their evolutionary significance, it is relevant to know the heritability of these traits and how they relate to reproductive success. This might give insights into selective processes that maintain variation as well as into potential trade-offs. We scored approach to novel objects of 530 captive zebra finches in a familiar environment. Scores were highly repeatable and showed substantial additive genetic variation. We measured reproductive success, promiscuity, and extrapair paternity rates under aviary conditions and calculated linear and nonlinear selection differentials based on fertilization success as well as effects on chick-rearing success of pairs. Approach to novel objects had little influence on these components of reproductive success. However, we found that the social environment (manipulated operational sex ratios) influenced the correlation between approach to a novel object and the proportion of extrapair paternity. We also found that the sex ratio manipulation affected measures of the intensity of sexual selection. Both effects were stronger in males than in females. We conclude that despite the lack of differences in overall reproductive success, approach to novel objects reflects variation in reproductive strategies. *Key words:* behavioral syndromes, extrapair paternity, fitness landscapes, mating preferences, operational sex ratio, reproductive success, sexual selection, temperament. *[Behav Ecol 22:126–134 (2011)]*

In humans and in other animal species, individuals from the same population differ consistently in their behavior toward the environment, and suites of traits describing these behaviors have been termed coping styles, behavioral syndromes, personality traits, or temperament (Wilson et al. 1994; Wilson 1998; Bolnick et al. 2003; Bell 2007; Réale et al. 2007; in this paper, we use the term "personality"). Personality traits are characterized by high within-individual consistency and low context specificity (Sih, Bell, and Johnson 2004; Sih, Bell, Johnson, and Ziemba 2004). This makes such traits particularly interesting for behavioral ecologists because they might mediate trade-offs, explain maladaptive behavior, and show unexpected cross-context correlations (Bell 2007).

A classical, probably very context-general trait is the tendency of individuals to approach novel objects. The presumed context generality makes it interesting to study the consequences of approach to novel objects in competitive situations. The trait can be readily measured by presenting novel objects in a familiar environment (Verbeek et al. 1994). Variation in the way how individuals approach a novel object is sometimes interpreted as variation in boldness (Smith and Blumstein 2008), whereas others have included it in the context of variation in exploration behavior (Stöwe et al. 2006; Réale et al. 2007). Because the terms "boldness" and "exploration" have often been used in other types of trials (exposure to risky situations and open field trials, respectively), we here prefer to use the more neutral term "neophilia" for approach

to a novel object. There is also an overlap with the measurement of fearfulness or neophobia. Neophobia is usually quantified by presenting highly artificial novel objects close to a food source (Coleman and Mellgren 1994; Webster and Lefebvre 2001; Mettke-Hofmann et al. 2005; Fox and Millam 2007; Herborn et al. 2010) so that subjects are forced to approach the novel object or risk starvation. The influence of fearfulness on approach to a novel object can be minimized (though not fully excluded) by presenting natural novel objects in a familiar environment in a neutral position (Murphy 1978; Mettke-Hofmann et al. 2002).

Relatively few studies have explored the covariance between personality traits and fitness (reviewed in Dingemanse and Réale 2005; Réale et al. 2007; Smith and Blumstein 2008). We here focus on traits that are related to the behavior of individuals in novel situations. Empirical studies correlating some aspect of fitness with measures of boldness, exploration, or neophilia have been conducted in squid *Euprymna tasmanica* (Sinn et al. 2006), guppies *Poecilia reticulata* (Godin and Dugatkin 1996), great tits *Parus major* (Both et al. 2005; van Oers et al. 2008; Quinn et al. 2009), and bighorn sheep *Ovis canadensis* (Réale et al. 2009). As a general trend, boldness seems to increase reproductive success but tends to impair survival, whereas exploratory tendencies seem to increase survival (Smith and Blumstein 2008). If novelty behavior or components thereof are heritable (as has been shown for boldness and exploratory behavior, for a review see van Oers et al. 2005), effects on fitness will lead to changes in allele frequencies linked to these traits.

If variation in novelty behavior indeed has fitness consequences and the trait is heritable, the question is how variation in the trait is maintained. There are 2 selective processes that have the potential to maintain personality variation. First, complex fitness surfaces with multiple optima might result in disruptive selection that leads to variation in (behavioral)

traits (Both et al. 2005; Sinn et al. 2006). Complex fitness surfaces might arise, for example, if the combination of personality traits within pair-bonds matters for reproductive performance. Second, fluctuating selection pressures due to environmental variability (Dingemanse et al. 2004; Boon et al. 2007) or due to negative frequency dependence might create and maintain variation in behavioral traits because different phenotypes are selected in different environments or at different times. Fluctuating selection pressures may be due to variability in food availability, in predator pressure, in population structure, or in the social environment. In this study, we explore associations between neophilia and reproductive success that would support such selective explanations. Alternatively, variation in personality traits might be maintained by nonselective processes like aspects of their genetic architecture (e.g., intra- or interlocus interactions, pleiotropic effects) or by abundant mutations that produce behavioral variation and that are counteracted by stabilizing selection (mutation–selection balance, Kingman 1978). These are not further discussed here.

The zebra finch is a suitable model species for studying the fitness consequences of novelty behavior because group compositions change frequently in this gregarious species (Zann 1996). Hence, the group structure constitutes a fluctuating social environment that has the potential to impose fluctuating selection pressures. This seems particularly relevant for sexual selection because the intensity of competition depends on the number of competitors. We studied the fitness consequences of variation in neophilia in domesticated zebra finches held under a constant physical environment but in different social environments. These environments were characterized by variation in the operational sex ratio, which leads to variation in intrasexual competition for mates (Burley and Calkins 1999). We present evidence that the sex ratio treatments indeed influenced reproductive behavior in our population. If variation in neophilia is related to mating success and reproduction and if it is maintained by fluctuations in the social environment, we expect different behavioral types to be most successful under different sex ratio treatments.

Previous studies suggest that novelty behavior correlates positively with reproductive success (Smith and Blumstein 2008), and we expect this effect to be stronger in competitive situations. Neophilia might relate to the exploitation of food and water resources, to defense against predators, dispersal, and dominance (Réale et al. 2007), but potentially also to the response to new potential mates. For example, we might expect that individuals that are more inclined to explore novel objects will also mate with a greater number of partners (elevated promiscuity). It seems also possible that neophobia affects mate choice, both the choice for the social mate and for extrapair mates. It has been suggested that subjects prefer explorative partners (Schütt et al. 2010) because they are likely to be successful in the search for food (social mate, direct benefits) or because their offspring would inherit genes for successful foraging (social and extrapair mates, indirect benefits). If a ubiquitous preference with respect to novelty behavior is present in the population, we would not expect strong differences between different social environments, but a similar advantage of individuals that score high on neophilia in all environments.

Our study has 3 aims. The first aim was to estimate proximate sources of variation in neophilia by quantifying additive genetic, maternal, and early-environmental effects using animal models (Kruuk 2004). The second and main aim was to explore the covariance between neophila and several components of fitness, including a measure of offspring growth that reflects parental quality. We did this by calculating standardized linear and nonlinear selection differentials (Arnold and

Wade 1984; Brodie et al. 1995), an approach that has been suggested for personality research (Dingemanse and Réale 2005; Réale et al. 2007). Linear selection differentials measure the directional selection effects on the phenotype (for larger or smaller trait values), whereas nonlinear selection differentials capture selection on the variance in the trait value (disruptive vs. stabilizing selection). Finally, we explored how variation in neophilia influenced levels of extrapair paternity (number of genetic partners and proportion of extrapair eggs laid/sired). This association would reflect variation in reproductive strategies. For all traits, we tested whether the sex ratio treatments changed the relationship between personality traits and fitness, that is, whether the performance of personality phenotypes depends on the social environment. This would be indicative of fluctuating selection imposed by the social environment.

MATERIALS AND METHODS

Subjects and housing

We studied wild-type zebra finches from a large captive breeding population held at the Max Planck Institute for Ornithology in Seewiesen, Germany. Although genetic diversity is somewhat lower in captive populations compared with wild populations, our population does not show substantially reduced genetic diversity (Forstmeier, Segelbacher, et al. 2007). Subjects belonged to 2 generations (referred to as F_1 and F_2). Eggs had been cross-fostered individually within 24 h after laying so that all subjects grew up with unrelated nest mates and were reared by unrelated foster parents (Schielzeth et al. 2008). The clutch size was close to the original clutch size of the host. Subjects were separated from their foster parents and siblings at day 35 (day 0 is the hatching date), when young zebra finches become independent from their parents. They were held in (same-sex or mixed-sex) peer groups between day 35 and day 100 (2–75 individuals, see Bolund et al. 2010, all birds involved in the aviary breeding experiment were raised in same-sex peer groups of 14–75 individuals). Assignment to peer groups was randomized, but we avoided having full siblings in the same peer group wherever possible. Peer group size did neither influence measures of reproductive success (results not shown) nor of personality scores (see below).

We measured the approach to a novel object of 156 males and 131 females from the F_1 generation and of 125 males and 118 females from the F_2 generation. Birds from the F_1 generation were 358 ± 32 days of age (mean ± standard deviation [SD]; range: 287–417) at the start of testing and birds from the F_2 generation were 412 ± 22 days old (range: 360–453). Only subjects from the F_1 generation were involved in the aviary breeding experiment (in total 68 males and 71 females). Most of these birds were tested again for approach to a novel object after 2 breeding seasons (62 males and 57 females, 985 ± 29 days of age, range: 930–1036).

Novel object trials

Setup of novel object trials

We tested subjects by presenting them with novel objects in their familiar environments. Before the trials, 2 same-sex individuals were held in a cage (120 × 45 × 40 cm, width × height × depth) with 2 wooden perches in each of 2 compartments. We provided food, water, and grit ad libitum in one of the compartments (A); the other compartment (B) was empty except for the 2 perches. Adjacent to compartment B there was a third compartment (C) of the same size that contained 2 perches but was not part of the bird's home cage. It was used for temporary housing of one of the birds, while the other was subjected to the novelty trial. We allowed individuals

to familiarize themselves with the cage (compartments A and B) for 3–4 days before testing them with novel objects. We housed birds in same-sex pairs before and between trials to avoid exposing these highly social birds to potentially stressful long-term isolation. Nevertheless, we tested subjects individually to minimize confounding effects of interactions during trials.

Just before each trial, we gently chased birds (without handling), one to compartment A and one to an identical third, adjacent compartment (C). We then used opaque dividers to separate the 3 compartments (A, B, and C). At the start of the trial, we placed a novel object at the far end of compartment B and removed the divider between A and B so that the focal bird in compartment A had access to the novel object in compartment B (compartments A and B were the familiar home cage). Note that birds were not forced to approach the novel object in cage B because food and water were provided in cage A. After the 5 min trial, we replaced the dividers and swapped the position of the 2 subjects (in compartments A and C) without handling. We then tested the second individual in the same way. We randomized the sequence of testing of the 2 birds and the orientation of the compartments.

We tested all individuals with 2 types of novel objects. The order of presentation of the different novel objects was the same for all birds. On day 1, we presented a bouquet of dry perennial herbs (height ca. 25 cm, width at top ca. 12 cm), which was placed upright in the far corner of compartment B. On day 3, we presented half an apple placed below the outer perch of compartment B with the cut surface showing upward. None of the subjects had previously had any contact with these kinds of objects, but all subjects had been kept in aviaries with natural tree branches for a few weeks. We additionally tested all individuals from the F_2 generation and all repeated trials with the F_1 generation (119 individuals) with a flashy colored toy ball (blue and orange, 7 cm in diameter). This was done to assess whether the reaction to a highly artificial novel object (ball) correlated with the reaction to more natural objects used in the first round (herbs and apple). Finally, we tested 48 individuals without a novel object as a neutral control and recorded behavior as in the herbs trials.

Data recording and analysis
During trials, we recorded all changes in position (between the perches, the floors of the 2 compartments, and the slat between compartments; i.e., 7 positions in total), using The Observer 4.1 software (Noldus Inc., Wageningen, The Netherlands) for live scoring. From the resulting log files, we calculated the time spent in each of the 7 positions.

We calculated an approach score that integrated approach distance and duration as follows. Each position was assigned a weight that expressed the relative distance to the novel object, and this weight was multiplied by the time spent in this position (time measured in fractions or multiples of 15 s). The weights for the bouquet and the ball trials were far perch in compartment A = 1, close perch in A = 2, far perch in compartment B = 3, close perch in B = 4, floor of compartment A = 1.5, slat between A and B = 2.5, and floor of B = 3.5. In the apple trials, the scores were the same except for the floor in compartment B (with a weight of 4 instead of 3.5) because the apple itself was placed on the floor. We took the square root of these weighted sums to reduce the skew in the distribution. We refer to the averages of the scores from the 2 novel object trials as the approach score of an individual. Although this calculation of the approach scores seems somewhat complicated, it had the advantage that it captures approach to the novel object integrated over time without focusing on singular events (like measures of latency would). We calculated analysis of variance (ANOVA)-based repeatabil-

ities (R_A) of the approach scores (Nakagawa and Schielzeth, 2010).

Quantitative genetics

We used animal models in REML-VCE 6.0.2 (Groeneveld et al. 2008) to partition the phenotypic variance in approach scores (averaged across 2 trials, one with apple and one with flower) into additive genetic, maternal, and early-environmental effects. We included 1) a pedigree that identifies the ancestors of each bird in the grand-parental, parental, and F_1 and F_2 generation ($n = 1066$ individuals) to estimate additive genetic components, 2) mother identity (not linked to the pedigree) to estimate maternal effects ($n = 114$ mothers), 3) foster parent identity (not linked to the pedigree) to estimate rearing environment effects ($n = 128$ foster families), 4) peer group identity to estimate peer group effects ($n = 59$ peer groups), and 5) individual identity to estimate permanent environment effects ($n = 530$). By calculating expected repeatabilities from multiple measurements (Falconer and Mackay 1996, p. 139), we further decomposed the residual variance into 6) measurement error and object specificity, $ME = [(1 - R_{obj})/n_{obj}]/[(1 - R_{obj})/n_{obj} + R_{obj}]$, where R_{obj} is the between-object repeatability and n_{obj} is the number of objects and 7) behavioral flexibility, $BF = Residual - ME$.

Reproductive success

Aviary breeding
We allowed 63 male and 63 female zebra finches to breed under seminatural conditions in aviaries. Birds that were sick (1 female and 1 male) or that died (5 birds died during and 6 between breeding seasons) were replaced, so the total number of individuals involved was 68 males and 71 females. All analyses were restricted to birds that were in the aviaries for at least one complete breeding round. We used 3 sex ratio treatments (population sex ratio 0.4, 0.5, and 0.6) with 3 replicate aviaries for each treatment (Figure 1) and allowed 2 breeding rounds of 3 months each (30 August–21 November 2005 and 31 March–26 June 2006). We swapped subjects between breeding rounds so that each subject experienced 2 of the 3 treatments (except for 9 males and 9 females that stayed in the male-biased and female-biased treatment, respectively).

We randomly assigned birds to hexagonal aviaries (size: 190 cm across, 192 cm high, Figure 1), ensuring that genetic or foster siblings did not share the same aviary. Variances and ranges of approach scores were similar for all 3 treatments in males (equal sex ratio 5.76 ± 0.70 [mean ± SD], male biased 5.96 ± 0.62, female biased 5.69 ± 0.65) and in females (equal sex ratio 5.91 ± 0.76, male biased 5.96 ± 0.67, female biased 5.90 ± 0.60). All tests for differences between treatments were nonsignificant (all $P > 0.14$). The novel object trials were conducted once before and once after the 2 breeding rounds (April/May 2005 and January 2007).

In total, experimental birds laid 801 fertile eggs in 2005 and 821 in 2006. We cross-fostered eggs to caged foster pairs within 24 h after they were laid. We sampled blood from all parents and offspring and took tissue samples from all dead chicks and embryos and genotyped all samples using 10 microsatellite makers (Forstmeier, Schielzeth, et al. 2007). Parentage was assigned by exclusion. We sampled 96.3% of all fertile eggs and unambiguously assigned 99.9% of these to a genetic father and a genetic mother. Additionally, we sampled 175 eggs from 19 females for hormonal analyses (for another study), and 170 of these were assigned to their putative parents (based on genetic parentage assignment from the remaining eggs of the clutch). We tested the validity of this assignment
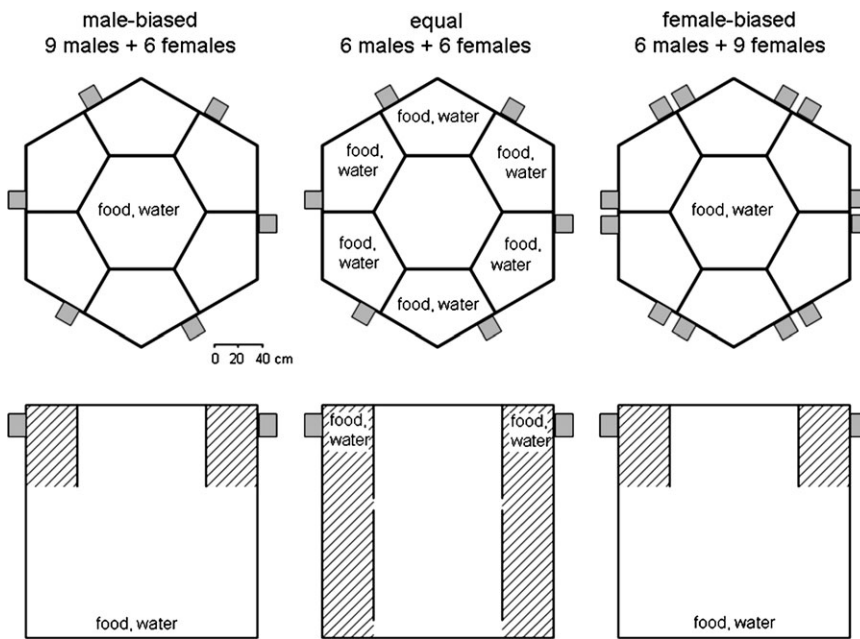
Figure 1
Setup of the breeding aviaries. Zebra finches were allowed to breed in hexagonal aviaries under 3 sex ratio treatments. The treatments also differed in spatial separation, distribution of food and water, and supply of nest-boxes. The upper row shows a top view of the aviaries. The lower row shows a cross-section that illustrates the spatial separation. Hatched sections indicate the separation between breeding compartments (by mesh wire); vertical lines indicate the separation between the outer breeding compartments and the central compartment. Nest-boxes are shown as gray squares.

by simulating the same assignment rules for clutches with complete parentage information and found that 14% of the assigned eggs were possibly misassigned (1.2% of the total). This introduces some measurement error but is still preferable over the exclusion of these eggs because this would introduce bias against some individuals. Hence, including the hormone eggs, the analyses are based on a total of 1727 eggs.

Fitness components and selection differentials
In the aviary breeding experiment, we measured 2 independent components of fitness.

Female fecundity and male fertilization success. We counted the number of fertile eggs produced (females) or fertilized (males). We used relative fecundity and fertilization success (absolute values divided by the mean of all individuals in the same aviary) to calculate standardized selection differentials by regressing these measures of relative fitness on standardized personality scores (Arnold and Wade 1984; Brodie et al. 1995). The slope of the linear term of the personality scores represents the linear selection differential S (directional selection), whereas the slope of the quadratic term represents the nonlinear selection differential C (positive = disruptive, negative = stabilizing selection). Models controlled for individual identity as a random intercept effect (n for females = 34, 35, and 50, n for males = 52, 35, and 34, sample sizes for the male biased, equal sex ratio, and female biased treatment, respectively).

Chick-rearing success. Monogamous pairs that occupied nest-boxes (there were always at least as many nest-boxes as breeding pairs, see Figure 1) and laid complete clutches received 2–3 eggs from randomly selected caged pairs (every egg from a different pair) after they had laid eggs themselves. On day 8 posthatching, we measured chick mass. Mass at day 8 varies greatly between individual chicks and a large proportion of this variation can be explained by foster pair identity (24%, Schielzeth et al. 2008). We used best linear unbiased predictors (BLUPs) for each aviary pair within seasons controlling for hatch order, egg volume, genetic parent identity, and genetic clutch identity (for details, see Schielzeth et al. 2008). We calculated standardized slopes by regressing these BLUPs (standardized to mean of zero and unit of variance) on foster female and foster male personality scores (linear, quadratic, and including the interaction between male and female score). These standardized slopes represent the differential success of low-scoring versus high-scoring individuals (linear term), intermediate versus extremes (quadratic term), and assortatively versus disassortatively paired individuals (interaction). Hence, in this analysis, we explored the pair combination effects of foster pairs because the behavioral types of the caring individuals (and not of the genetic parents) seem most relevant for the chick condition (see also variance component analysis in Schielzeth et al. 2008). Models controlled for pair identity as a random intercept effect (n for pairs for the 3 treatments: 24, 19, and 25).

Extrapair paternity
We measured 2 traits that represent reproductive strategies.

Promiscuity. We counted the number of partners with whom a particular individual produced eggs within a given breeding season. We used a Poisson model with log link to model the number of mating partners for all individuals. Sample sizes were as for total fecundity and fertilization success. Models controlled for individual identity as a random intercept effect.

Levels of extrapair paternity. We calculated the number of eggs sired within the pair-bond and the number of eggs sired outside the pair-bond for each individual male that was paired monogamously for an entire season. Monogamous pairing was inferred from daily observations (for details, see Schielzeth and Bolund 2010). The odds of the 2 egg counts express a male's tendency to be successful outside the pair-bond relative to success within the pair-bond (independent of the number of eggs sired). We also analyzed the absolute number of extrapair eggs sired. However, because this measure is strongly correlated with the total fertilization success ($r = 0.73$, $t_{103} = 10.94$, $P < 10^{-15}$), results are very similar to the results of the analysis of total fertilization success. Similarly, we calculated the levels of extrapair paternity for all females that were paired monogamously for an entire season by counting the number of eggs sired by the social mate and the number of eggs sired by extrapair males. We used a Binomial model with logit link to model the within-female proportion of extrapair eggs ($n = 27$, 29, and 28, for the male biased, equal sex ratio,

and female-biased treatment, respectively) and the within-male proportion of extrapair eggs ($n = 26$, 29, and 27, respectively). Models controlled for individual identity as a random intercept effect.

Statistical analysis

Generalized linear mixed-effects models were fitted in R 2.11.1 (R Development Core Team 2010) using the function lmer from the lme4 package (Bates and Maechler 2010). The function lmer provides P values for fixed effects for Binomial and Poisson model but not for Gaussian mixed model. For the latter, we approximate P values from t values (the ratios of point estimates to standard errors [SEs]) assuming t distributions with the degrees of freedom equal to the number of groups (individuals or pairs). The significance of the combined treatment effect (across all 3 levels) was determined by likelihood ratio tests. We denote linear standardized slopes as b_x, second-degree polynomials as b_{x2}, and interaction term estimates as b_{int}. Average effects across all treatments were calculated as the main effects of personality traits while controlling for treatment as a set on centered treatment indicators (Schielzeth 2010).

RESULTS

Behavioral response to the novel object

The zebra finches appeared to realize the novelty of the situation immediately after trials started. Many individuals leaned forward inspecting the object from a distance. Individuals then typically started hopping back and forth between the perches in cage A. In 61% of the trials, subjects entered cage B (where the novel object was placed). Subjects typically made only brief entries into cage B, but many started hopping back and forth between perches in cage A and in cage B. Nine individuals made physical contact with the novel object. Approach scores varied between 4.47 and 7.85 (possible range: 4.47–8.94) and showed a continuous and unimodal distribution. Sexes did not differ in their approach scores (ANOVA: $F_{1,528} = 0.01$, $P = 0.93$). The sequence of testing the 2 birds within dyad did not affect approach scores (ANOVA: $F_{1,528} = 0.03$, $P = 0.85$, apple: $F_{1,528} = 0.67$, $P = 0.41$). A subset of 48 individuals were additionally tested without a novel object. "Approach scores" without novel objects were significantly higher than approach scores with a novel object (paired t tests: flower: $t_{47} = 5.40$, $P < 10^{-6}$, apple: $t_{47} = 5.73$, $P < 10^{-6}$, ball: $t_{47} = 6.82$, $P < 10^{-8}$). This clearly shows that birds reacted to the presence of novel objects.

Repeatability of approach to novel objects

Subjects achieved higher approach scores in the trials with an apple as the novel object than in those with dry herbs (paired t test: $t_{529} = 2.70$, $P = 0.007$). Scores measured later with the flashy colored ball as the novel object correlated significantly with scores measured when presenting more natural objects (approach score: flower–ball $r = 0.45$, $P < 10^{-15}$, apple–ball $r = 0.52$, $P < 10^{-15}$). Approach scores measured with a flashy ball were significantly lower as compared with the apple trials (paired t test: $t_{362} = -3.58$, $P = 0.0004$) but did not differ significantly from trials with a bouquet of flowers (paired t test: $t_{362} = 1.42$, $P = 0.16$).

The between-object repeatability of approach score was high ($R_A = 0.43 \pm 0.04$, $P < 10^{-25}$, based on $n = 530$ individuals measured with 2 novel objects). The between-year repeatability (i.e., after averaging between the 2 objects) was also high ($R_A = 0.45 \pm 0.07$, $P < 10^{-6}$, based on $n = 119$ individuals measured twice with a 1.5 years interval). To reduce measure-ment error and because the majority of individuals involved in the breeding experiment were tested twice, we used mean scores for all individuals in the fitness analyses. There was no significant difference in approach score before and after the breeding round (paired t test: females: $t_{54} = -1.08$, $P = 0.28$; males: $t_{57} = -1.04$, $P = 0.30$). Furthermore, aviary breeding treatments did not significantly affect approach scores, neither when considering treatment order (ANOVA using change in approach scores as response and all distinct combinations of 2 treatments as predictors; females: $F_{6,43} = 0.64$, $P = 0.70$; males: $F_{6,47} = 0.90$, $P = 0.50$) nor when ignoring treatment order (ANOVA: females: $F_{3,46} = 0.85$, $P = 0.48$; males: $F_{3,50} = 0.47$, $P = 0.71$).

Quantitative genetics of approach to novel objects

Approach scores (averaged across 2 novel object trials) showed a substantial amount of heritable variation ($h^2 = 0.27 \pm 0.09$). Maternal effects were low, and nonsignificant and foster environment effects and peer group effects were close to zero (Table 1). There was also no correlation between approach scores and peer group size ($r = 0.06$, $P = 0.16$). There was evidence of lasting permanent environment effects (i.e., nonshared environmental effects and possibly nonadditive genetic effects) as well as long-term behavioral flexibility. This analysis is based on averages across 2 different novel objects (apple and flower). The trait used in the fitness analysis is based on averages across 4 trials (most individuals were measured before and after the breeding season). Because averaging across 2 sessions (of 2 trials each) will reduce measurement error, the expected heritability of the approach scores used in the fitness analysis is $V_{A(means)} = V_A/(V_M + V_F + V_P + V_{PE} + V_R/2)$, where V_A, V_M, V_P, V_{PE}, V_R are the additive genetic, maternal, peer, permanent environment, and residual variance components (as in Table 1), respectively. The extrapolated heritability of the trait used in the fitness analysis is therefore $h^2 = 0.38$.

Neophilia and social pairing

Birds were free to pair and mate with other birds in the aviary. Partner choice occurred simultaneously, so it is difficult to

Table 1

Variance component analysis of the approach scores (averages across 2 trials with 2 different novel objects)

Variance component	Estimate $\pm$ SE
Additive genetic effects	0.274 ± 0.089
Maternal effects	0.063 ± 0.041
Foster environment effects	0.005 ± 0.023
Peer group effects	0.005 ± 0.014
Permanent environment effects	0.097 ± 0.084
Residual	0.556 ± 0.067
Behavioral flexibility (long term)	0.157
Object specificity, short-term behavioral flexibility, and measurement error	0.399

Additive genetic, maternal, foster environment, peer group, and permanent environment (incl. nonadditive genetic) effects were estimated from an animal model with 4 generations of pedigree information. Variance components (total variance scaled to unity) and their SEs are shown. Based on the between-object repeatability, the residual component was further decomposed into a short-term behavioral flexibility (that includes object specificity and measurement error) and a long-term behavioral flexibility component.

disentangle mate preferences and effects of competition. Pairs mated randomly with respect to approach scores (correlation between approach scores of partners that were mated for at least one complete season: $r = -0.03$, $P = 0.76$).

Neophilia and number of fertilized eggs

Female fecundity ranged between 0 and 37 and was very similar in all 3 sex ratio treatments (mean ± SE: male biased 13.0 ± 1.2, equal sex ratio 14.3 ± 1.0, female biased 14.9 ± 1.0; ANOVA: $F_{2,116} = 0.80$, $P = 0.45$). Male fertilization success ranged from 0 to 54 and increased strongly with increasing sex ratio (mean ± SE: male biased 8.7 ± 1.2, equal sex ratio 14.3 ± 1.8, female biased 21.9 ± 2.2; ANOVA: $F_{2,118} = 16.28$, $P < 10^{-6}$). For males, the opportunity for selection (variance in relative fitness) based on the number of fertilized eggs differed significantly among treatments (mean ± SE: male biased: $I = 1.1 ± 0.11$, equal sex ratio $I = 0.62 ± 0.29$, female biased: $I = 0.37 ± 0.09$; ANOVA: $F_{2,15} = 3.8$, $P = 0.046$). In females, the opportunity for selection was overall lower than in males and did not vary much between the treatments (male biased: $I = 0.19 ± 0.09$, equal sex ratio: $I = 0.27 ± 0.06$, female biased $I = 0.24 ± 0.07$; ANOVA: $F_{2,15} = 0.30$, $P = 0.74$).

Selection differentials for approach scores were low and non-significant in males (overall $S = -0.10 ± 0.09$, $P = 0.28$, overall $C = 0.02 ± 0.08$, $P = 0.71$) as well as in females (overall $S = 0.02 ± 0.05$, $P = 0.67$, overall $C = -0.03 ± 0.04$, $P = 0.42$). Selection differentials differed little among the 3 sex ratio treatments (Figure 2).

Neophilia and chick-rearing success

Our sex ratio treatment did not affect the average mass of chicks at day 8 (mean ± SE, male biased: 7.78 ± 0.10, equal sex ratio: 7.93 ± 0.10, female biased: 7.90 ± 0.08; ANOVA: $F_{2,65} = 0.77$, $P = 0.47$) or the variation in chick mass between pairs (Levene test: $F_{2,65} = 0.54$, $P = 0.58$). Overall, approach scores did not explain chick-rearing success neither in males ($b_x = 0.23 ± 0.29$, $P = 0.43$, $b_{x2} = 0.41 ± 0.29$, $P = 0.16$) nor in females ($b_x = 0.22 ± 0.27$, $P = 0.42$, $b_{x2} = 0.08 ± 0.14$, $P = 0.57$). There was no evidence for an interaction between female and male approach scores to explain chick-rearing
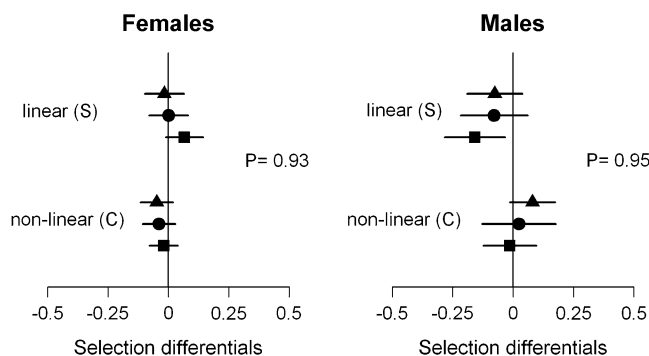

Figure 2
Linear and nonlinear selection differentials (means ± SE) on approach scores based on total fecundity (in females) and fertilization success (in males). S = linear selection differential (directional selection), C = nonlinear selection differential (selection on the variance, i.e., $C > 0$ means disruptive selection, $C < 0$ means stabilizing selection). The 3 sex ratio treatments are marked as male biased (triangles), equal (circles), and female biased (squares). Differences between treatments (in the linear and quadratic term combined) were tested by likelihood-ratio tests.
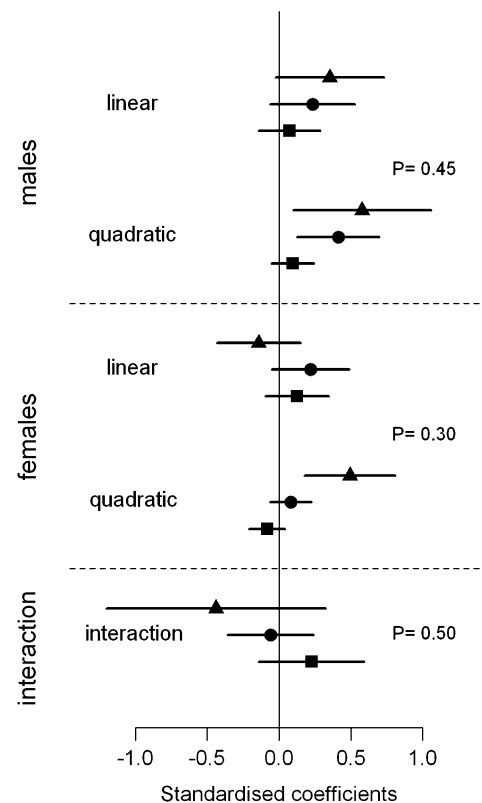
success ($b_{int} = -0.06 ± 0.30$, $P = 0.84$). There were no significant differences between treatments in how approach score affected chick-rearing success (Figure 3).

Neophilia and number of partners

Females laid eggs that were sired by 1–4 (mean: 2.1) partners, except for one individual that reproduced with 5 males in the female biased treatment. The mean number of sires did not differ among treatments (Poisson generalized linear model [GLM]: $F_{2,116} = 0.09$, $P = 0.91$). In males, the number of females with whom they sired eggs differed significantly between treatments (male biased: 0–5, mean = 1.4; equal sex ratio: 0–6, mean = 2.0; female biased: 0–7, mean = 3.3; Poisson GLM: $F_{2,118} = 18.02$, $P < 10^{-6}$).

Overall, the effect of approach scores on the number of partners was low and clearly nonsignificant in females ($b_x = -0.02 ± 0.07$, $P = 0.68$, $b_{x2} = -0.02 ± 0.05$, $P = 0.66$) and in males

Figure 3
Standardized coefficients (means ± SE) for approach scores as predictors of rearing success measured as mean mass of chicks at day 8. Standardized coefficients represent the change in rearing success in response to changes in the personality trait, both in units of standard deviations. The 3 sex ratio treatments are marked as male biased (triangles), equal (circles), and female biased (squares). Differences between treatments were tested by likelihood-ratio tests. Hence, the 3 P values test for between-treatment differences in curves for female contribution (linear and quadratic term combined), male contribution (linear and quadratic term combined), and the interaction between the sexes, respectively. The linear term captures directional effects, whereas the quadratic term captures nonlinear effects ($b_{x2} > 0$ means extremes produces higher weight offspring, $b_{x2} < 0$ means intermediates produce higher weight offspring) and the interaction measures the nonadditive interaction between female and male scores ($b_{int} > 0$ means assortatively mated pairs produce higher weight offspring, $b_{int} < 0$ means disassortatively mated pairs produces higher weight offspring).

Figure 4
The number of (genetic) partners in aviaries as a function of approach scores (first and second polynomial). Functions were estimated using a Poisson generalized linear mixed-effects model and back-transformed for display. The 3 sex ratio treatments are marked as male biased (triangles and dashed lines), equal (circles and continuous lines), and female biased (squares and dotted lines). Differences between treatments (linear and quadratic term combined) were tested by likelihood-ratio tests.
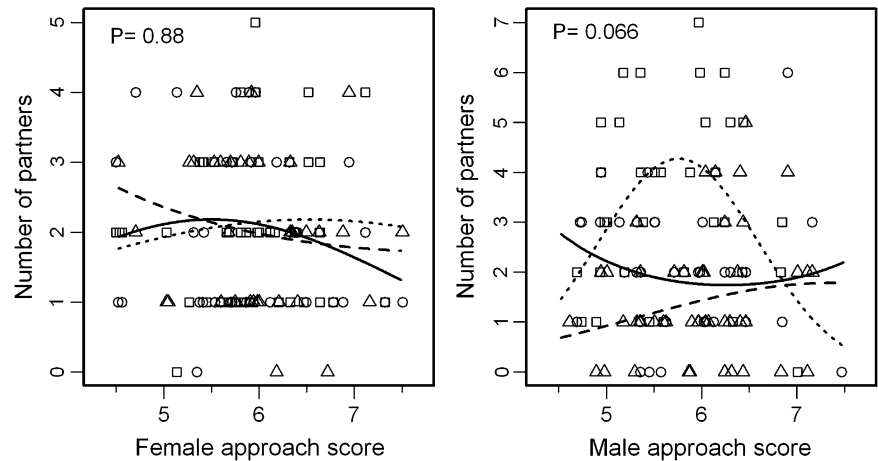
$(b_x = 0.05 \pm 0.09, P = 0.55, b_{x2} = -0.10 \pm 0.08, P = 0.23)$. The effect of approach score differed little between treatments, although there were indications that intermediate males had more (genetic) partners in the female-biased treatment but not in the other treatments (Poisson model, likelihood ratio test [LRT]: $\chi^2_4 = 8.82$, $P = 0.066$, Figure 4).

Neophilia and extrapair paternity

In females, the proportion of eggs sired by extrapair males (levels of extrapair paternity in females) did not differ between treatments (Binomial GLM: $F_{2,81} = 0.09$, $P = 0.92$, means per treatment: male biased: 0.30, equal sex ratio: 0.27, female biased: 0.26), whereas in males, the proportion of eggs sired with extrapair females (levels of extrapair paternity in males) did differ between treatments (Binomial GLM: $F_{2,79} = 6.18$, $P = 0.0032$ means per treatment: male biased: 0.26, equal sex ratio: 0.27, female biased: 0.46).

Overall, that is, averaged across the 3 sex ratio treatments, approach score did not predict significantly the levels of extrapair paternity rate in males ($b_x = 0.32 \pm 0.19$, $P = 0.086$, $b_{x2} = 0.20 \pm 0.19$, $P = 0.27$) and in females ($b_x = -0.45 \pm 0.40$, $P = 0.25$, $b_{x2} = -0.35 \pm 0.28$, $P = 0.20$). However, there were significant differences among treatments in the effect of neo-

philia on levels of extrapair paternity. Males with intermediate approach scores showed higher levels of extrapair paternity in the female-biased treatment (the one with the highest level of extrapair paternity), whereas in the equal sex ratio treatment males with extreme approach scores had higher levels of extrapair paternity (Binomial model, LRT: $\chi^2_4 = 20.69$, $P < 0.0004$, Figure 5). The linear term in the male biased ($z = 2.49$, $P = 0.013$) and the quadratic term in the equal sex ratio treatment ($z = 3.47$, $P = 0.00051$) were significant, whereas all other terms were nonsignificant ($|z| < 1.40$, $P > 0.16$).

In females, differences between treatments were less strong. Females with high approach scores tended to have lower levels of extrapair paternity in the equal sex ratio and in the male-biased treatment but not in the female-biased treatment (Binomial model, LRT: $\chi^2_4 = 8.76$, $P = 0.067$, Figure 5). None of the individuals terms were significant ($|z| < 1.55$, $P > 0.12$), except for linear term in female-biased treatment, which approached significance, $z = -1.84$, $P = 0.066$.

DISCUSSION

Our study explores the consequences of neophilia for reproductive performance under seminatural breeding conditions in aviaries. First, we show that approach scores as measured

Figure 5
Levels of extrapair paternity in males (proportion of all fertilized eggs sired with extrapair females) and in females (proportion of all laid eggs sired by extrapair males) in aviaries as a function of approach scores (first and second polynomial). Functions were estimated using a binomial generalized linear mixed-effects model and back-transformed for display. The 3 sex ratio treatments are marked as male biased (triangles and dashed lines), equal (circles and continuous lines), and female biased (squares and dotted lines). Differences between treatments (linear and quadratic term combined) were tested by likelihood-ratio tests.
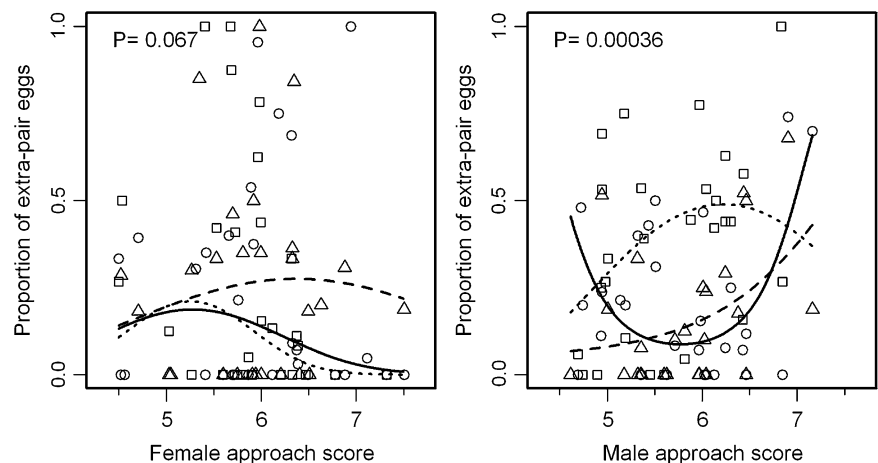
in the novel object trials were consistent within individuals even over extended periods of time. Second, we show that approach to the novel object had an additive genetic basis. Third, we show that our sex ratio treatment was effective in manipulating reproductive performance of males but less so of females. Fourth, we found little evidence for overall fitness consequences under seminatural aviary breeding conditions. Lastly, we found that the sex ratio treatments affected the relationship between personality scores and extrapair paternity but not overall fertilization or chick-rearing success. This suggests that variation in personality traits might reflect variation in reproductive strategies that depend on the social environment.

Our sex ratio treatment was successful in manipulating the opportunity for selection in males. The more competitive the environment (i.e., the more male biased the sex ratio), the larger the between-male variation in fertilization success. On the other hand, our treatments did not affect chick-rearing success. In agreement with this, zebra finch males increased nest defense in a male-biased treatment but did not alter their parental-care behavior (Burley and Calkins 1999). This suggests that the sex ratio treatment influenced mainly sexual selection (intrasexual competition and possibly mate choice) and less so natural selection (parental qualities). The absence of an effect on chick-rearing success may be a consequence of the aviary conditions, with ad libitum access to high-quality food (see also below). In females, the treatment did not influence the opportunity for selection as measured by the number of fertile eggs they produced, and it had little influence on the number of genetic partners and the proportion of eggs sired by extrapair males. In other words, the promiscuity of socially monogamously paired females was unaffected by the operational sex ratio. In contrast, the treatment clearly affected the level of promiscuity in males: in the female-biased treatment, where there were unpaired females that would still produce eggs (egg dumping and conspecific brood parasitism, Schielzeth and Bolund 2010), males sired eggs with a greater number of females and overall they had a higher siring success outside their monogamous pair-bonds. In summary, these data show that the sex ratio treatment had a profound effect on males but less effect on female reproductive success.

Treatments did not only differ in operational sex ratios but also in the distribution of food and water resources, overall density of birds (15 birds in male- and female-biased treatment, 12 birds in equal sex ratio treatment) and in the total number of nest-boxes (6 in male-biased and equal sex ratio treatment, 12 in female-biased treatment). All these differences might have influenced some of our results. However, the largely linear effects on measures of reproductive success strongly indicate that our treatment effect was dominated by the sex ratio effect. There is no indication that the different spatial arrangement of the equal sex ratio treatment (Figure 1) had a strong impact on the results, otherwise we would have expected that the equal sex ratio treatment differs significantly (in measures of reproductive success or in correlations between neophilia and fitness) from the 2 treatments with biased sex ratios.

Overall, variation in neophilia explained little of the variation in fertilization success/fecundity and chick-rearing success. The weak effects of neophilia on fitness in this study might be a consequence of the ad libitum food availability. This is particularly likely for the effect on parental qualities. Differences in parental quality due to more or less effective cooperation might become evident only if resources are limited or if resource availability fluctuates in space and/or time (Dingemanse et al. 2004; Boon et al. 2007). Studying the effectiveness of cooperation between pairs of different personalities in variable environments may be a promising avenue to find effects of neophilia on repro-

ductive success and to understand the underlying causes of these effects (Dingemanse et al. 2004; Both et al. 2005).

The sex ratio treatment had a more profound effect on the correlations between approach scores and levels of extrapair paternity than on the correlations between approach scores and overall reproductive success (see above). As predicted, males with the highest scores for approach to the novel object sired the highest proportion of extrapair eggs in the high competition (male biased) treatment. In the female-biased treatment, males with intermediate scores had higher extrapair success and in the equal sex ratio treatment males with extreme scores had highest success (Figure 5). In a number of species, exploration and boldness are correlated with aggression (e.g., in crickets: Kortet and Hedrick 2007, sticklebacks: Bell 2005, great tit: Verbeek et al. 1996), and this might explain the success of individuals with high approach scores in the male-biased treatment. However, increased aggression does not always translate into higher dominance ranks (great tit: Verbeek et al. 1999).

The effects of the social environment on the correlations between approach scores and reproductive performance were weaker in females, perhaps because our sex ratio treatment was less successful in manipulating female reproductive success and behavior in a measurable way. Clearly, however, there was no positive correlation between female approach to novel objects and extrapair paternity, in contrast to what one might have expected if neophilia influence the frequency of interactions with extrapair males.

In summary, most of the effects of neophilia on fitness-related traits we found were relatively weak and nonsignificant and given the large number of parameters we estimated, a few significant effects should not be overinterpreted. However, it is remarkable that differences between treatments are apparent when considering extrapair paternity but not when looking at overall success (in producing eggs or in rearing chicks). Hence, it seems likely that the response to novel objects in zebra finches is related to variation in reproductive strategies. Interestingly, these effects depend on the social environment as shown by the effects of the social environment on the correlations between personality traits and extrapair paternity rates. It remains to be seen whether personality traits mainly influence performance in intrasexual competition or whether they also influence (extrapair) mate choice.

REFERENCES

Arnold SJ, Wade MJ. 1984. On the measurement of natural and sexual selection: theory. Evolution. 38:709–719.

Bates D, Maechler M. 2010. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-35. http://CRAN.R-project.org/package=lme4.

Bell AM. 2005. Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). J Evol Biol. 18: 464–473.

Bell AM. 2007. Future directions in behavioural syndromes research. Proc R Soc B Biol Sci. 274:755–761.

Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML. 2003. The ecology of individuals: incidence and implications of individual specialization. Am Nat. 161:1–27.

Bolund E, Schielzeth H, Forstmeier W. 2010. No heightened condition dependence of zebra finch ornaments: a quantitative genetic approach. J Evol Biol. 23:586–597.

Boon AK, Réale D, Boutin S. 2007. The interaction between personality, offspring fitness and food abundance in North American red squirrels. Ecol Lett. 10:1094–1104.

Both C, Dingemanse NJ, Drent PJ, Tinbergen JM. 2005. Pairs of extreme avian personalities have highest reproductive success. J Anim Ecol. 74:667–674.

Brodie ED, Moore AJ, Janzen FJ. 1995. Visualizing and quantifying natural selection. Trends Ecol Evol. 10:313–318.

Burley NT, Calkins JD. 1999. Sex ratios and sexual selection in socially monogamous zebra finches. Behav Ecol. 10:626–635.

Coleman SL, Mellgren RL. 1994. Neophobia when feeding alone or in flocks in zebra finches, Taeniopygia guttata. Anim Behav. 48: 903–907.

Dingemanse NJ, Both C, Drent PJ, Tinbergen JM. 2004. Fitness consequences of avian personalities in a fluctuating environment. Proc R Soc Lond B Biol Sci. 271:847–852.

Dingemanse NJ, Réale D. 2005. Natural selection and animal personality. Behaviour. 142:1159–1184.

Falconer DS, Mackay TFC. 1996. Introduction to quantitative genetics. 4th ed. Harlow (UK): Prentice Hall.

Forstmeier W, Schielzeth H, Schneider M, Kempenaers B. 2007. Development of polymorphic microsatellite markers for the zebra finch (Taeniopygia guttata). Mol Ecol Notes. 7:1026–1028.

Forstmeier W, Segelbacher G, Mueller JC, Kempenaers B. 2007. Genetic variation and differentiation in captive and wild zebra finches (Taeniopygia guttata). Mol Ecol. 16:4039–4050.

Fox RA, Millam JR. 2007. Novelty and individual differences influence neophobia in orange-winged Amazon parrots (Amazona amazonica). Appl Anim Behav Sci. 104:107–115.

Godin JGJ, Dugatkin LA. 1996. Female mating preference for bold males in the guppy, Poecilia reticulata. Proc Natl Acad Sci U S A. 93:10262–10267.

Groeneveld E, Kovač M, Mielenz N. 2008. VCE4 user's guide and reference manual. Software package version 6.0.2. Mariensee (Germany): Institute of Farm Animal Genetics, Friedrich-Loeffler Institute.

Herborn KA, Macleod R, Miles WTS, Schofield ANB, Alexander L, Arnold KE. 2010. Personality in captivity reflects personality in the wild. Anim Behav. 79:835–843.

Kingman JFC. 1978. Simple-model for balance between selection and mutation. J Appl Probab. 15:1–12.

Kortet R, Hedrick A. 2007. A behavioural syndrome in the field cricket Gryllus integer: intrasexual aggression is correlated with activity in a novel environment. Biol J Linn Soc. 91:475–482.

Kruuk LEB. 2004. Estimating genetic parameters in natural populations using the 'animal model'. Philos Trans R Soc Lond B Biol Sci. 359:873–890.

Mettke-Hofmann C, Ebert C, Schmidt T, Steiger S, Stieb S. 2005. Personality traits in resident and migratory warbler species. Behaviour. 142:1357–1375.

Mettke-Hofmann C, Winkler H, Leisler B. 2002. The significance of ecological factors for exploration and neophobia in parrots. Ethology. 108:249–272.

Murphy LB. 1978. The practical problems of recognizing and measuring fear and exploration behavior in domestic fowl. Anim Behav. 26:422–431.

Nakagawa S, Schielzeth H. Forthcoming. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev. 85:935–956.

van Oers K, de Jong G, van Noordwijk AJ, Kempenaers B, Drent PJ. 2005. Contribution of genetics to the study of animal personalities: a review of case studies. Behaviour. 142:1185–1206.

van Oers K, Drent PJ, Dingemanse NJ, Kempenaers B. 2008. Personality is associated with extrapair paternity in great tits, Parus major. Anim Behav. 76:555–563.

Quinn JL, Patrick SC, Bouwhuis S, Wilkin TA, Sheldon BC. 2009. Heterogeneous selection on a heritable temperament trait in a variable environment. J Anim Ecol. 78:1203–1215.

R Development Core Team. 2010. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.

Réale D, Martin J, Coltman DW, Poissant J, Festa-Bianchet M. 2009. Male personality, life-history strategies and reproductive success in a promiscuous mammal. J Evol Biol. 22:1599–1607.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biol Rev. 82:291–318.

Schielzeth H. 2010. Simple means to improve the interpretability of regression coefficients. Methods Ecol Evol. 1:103–113.

Schielzeth H, Bolund E. 2010. Patterns of conspecific brood parasitism in zebra finches. Anim Behav. 79:1329–1337.

Schielzeth H, Burger C, Bolund E, Forstmeier W. 2008. Sexual imprinting on continuous variation: do female zebra finches prefer or avoid unfamiliar sons of their foster parents? J Evol Biol. 21: 1274–1280.

Schütt W, Tregenza T, Dall SRX. 2010. Sexual selection and animal personality. Biol Rev. 85:217–246.

Sih A, Bell A, Johnson JC. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evol. 19:372–378.

Sih A, Bell AM, Johnson JC, Ziemba RE. 2004. Behavioral syndromes: an integrative overview. Q Rev Biol. 79:241–277.

Sinn DL, Apiolaza LA, Moltschaniwskyj NA. 2006. Heritability and fitness-related consequences of squid personality traits. J Evol Biol. 19:1437–1447.

Smith BR, Blumstein DT. 2008. Fitness consequences of personality: a meta-analysis. Behav Ecol. 19:448–455.

Stöwe M, Bugnyar T, Loretto MC, Schloegl C, Range F, Kotrschal K. 2006. Novel object exploration in ravens (Corvus corax): effects of social relationships. Behav Process. 73:68–75.

Verbeek MEM, Boon A, Drent PJ. 1996. Exploration, aggressive behavior and dominance in pair-wise confrontations of juvenile male great tits. Behaviour. 133:945–963.

Verbeek MEM, de Goede P, Drent PJ, Wiepkema PR. 1999. Individual behavioural characteristics and dominance in aviary groups of great tits. Behaviour. 136:23–48.

Verbeek MEM, Drent PJ, Wiepkema PR. 1994. Consistent individual differences in early exploratory behavior of male great tits. Anim Behav. 48:1113–1121.

Webster SJ, Lefebvre L. 2001. Problem solving and neophobia in a columbiform–passeriform assemblage in Barbados. Anim Behav. 62:23–32.

Wilson DS. 1998. Adaptive individual differences within single populations. Philos Trans R Soc Lond B Biol Sci. 353:199–205.

Wilson DS, Clark AB, Coleman K, Dearstyne T. 1994. Shyness and boldness in humans and other animals. Trends Ecol Evol. 9: 442–446.

Zann RA. 1996. The zebra finch: a synthesis of field and laboratory studies. Oxford: Oxford University Press.