Quantitative genetics of behavioural reaction norms: genetic correlations between personality and behavioural plasticity vary across stickleback populations

N. J. Dingemanse†, I. Barber‡§, J. Wright¶ & J. E. Brommer**

†Department of Behavioural Ecology & Evolutionary Genetics, Max Planck Institute for Ornithology, Seewiesen, Germany
‡Animal Ecology Group, Centre for Ecological and Evolutionary Studies & Department of Behavioural Biology, Centre for Behaviour and Neurosciences, University of Groningen, Harren, The Netherlands
§Department of Biology, University of Leicester, Leicester, UK
¶Institute of Biological Sciences, University of Wales Aberystwyth, Aberystwyth, Wales, UK
**Bird Ecology Unit, Department of Biosciences, University of Helsinki, Helsinki, Finland

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Abstract

Behavioural ecologists have proposed various evolutionary mechanisms as to why different personality types coexist. Our ability to understand the evolutionary trajectories of personality traits requires insights from the quantitative genetics of behavioural reaction norms. We assayed >1000 pedigreed stickleback for initial exploration behaviour of a novel environment, and subsequent changes in exploration over a few hours, representing their capacity to adjust their behaviour to changes in perceived novelty and risk. We found heritable variation in both the average level of exploration and behavioural plasticity, and population differences in the sign of the genetic correlation between these two reaction norm components. The phenotypic correlation was not a good indicator of the genetic correlation, implying that quantitative genetics are necessary to appropriately evaluate evolutionary hypotheses in cases such as these. Our findings therefore have important implications for future studies concerning the evolution of personality and plasticity.

Introduction

Behavioural ecologists have long been interested in the adaptive nature of within-individual changes in behaviour (plasticity) in response to environmental change (Krebs & Davies, 1997; Piersma & Drent, 2003). Classic behavioural ecology theory would, for example, predict that natural selection favoured individuals that varied their boldness in response to changes in immediate predation risk (Krebs & Davies, 1997; Piersma & Drent, 2003), particularly in cases where (i) bold behaviour would facilitate resource acquisition in the absence of predators but otherwise increase the risk of predation (Sih et al., 2003), (ii) individuals would be regularly exposed to alternative predation environments, (iii) individuals would be capable of reliably predicting changes in predation risk and (iv) individuals would be able to respond within a behavioural timeframe (DeWitt et al., 1998; Gabriel et al., 2005). This function, describing how behaviour changes over an environmental gradient within a single individual, can be viewed as its behavioural reaction norm (BRN) (Smiseth et al., 2008; Dingemanse et al., 2010), which is characterized by a certain combination of behavioural elevation (its average level of behaviour) and (e.g. linear) slope (its behavioural plasticity). Traditionally, behavioural ecologists have assumed that all individuals can be characterized by the same BRN, often attributing individual variation within the same population to noise around an adaptive mean (Wilson, 1998; Dall et al., 2004; Sih et al., 2004b). Accumulating evidence for substantial variation in
average level of behaviour between individuals, commonly explaining > 30% (repeatability, assuming zero-order reaction norms) of the behavioural variation present within populations (Bell et al., 2009), has recently stimulated the consideration of alternative (i.e. adaptive) explanations for individual variation (Réale et al., 2007, 2010). Research into the evolutionary causation of individual variation has also been prompted by a growing awareness that the existence of consistent individual variation in behaviour across time or contexts (i.e. individual variation in the BRN elevation; Martin & Réale, 2008; Dingemanse et al., 2010), a phenomenon called ‘animal personality’ in the recent behavioural ecology literature (Dingemanse et al., 2010; Réale et al., 2010), might be the rule rather than the exception in a wide variety of animal taxa (Réale et al., 2007; Bell et al., 2009). It thus appears that populations in general might be comprised of individuals with different BRNs, where the average individual shows limited (though not necessarily a total lack of; Sih et al., 2004a; Dingemanse et al., 2010) behavioural plasticity. A host of evolutionary explanations has recently been put forward to explain why animals might have different personalities, invoking either constraints (Bell, 2005; Duckworth, 2010; Sih et al., 2004a,b) or adaptive arguments (reviewed by Dingemanse & Wolf, 2010; Wolf & Weissing, 2010).

There have recently been repeated calls to study animal personality variation within a BRN framework (Martin & Réale, 2008; Dingemanse et al., 2010; Stamps & Groothuis, 2010). The application of RN approaches has been advocated for three main reasons. First, this approach enables detailed decomposition of ‘raw’ phenotypic variances into key components, namely variance in average level of behaviour (BRN elevation), between-individual variance in behavioural plasticity (BRN slope), covariance between these RN components, and error (Nussey et al., 2007; Dingemanse et al., 2010; Westneat et al., 2011). Second, fitness studies applying this framework can document the patterns of selection that would otherwise remain hidden (Nussey et al., 2005; Duckworth & Kruuk, 2009; Dingemanse & Réale, in press), for example selection acting specifically on average level of behaviour, behavioural plasticity or the correlation between the two (Dingemanse et al., 2010). Third, quantitative genetics studies applying RN approaches can help better predict evolutionary repercussions of selection acting on behavioural variation. Specifically, the amount of heritable variation in both average level of behaviour and behavioural plasticity, and the genetic correlation between the two RN components, provides insightful information on the potential for constraints in adaptive evolution (Roff, 1997).

We use here a BRN approach to estimate the quantitative genetics parameters of a behavioural trait commonly used in animal personality research. BRN approaches are increasingly applied in personality research although primarily at the phenotypic level (reviewed by van Oers et al., 2005; Dingemanse et al., 2010). One reason for the absence of quantitative genetics information on BRNs within this field is that the estimation of genetic components of RNs requires information from a large number of individuals within pedigreed populations with substantial variation in relatedness among assayed individuals (Nussey et al., 2007; Brommer et al., 2008; Charmantier et al., 2008; Husby et al., 2010). In this study, we report the estimates of genetic variation in BRN elevation and slope based on an experimental study specifically designed to face this challenge. First, we used in vitro fertilization techniques to induce the variation in relatedness among three-spined stickleback Gasterosteus aculeatus bred within a partial North Carolina II breeding design (Dingemanse et al., 2009). Second, we screened large numbers of pedigreed offspring (> 1000) for behaviour over an environmental gradient. Third, we tested for the presence of heritable variation in average level of behaviour and behavioural plasticity using powerful random regression animal models (Schaeffer, 2004). Offspring used for this experiment were bred from wild-caught parents, which thereby provided insight into the presence of heritable variation in behavioural plasticity in natural populations. Finally, the experiment was conducted for two populations. This enabled us to examine whether any reported patterns of genetic structure of behaviour were population specific, or more general (Kelly, 2006).

We focussed on exploration behaviour in novel environments, a key animal personality trait (Réale et al., 2007) for which repeatable and heritable variation has been recorded for many animal taxa (Bell et al., 2009). We exposed our pedigreed stickleback fry to a novel aquarium containing five large stones positioned strategically such that information about the environment could only be gained by moving around the tank (Dingemanse et al., 2007, 2009). We quantified the amount of area covered (‘exploration’) following an individual’s introduction into this novel environment. Sticklebacks typically respond to such experiments by showing an initial burst of active exploration followed by a gradual return to normal activity levels within few hours (Dingemanse et al., 2009). This initial active form of exploration enables these fish to acquire information about the unfamiliar environment, but might otherwise increase the detection by predators (Sih et al., 2003) and incur energetic costs (Biro & Stamps, 2010). Because the marginal net fitness benefits per unit effort of exploration presumably decrease as more information about the environment is gathered, active exploration decreases over time (Dingemanse et al., 2007, 2009), leading to a form of adaptive behavioural plasticity commonly known as ‘acclimation’ (Archer, 1973; O’Keefe & Nadel, 1978).

We here view acclimation as a specific type of behavioural plasticity, because the individual’s perception of the novelty and risk of the same physical environment changes over time; time since introduction therefore...
represents an environmental axis of variation in the broad sense (for further discussion, see Réale et al., 2007; Martin & Réale, 2008; Dingemanse et al., 2012).

Exploration–acclimation RNs are interesting within an evolutionary context. According to theory, adaptive within-individual plasticity would be favoured when certain key conditions are met, which include (i) high environmental predictability, (ii) high incidence of repeated exposure to alternative environments (although individuals may have strategies to avoid environmental perturbation) and (iii) the ability of individuals to quickly alter their phenotype (DeWitt et al., 1998; Gabriel et al., 2005). These conditions are all likely fulfilled in the case of variation in novelty experienced by acclimating animals. Specifically, (i) novelty is expected to change predictably with unit time spent exploring (Archer, 1973), (ii) wild animals are continuously exposed to novel situations as part of everyday activities (e.g. during foraging or territory defence; Réale et al., 2007; Martin & Réale, 2008) and (iii) individuals of a diverse array of taxa, such as birds (Dingemanse et al., 2002: Ellenberg et al., 2009), fish (Peeke, 1995) and mammals (Pouret al., 1988; Martin & Réale, 2008), are known to acclimate rapidly to novel situations. For this reason, information on the presence of genetic variation in plasticity (acclimation) would be insightful, because if it were absent, there is the potential for constraints in the evolution of this behaviour.

The main findings reported here are that both elevations (personality) and slopes (behavioural plasticity) of exploration–acclimation BRNs contained genetic variation within both populations of stickleback fish, whereas the genetic correlation between those RN components was population specific. The phenotypic correlation was not a good indicator of the genetic correlation, implying that evolutionary response to selection acting on this variation cannot readily be predicted without insight into the quantitative genetics structure of behaviour.

Materials and methods

Study populations

The two populations inhabit man-made lakes situated 5.5 km apart on the island of Anglesey (North Wales, UK). Llyn Alaw (N50°20′23″ W04°26′20″) is a 3.09 km² reservoir created in 1966 by the impoundment of the River Alaw, whereas Cae Mawr (N50°17′06″ W04°23′31″) is a 214-m² pond constructed ca. 1980 with no natural input or outflow. Stickleback in the two populations experience different predation regimes (Dingemanse et al., 2007). Predatory fish (perch Perca fluviatilis and rainbow trout Oncorhynchus mykiss) were introduced into Llyn Alaw immediately after impoundment, and these populations have since been maintained at high densities through stock exchange programmes. In contrast, predatory fish have not been introduced into Cae Mawr and have never been observed during ecological surveys.

Experimental protocol

We provide here a summary of the full experimental protocol that has been described elsewhere (Dingemanse et al., 2009). In short, adult stickleback used as parents for our laboratory experiments were captured from both populations in May 2006 and housed in the laboratory at Aberystwyth University, UK. We applied two types of standard crossing schemes using standard split-clutch in vitro fertilization techniques and egg husbandry protocols (Barber & Arnott, 2000), Partial North Carolina II breeding designs and full crosses (Lynch & Walsh, 1998), using a total of 22 males and 15 females (Llyn Alaw) and 30 males and 28 females (Cae Mawr) for breeding (Dingemanse et al., 2009). Following egg fertilization (day 0), each portion of the split-clutch (hereafter called a ‘full-sib family’) was incubated in isolation. At hatching (day 8), fry in each full-sib family were counted and divided equally between two 30 × 20 × 20 cm tanks (hereafter, ‘holding tanks’; mean (±SE) number of fry per holding tank was 14.72 ± 0.466). One of the two holding tanks was then randomly assigned to a control treatment and the other to a predator-exposed treatment (for full details on predator-exposure treatment, see Dingemanse et al., 2009).

Behavioural assays

At ages 44, 46, 48, 50 and 52 days, one or two randomly selected individuals were captured from each holding tank without replacement (i.e. each individual was assayed once at a specific age), transferred alone to a ‘full-sib family’) was incubated in isolation. At hatching (day 8), fry in each full-sib family were counted and divided equally between two 30 × 20 × 20 cm tanks (hereafter, ‘holding tanks’; mean (±SE) number of fry per holding tank was 14.72 ± 0.466). One of the two holding tanks was then randomly assigned to a control treatment and the other to a predator-exposed treatment (for full details on predator-exposure treatment, see Dingemanse et al., 2009).

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analyses (PCA) followed by varimax rotation (Tabachnick & Fidell, 2001) summarized these two variables in a single component (Eigenvalue: 1.627; loading for both variables: 0.902) explaining the majority (81.3%) of variance in the two measures.

Statistical analyses

Animal models

We used random regression animal models (Schaeffer, 2004) to estimate the variation in BRNs (see Appendix S1 for an alternative statistical analysis of these data following the character state approach). We modelled exploration behaviour as a function of log-transformed time since introduction, because acclimation effects decay over time and were therefore expected to be nonlinear (Dingemanse et al., 2002, 2012). The initial model was population specific, including information on all individuals that are descendants from individuals sampled in either population. We considered the hierarchical mixed models for the exploration E of individual i at time = log(t):

\[
E_{i,\text{time}} = \mu + \text{TREATMENT}_i + \text{AGE} + \text{TIME}_i + f(\text{ind}_i, \text{time}) + e_{i,\text{time}}
\]  

\[
E_{i,\text{time}} = \mu + \text{TREATMENT}_i + \text{AGE} + \text{TIME}_i + f(\text{a}_i, \text{time}) + f(\text{pe}_i, \text{time}) + e_{i,\text{time}}.
\]

The first model (eqn 1a) allows between-individual variance around the population fixed-effect mean change in exploration over time. Importantly, the mean change (Fig. 1) is described by including time as a factorial (F) fixed effect (TIME, 0, 2, 4 h), which ensures that any nonlinearity is fully described. The individual-specific deviation from the fixed-effect means is modelled as a random effect function over the continuous variable time, f(\text{ind}_i, \text{time}). This function thus describes the ‘ranking’ of exploration (relative to the time-specific mean) across individuals over time. The between-individual variation was then partitioned (eqn 1b) into an additive genetic component f(\text{a}_i, \text{time}) and a permanent environment (PE) component f(\text{pe}_i, \text{time}). The former was estimated using an animal model, with pedigree data allowing the relatedness matrix among individuals to be specified (Lynch & Walsh, 1998; Kruuk, 2004).

The individual-specific random effects (\text{ind}_i, \text{a}_i, \text{pe}_i) were specified as continuous functions of time. We compared the model fits with different polynomial functions for how the effect varies across time of order x, namely constant (x = 0) and linear (x = 1) forms of the RNs. (Polynomial random regression functions (x = 2) were not fitted because with three points in time, such models do not allow the estimation of residual variances and instead attribute all variation to the among-individual reaction norm (co)variance component). For example, fitting a zero-order function for \text{a}_i (eqn 1b) results in breeding values, and hence, genetic variance values constrained to be constant across time periods (no genotype by time interaction), i.e. a ‘standard’ repeated measures animal model. This model was then compared with a first-order polynomial function (\text{a}_i + \text{a}_i \text{time}) in which the breeding value (considered as the deviation from the time-specific mean exploration, eqn 1) was modelled as a linear RN model across time such that variances in elevation (\text{a}_i) and slope (\text{a}_i \text{time}) are estimated, as well as the covariance between these components. Eqn 1 was solved using maximum-likelihood procedures in the program ASReml v2.0 (VSN International, Hemel Hempstead, UK). ASReml standardizes the covariate time prior to analyses such that the lowest value is −1 and the scaled values (Fig. 1)

Exploration behaviour (±SE) as a function of (a) log-transformed time since introduction into a novel tank, (b) age of testing and (c) treatment group. Open dots are for the predator-naïve Cae Mawr, and filled dots for the predator-sympatric Llyn Alaw, population of sticklebacks. We give here the raw phenotypic data; a random regression phenotypic model showed that time since introduction affected exploration behaviour (F(1,1000) = 71.1, P < 0.001) but not age (F(2,1000) = 0.03, P = 0.97) or predator-exposure treatment during development (F(2,1000) = 2, P = 0.14).
covariate has a mean of zero. In this case, data scale values for time were 0, 0.301 and 0.477, which were scaled to $-1$, 0.1607 and 0.8393, respectively (i.e. $-1 + 3.856 \times \text{time}$). Variance in elevation ($\text{ind}_{00}, a_0, p_{00}$) can be interpreted as the among-individual variance at the mean scaled covariate value of 0. It should be noted that variances of random regression slopes ($\text{ind}_{10}, p_{10}$, $a_{10}$) are specific to the scaling of the covariate (Schaefer, 2004).

Random effects, and residual errors, were modelled as normally distributed with zero means and variances to be estimated. Visual inspection of plots of residual vs. fitted values revealed satisfactory fit. Residual errors ($e_{ij}$, time) were assumed to be time specific (three time-specific error variances were estimated). Apart from time, additional fixed effects included in all the models were predator-exposure treatment (TREATMENT, a factorial fixed effect denoting whether individuals were exposed during ontogeny to predators or not) and mean-centred AGE (in days) of the individual when assayed for exploration behaviour. It should be noted that environmental maternal and holding tank effects were modelled elsewhere and not detected (Dingemanse et al., 2009) and are therefore not included in the analyses presented here.

Pedigree information

Eqn 1b can be solved for the genetic (co)variance function by using information on the coefficient of ancestry $\Theta_{ij}$ between individuals $i$ and $j$, which is directly obtained from the pedigree. In all analyses, we included the pedigree information, where the base parents derived from the two different populations were entered as separate genetic groups. To solve eqn 1b, the additive genetic effects on individual $i$, $a_{ii}$, were modelled as a mean of zero and normally distributed additive genetic variance of $\sigma_{A}^2$ (the variance in $a_{ii}$). This variance (and the additive genetic covariance between all $a_{ii}$ if $x > 0$) was estimated from the variance–covariance matrix of additive genetic effects which is equal to $A \sigma_{A}^2$, where $A$ has elements $A_{ij} = 2 \Theta_{ij}$. For details on random regression animal models, see Schaeffer (2004).

Model comparison and inference

We established the most parsimonious population-specific models following eqn 1. In order to allow the comparison of variance components across populations, we standardized the measure of exploration by its population-specific standard deviation. Eqn 1 allows for a hierarchical stepwise forward approach to assess the statistical significance of all the random effects by a likelihood ratio test (LRT). The test statistic is twice the difference in log-likelihood between hierarchical models and is distributed as $\chi^2$ with degrees of freedom equal to the difference in the number of (co)variance parameters estimated. First, we tested for phenotypic variance in the parameters describing individual exploration over time (eqn 1a). We started with a model that included only fixed effects and time-specific residuals, with residuals that were assumed to be uncorrelated across time (model 1), then tested for differences among individuals by sequentially fitting individual effects as zero-order (model 2, $\text{ind}_{0i}$) and first-order (model 3, $\text{ind}_{0i} + \text{ind}_{1i} \times \text{time}$) (Table 1). Significance of each increase in order of the polynomial function $f(\text{ind}_{0i}, \text{time})$ was evaluated using a LRT test. Because we detected significant variation in RN elevation and slopes (Table 1), we then continued by testing whether partitioning variance in RN elevation $\text{ind}_{0i}$ into additive and permanent environmental effects ($a_0$ and $p_{0i}$) significantly improved the model (while retaining the random regression across individuals; $a_{0i} + p_{0i} + f(\text{ind}_{0i}, \text{time})$; $n > 0$). This model tested the hypothesis that exploration is heritable, while allowing individual-specific variance to vary with time. We then allowed additive genetic variance to vary with time by testing for first-order polynomials of time-specific additive genetic and permanent environment effects. See Brommer et al. (2008) for an equivalent modelling approach and Nussey et al. (2007) for details on the philosophy of exploring variation in plasticity in this fashion.

The RNs of the models were visualized (Fig. 2) by extracting the best linear unbiased predictor (BLUP) value for each individual’s $\text{ind}_{0i},a_{0i}$ and $p_{0i}$ and by plotting the appropriate random effect function (eqn 1) on the data scale (scaling described above). These RNs were thus defined as deviations from the fixed-effect means.

Comparison of G matrices across populations

The structure of the variance–covariance matrix of the random regression terms was estimated for both populations simultaneously by constructing a multivariate version of eqn 1 and assuming homogeneous population-specific residuals (homogenous residuals were assumed because heterogeneous ones cannot easily be implemented in bivariate random regression models). For example, in considering zero- and first-order terms on the genetic ($a_0$, $a_1$) and nongenetic levels ($p_{0i}$, $p_{1i}$) in both populations, a bivariate random regression can be constructed estimating population-specific matrices for genetic (co)variances and nongenetic (co)variances between these terms. Because individuals were not cross-bred between the two populations, the covariances across populations are not available to estimation: on the genetic level, we could only estimate $G = \begin{bmatrix} G_1 & 0 \\ 0 & G_2 \end{bmatrix}$, where $G_i$ is a matrix containing the genetic variances ($V$) and covariances (COV) in $a_0$ and $a_1$ for population $i$ 

\begin{align*}
G_i &= \begin{bmatrix}
V(a_{0i}) & \text{COV}(a_{0i}, a_{1i}) \\
\text{COV}(a_{0i}, a_{1i}) & V(a_{1i})
\end{bmatrix},
\end{align*}

and and $[0]$ is a matrix of the same size as $G_i$ containing zeros). An analogous matrix PE was estimated for the population-specific permanent environment (co)variance matrices $\text{PE}_1$ and $\text{PE}_2$ specifying the (co)variances in the perma-
Table 1 Hierarchical models describing exploration behaviour of three-spined stickleback over three time steps (0, 2, 4 h) as a function of log (time since introduction (h) + 1) in a novel environment tank. (a) Cae Mawr population; analysis based on 1698 exploration tests of 366 individuals. (b) Llyn Alaw population; analysis based on 1338 exploration tests of 446 individuals. The estimated variance with standard error in parentheses is given for terms (as specified in eqns 1a and 1b) that were included in the model, with ‘-’ indicating terms that were not included. Models are nested with increasingly higher-order terms, where the significance of each higher-order term is based on the increase in log-likelihood (log L), starting with the model with environment-specific residuals only (Model 1) and ending with model 5 which includes all terms in eqn (1b). Terms are a function of either the phenotypic variance across individuals (’ind’) or its components, the additive genetic effect (’a’) and permanent environment effect (’pe’) as a polynomial function with the order (0 or 1) indicated by subscript. For all the models, the number of estimated (co)variance terms is provided with the likelihood ratio test (LRT) (χ²-statistic with associated d.f.) between the given model and the model that is one hierarchical step higher (i.e. 2 vs. 1, 3 vs. 2, etc.). Model 5 is the most parsimonious model for both populations and has the following residual variances for (a) Cae Mawr 0 h: 0.33 ± 0.082; 2 h: 0.46 ± 0.036; 4 h: 0.17 ± 0.050 and for (b) Llyn Alaw: 0 h: 0.52 ± 0.093; 2 h: 0.41 ± 0.037; 4 h: 0.18 ± 0.054.

(a) Random regression model terms* Test

<table>
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<tr>
<th>Model</th>
<th>I (ind)</th>
<th>I × E (ind)</th>
<th>G (a)</th>
<th>G × E (a)</th>
<th>PE (pe)</th>
<th>PE × E (pe)</th>
<th>log L</th>
<th>χ²</th>
<th>d.f.</th>
<th>P*</th>
</tr>
</thead>
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<tr>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>–780.13</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>0.41 ± 0.037</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–704.75</td>
<td>230.77</td>
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<tr>
<td>3</td>
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<td>0.33 ± 0.042</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–662.70</td>
<td>84.10</td>
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</tr>
<tr>
<td>4</td>
<td>–</td>
<td>0.33 ± 0.042</td>
<td>0.07 ± 0.033</td>
<td>–</td>
<td>0.37 ± 0.040</td>
<td>–</td>
<td>–653.36</td>
<td>18.68</td>
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<tr>
<td>5</td>
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<td>–</td>
<td>0.07 ± 0.031</td>
<td>0.05 ± 0.026</td>
<td>0.38 ± 0.040</td>
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(b)

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<th>I × E (ind)</th>
<th>G (a)</th>
<th>G × E (a)</th>
<th>PE (pe)</th>
<th>PE × E (pe)</th>
<th>log L</th>
<th>χ²</th>
<th>d.f.</th>
<th>P*</th>
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<td>–</td>
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<td>–645.68</td>
<td>–</td>
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<tr>
<td>2</td>
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<td>0.14 ± 0.056</td>
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<td>0.28 ± 0.049</td>
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<td>24.49</td>
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*We note that variances are non-normally distributed by nature (Hallgrímsson & Hall, 2005); printed standard errors should therefore be interpreted with caution as significance of random effects should be derived from LRTs between nested models (Hallgrímsson & Hall, 2005).

†Model selection procedures based on the Akaike’s information criterion (AIC) (Akaike, 1973) confirmed that, for both populations, the full model (model 5) fitted the data best: ΔAIC values for models 1–5 were, respectively, (a) 340.74, 111.98, 31.88, 15.20, 0.00; (b) 335.74, 122.86, 25.84, 20.48, 0.00.

Results

Heritable variation in BRN components

On introduction into the novel environment, laboratory-raised offspring from both parental stickleback populations showed substantial levels of exploration followed by a period of acclimation, in which exploration levels were reduced (Fig. 1). However, the BRN of the average individual differed markedly between the two populations: stickleback offspring from Llyn Alaw – a large reservoir – were more explorative at all sampled time points as compared with stickleback from Cae Mawr, a small, isolated pond situated 5.5 km from Llyn Alaw (Dingemanse et al., 2007). At the same time, the pattern of acclimation (behavioural plasticity) did not differ between the populations (Fig. 1). In other words, population–average exploration–acclimation RNs differed in elevation, but not in slope, because there was between-population genetic variation in exploration behaviour.

Both populations harboured significant amounts of heritable variation in the average level of behaviour and behavioural plasticity, based on a four-step hierarchical comparison of statistical models describing sources of variation in exploration behaviour over log-transformed time since introduction in the novel environment (Table 1). First, inclusion of individual-specific BRN elevations (’I’ for individual, Nussey et al., 2007; Model 2) significantly improved model fit compared with a model containing time-specific residuals alone (Model 1), implying that individuals differed consistently in their average level of exploration behaviour over the three time periods. Adjusted repeatability values (r) ± SE, values of r after controlling for the fixed effects in the
model (Nakagawa & Schielzeth, 2010), were 0.41 ± 0.037 for Cae Mawr (Model 2; Table 1a) and 0.45 ± 0.043 for Llyn Alaw (Model 2; Table 1b). Second, model fit improved further when we subsequently included individual-specific slopes (‘I × E’ for individual by environment interaction; Nussey et al., 2007), meaning that individuals also differed in behavioural plasticity (Model 3; Table 1a,b). Third, splitting I into its additive genetic (‘G’) and environmental (‘PE’ for permanent environment; Nussey et al., 2007) components (Models 4; Table 1a,b) further improved model fit for both populations, implying that the average level of exploration behaviour was partly heritable (narrow-sense heritability \( h^2 \) ± SE of behavioural elevation was 0.07 ± 0.033 (Model 4; Table 1a) for Cae Mawr and 0.14 ± 0.056 (Model 4; Table 1b) for Llyn Alaw). (We note that ‘I’ is statistically split into ‘PE’ and ‘G’ by fitting an extra random effect (see the Materials and Methods), namely the additive genetic variance in BRN elevation \( a_0 \); a significant improvement of model fit therefore implies \( h^2 > 0 \).) Finally, splitting I × E into its genetic (‘G × E’) and permanent environmental (‘PE × E’) components again further improved model fit for both populations (Models 5; Table 1a,b), which shows that the variation between individuals in behavioural plasticity had a heritable basis (i.e. \( G \times E > 0 \)).

Fig. 2 Behavioural reaction norm (BRN) plots for exploration behaviour over log-transformed time since introduction into a novel tank for the predator-naïve (panel a) and predator-sympatric (panel b) population of sticklebacks. Best linear unbiased predictors (BLUPs) are printed at the individual (ind), permanent environment (pe) and additive genetic (a) level. (We note that, following Hadfield et al. (2009), BLUPs are used here for illustrational reasons only; the statistical analysis was not based on them.) Elevation–slope correlations (±SE) are reported above each figure. Variances increase over time (‘fanning-out’) where the elevation–slope correlation was positive, but decrease over time (‘fanning-in’) for cases where it was negative. The reaction norms are here defined relative to the fixed-effect mean (indicated by ‘0’ on the y-axis) for each of the three time steps when exploration was measured and thus ignores the large overall decrease in activity (Fig. 1).
Genetic covariance between BRN components

Average level of exploration behaviour (BRN elevation) and behavioural plasticity (BRN slope) was positively correlated at the level of the individual (elevation–slope correlation $r_{\text{ind,ind}}$) ± SE was 0.21 ± 0.08 for Cae Mawr and 0.42 ± 0.11 for Llyn Alaw: the amount of between-individual variation in exploration behaviour increased with time since introduction in the novel environment for both populations (Figs 2 and 3a,b). Decomposition of $r_{\text{ind,ind}}$ into its permanent environment ($r_{\text{pe,pe}}$) and additive genetic components ($r_{\text{G,ind}}$) revealed population differences in the genetic structure of behaviour. First, $r_{\text{G,ind}}$ was negative in Cae Mawr (where the expression of additive genetic variation decreased with time since introduction; $r_{\text{G,ind}}$ ± SE = −0.64 ± 0.32; Figs 2a and 3c) but positive in Llyn Alaw (where the expression of genetic variation instead slightly increased with time since introduction; $r_{\text{G,ind}}$ ± SE = 0.44 ± 0.26; Figs 2b and 3d; statistical analyses using character state approaches led to the same interpretation, see Appendix S1). Second, a test for equality of the $G$ matrix across the two populations revealed significant population differences ($\chi^2 = 9.22, P = 0.027$). Such population variation in $G$ matrix structure might result from differences in additive genetic variation for elevation ($v_0$), additive genetic variation for slope ($v_1$) or elevation–slope covariance ($COV_{v_0,v_1}$). Post hoc tests revealed that neither $v_0$ ($\chi^2 = 1.92, P = 0.17$) nor $v_1$ ($\chi^2 = 0.62, P = 0.43$) differed significantly between the two populations, whereas $COV_{v_0,v_1}$ did ($\chi^2 = 6.00, P = 0.014$). Hence, variation in $G$ resulted from population differences in how BRN elevation and slope were genetically correlated rather than reflecting differences in the amount of genetic variation per se.

Taken together, these findings imply that although the genetic correlation structure might potentially constrain evolution within these populations at the current time, population differences in sign of the genetic correlation between elevation and slope suggest that such constraints would be ‘relative’ (i.e. short-term) rather than ‘absolute’ (Klingenberg, 2005). Moreover, stickleback are known to have occupied these freshwater habitats only since the last ice age, and populations in close proximity, like the ones studied here, are normally derived from the same marine ancestral population (Määkinen et al., 2006; Malhi et al., 2006). This implies that any genetic differentiation between our two populations must have occurred over short rather than long evolutionary time scales.

Nongenetic covariance between BRN components

In both populations, a substantial percentage of the between-individual variance in BRN elevation ($\text{ind}_0$) and slope ($\text{ind}_1$) was caused by permanent environment effects (i.e. variance in $pe_0$ and $pe_1$, respectively; Table 1): 84% (Cae Mawr) and 65% (Llyn Alaw) of the variance in $\text{ind}_0$, and 85% (Cae Mawr) and 63% (Llyn Alaw) of the variance in $\text{ind}_1$, was due to permanent environmental effects. Permanent effects occur when individuals differ consistently from each other for other reasons than additive genetic effects (Lynch & Walsh, 1998), for example because of variation in previous experience with certain stimuli, ‘condition’ (e.g. hunger level at the onset of the experiment) or maternal effects (Mousseau & Fox, 1998). Such a low heritable determination of BRN components implies that the individual-level elevation–slope correlation ($r_{\text{ind,ind}}$) will have been shaped primarily by the permanent environment ($r_{\text{pe,pe}}$) and not by the additive genetic correlation ($r_{\text{G,ind}}$) (Roff, 1997). Indeed, as expected on the basis of our positive estimates of $r_{\text{G,ind}}$, $r_{\text{pe,pe}}$ was also positive in both populations ($r_{\text{pe,pe}}$ ± SE = 0.35 ± 0.11 for Cae Mawr and 0.40 ± 0.19 for Llyn Alaw). The increase in individual variation in exploration with increasing time since introduction (Fig. 3a,b) therefore arose primarily through an increase in permanent environment variation (Figs 2 and 3c,d). For example, individual differences in internal state (e.g. energy reserves, hunger level) may have become more pronounced with time since introduction in the novel environment. At the same time, because the two populations were exposed to the same standardized laboratory conditions, $r_{\text{pe,pe}}$ was expected to be similar in sign and magnitude in both populations. PE matrices did indeed not differ significantly between the populations ($\chi^2 = 4.52, P = 0.21$).

Discussion

Population variation in genetic structure of behaviour

The presence of heritable variation in RN elevations and slopes (Table 1), as well as the existence of distinct population differences in $G$ matrix structure due to the population-specific genetic correlations between RN components (Fig. 2), implies that either stochastic processes (Lande, 1992; Whitlock et al., 2002), migration or adaptive population differences (Lande, 1979, 1986) have given rise to the observed patterns of population variation in genetic correlation structure of BRN components. Genetic drift or (restrictions in) migration may have played a major role particularly through founder effects, because one of the populations (Cae Mawr) is situated in a small isolated pool with no natural input or outflow, whereas the other inhabits a large reservoir (Llyn Alaw) with ample connections to other stickleback populations nearby (Dingemanse et al., 2007, 2009). An exciting alternative explanation for the observed differences in $G$ matrix structure is that the ecological characteristics of these populations have led to local adaptation (Dingemanse et al., 2007, 2009). The decrease in additive genetic variance for exploration behaviour with time spent in a novel environment that we observed for the Cae Mawr population (Figs 2 and 3c) is consistent with
an adaptive scenario where selection in more familiar environments (e.g. ≥ 2 h after introduction in our experiment; Fig. 2) strongly favours a single optimal response. Over evolutionary time, this type of interaction between the level of familiarity and strength of selection would presumably lead to (1) the loss of RN genotypes that fail to restrict their activity to the optimum level of exploration once the novelty of the environmental challenge has worn off and (2) the evolution of a negative genetic correlation between elevations and slopes of exploration–acclimation RNs. In contrast, large lakes with predators are likely to offer both safe and dangerous habitats (e.g. vegetated vs. open waters) that would not select for a single optimal level of ongoing exploratory activities in familiar environments. Within such populations, selection on the level of ongoing exploration behaviour in familiar environments might vary across habitat types and would therefore not favour the evolution of a negative genetic correlation between exploration–acclimation RN components. Whether or not ecological factors of this kind generate such patterns of local adaptation cannot be determined by the type of data we present here, and requires specific experimental testing. Nevertheless, it appears that the genetic structure of exploration–acclimation RNs can readily evolve because genetic variation in different RN components is present, and evolutionary processes appear to have generated considerable genetic differentiation on small spatial scales across these stickleback populations.

**Cheverud’s conjecture**

Our decomposition of \( r_{\text{ind},\text{ind}} \) into its permanent environment \( (r_{\text{pe},\text{pe}}) \) and additive genetic components \( (r_{\text{a},\text{a}}) \) warrants the conclusion that phenotypic information on the correlation structure of BRN components may not generally predict genetic correlation structure: Although \( r_{\text{ind},\text{ind}} \) closely resembled \( r_{\text{a},\text{a}} \) in Llyn Alaw, this was not the case in Cae Mawr, where \( r_{\text{ind},\text{ind}} \) was positive but \( r_{\text{a},\text{a}} \) was instead negative (Fig. 2). Cheverud’s conjecture (Cheverud, 1988), which poses that phenotypic correlations reflect genetic correlations (i.e. the ‘phenotypic gambit’ from the behavioural ecology literature; Grafen, 1984), thus appears invalid in the context of BRN structure in sticklebacks and obscures important differences between the populations. This is primarily because the (co)variances at the permanent environment level are relatively large and thereby drive the positive correlation on the phenotypic level even when the genetic correlation is negative, a situation that might well apply generally to behavioural variation (Hadfield et al., 2007; Dochtermann, 2011). Although heritable effects may cause only a relatively small part of the phenotypic (co)variance, they alone determine the evolutionary dynamics because permanent environmental effects are not inherited by an individual’s descendants. Experimental designs that enable the decomposition of genetic vs. environmental effects, as applied in this study, will therefore be necessary to appropriately evaluate...
evolutionary hypotheses concerning animal personality variation (Dochtermann & Roff, 2010; Dochtermann, 2011). At the same time, estimates of genetic structure of behaviour derived directly from pedigreed natural populations are perhaps needed to further assess the validity of Cheverud’s conjecture to verify that the patterns observed in these stickleback populations do not represent an exception to the rule.

**Missing pieces of the puzzle**

Although these findings imply that there is indeed the raw genetic material present within stickleback populations that would enable exploration-acclimation BRN elevations and slopes to evolve, two remaining key facets necessary for predicting how these RNs have evolved await future study. First, information regarding the selection pressures acting on exploration-acclimation RNs in these natural populations would help reveal whether optimal BRNs are currently present in these populations. This would, for example, be the case if selection favoured intermediate breeding values for RN elevations and slopes within populations, or selection on behavioural plasticity would be frequency dependent (Mathot et al., 2011). Selection pressures acting on exploration behaviour have recently been quantified in wild birds (Dingemanse et al., 2004; Quinn et al., 2009), but estimates of selection acting on behavioural plasticity in natural populations are generally missing from the empirical literature (Duckworth & Kruuk, 2009; Dingemanse et al., 2010). Second, planned artificial selection experiments for certain combinations of elevation and scope would provide further information on whether these populations can ultimately respond to (correlational) selection, and reveal whether there would be no response to selection (i.e. constrained evolution) for certain combinations of elevation and scope (for a worked example, see Beldade et al., 2002).

**Conclusions**

Our findings indicate that the existence of divergent ‘animal personalities’ (Dall et al., 2004; Dingemanse et al., 2010; Réale et al., 2010), applied to exploration behaviour in the context of acclimation to novel environments, may evolve in response to selection. Repeatable and heritable differences in exploration were evident in our two stickleback populations, both of which evidently harboured sufficient heritable variation in behavioural plasticity for selection to act upon (Table 1). We therefore argue that future research should focus more explicitly upon why consistent individual variation in both the average level of behaviour (animal personality) and behavioural plasticity is maintained, simultaneously (Dingemanse & Wolf, 2010; Dingemanse et al., 2010, 2012). For example, theoreticians have recently proposed that consistent individual differences in behavioural plasticity might be maintained by a combination of negative frequency-dependent selection (maintaining variation in plasticity) and positive feedback mechanisms reducing the costs of plasticity (favouring consistent individual differences in plasticity) (Wolf et al., 2008). More generally, a range of novel adaptive explanations for why animals have personalities (Dingemanse & Wolf, 2010; Wolf & Weissing, 2010), and why animal personality and behavioural plasticity might be linked (Wolf et al., 2008, 2011; Botero et al., 2010; Mathot et al., 2011), have recently been proposed, providing ample opportunities for experimental testing by empiricists interested in understanding the patterns of consistent individual variation in the wild.

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**References**


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