Heritability and fitness-related consequences of squid personality traits

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Abstract

Dumpling squid, *Euprymna tasmanica*, show consistent individual differences in behaviour that can be classified according to indices reflecting shy–bold, activity and reactivity responses. Using crosses of wild-caught single males to multiple females with known behavioural phenotypes, this study estimated patterns of additive genetic and residual variance in these behavioural traits from offspring of squid in two contexts, a threat (antipredator) and feeding (foraging) test. Genetic contributions to behavioural expression were dependent on test context. Behaviours in antipredator contexts had significant heritabilities ($h^2 = 0.2$–0.8) while behaviours from foraging contexts had lesser additive genetic and greater residual components ($h^2 = 0.05$–0.08). Personality trait variation in females was not related to her fecundity. Female boldness in foraging situations, which co-varied with body size, explained small but significant variation ($\sim$21%) in brood hatching success, while successful fertilization was determined by positive assortion of mate pairs according to their shy–bold phenotype. These results are discussed in terms of the ecological and evolutionary significance of animal 'personality' traits in wild populations of animals.

Introduction

Recently there has been increased interest in the evolutionary significance of a class of continuous behavioural variation in animals, variously referred to as 'personality traits' (Gosling, 2001) or 'behavioural syndromes' (Sih *et al.*, 2004b). Both terms refer to the suite of behaviours which make up an individual's unique behavioural phenotype; this individual variation is usually described along a number of axes, such as shyness/boldness (e.g. Wilson *et al.*, 1994), activity (e.g. Werner & Anholt, 1993), reactivity or fear (e.g. Boissy, 1995) and exploratory behaviour (e.g. Verbeek *et al.*, 1994). Animals from a variety of taxa show consistent individual differences in these behaviours in a number of contexts related to fitness, including mating, antipredator and foraging situations (reviewed in: Gosling, 2001). Thus, animal

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personality traits should be important in understanding alternative life history strategies in animals and contribute to our understanding of the mechanisms related to the maintenance of phenotypic and genotypic variation within animal populations (Dall *et al.*, 2004). Widespread phenotypic variation in animal personality traits in a number of populations implies that, instead of eroding variation around an adaptive mean, variation amongst traits in wild populations of animals has been maintained by natural selection (Clark & Ehlinger, 1987; Wilson, 1998). Few studies, however, have quantified both genetic parameters and direct fitness consequences for these behavioural traits in wild populations of animals (for an exception, see: Dingemanse *et al.*, 2004).

For any trait to respond to selection there must be phenotypic variation for that trait amongst individuals, a mechanism for its inheritance and fitness-related consequences related to trait expression (Endler, 1986). The genetic basis for personality variation has been well-documented in humans and other primates (McGue & Bouchard, 1998; Weiss *et al.*, 2000). However, these studies have been limited in their evolutionary

applications (i.e. fitness) because of the long life span of their subjects. On the other hand, heritable behavioural variation occurs in a number of well-known laboratory populations of mammals (e.g. Miczek *et al.*, 2001), insects (e.g. Hoffmann, 2000) and domesticated animals (e.g. Gauly *et al.*, 2001) but these estimates may be affected by domestication or long-term maintenance of populations under laboratory conditions, as environmental variation is normally controlled.

Conversely, there have been fewer studies examining the genetic influence on behavioural traits in wild populations of animals (Boake *et al.*, 2002; Stirling *et al.*, 2002). Evidence of heritability of personality traits in wild populations have been limited to two vertebrate study systems, great tits (exploratory behaviour and risk taking: Dingemanse *et al.*, 2002; van Oers *et al.*, 2004b) and stickleback fish (aggression, activity and boldness: Bakker, 1986; Bell, 2004). Thus, the behavioural genetics of animal personality traits is in its infancy and there is a basic need to address whether there is a genetic influence on these behaviours in wild populations across a wider range of taxa. Furthermore, information on the genetic influence on animal personality traits and their fitness-related consequences within the same animal system is rare and largely unknown, despite the requirement of these factors in understanding trait evolution (Dingemanse & Reale, 2005).

An animal's evolutionary fitness is determined by the number of reproductive descendants it produces and some studies have now begun to document the survival costs and reproductive correlates of animal personality traits (Reale & Festa-Bianchet, 2003; Dingemanse *et al.*, 2004). For example, in a multi-year study on marmots, boldness in female marmots was not directly related to reproductive output, but was associated with the recruitment of female yearlings (Armitage, 1986; Armitage & Van Vuren, 2003) who increase the chances of survival for young in the group (Armitage & Schwartz, 2000). Perhaps the most well studied example comes from studies on exploratory behaviour in birds (reviewed in Groothuis & Carere, 2005). Individual great tit reproductive success is a function of its exploratory behaviour, but during different years different phenotypes are more successful than others and this also varies for each of the sexes across years (Dingemanse *et al.*, 2004). Mating dynamics in the great tit system can also be influenced by personality trait variation. During some years, great tit mate pairs, which are positively matched for exploratory phenotypes also produce offspring in the best body condition (Both *et al.*, 2005). This influence of personality type variation (e.g. reactivity, anxiety and fear) on mating dynamics has also been reported in many domestic and zoo animals (Boissy, 1995; Wielebnowski, 1999).

In previous studies, we identified consistent phenotypic differences in behavioural tendencies between individual dumpling squid (*Euprymna tasmanica*, Pfeffer,

1884) along three axes in two different contexts (Sinn & Moltschaniwskyj, 2005). Individual squid display consistent behavioural types with regards to their expression of behaviours reflecting indices of boldness, activity and reactivity in tests simulating predator threat (threat test) and foraging opportunity (feeding test). In threat tests, bolder squid are ambivalent towards or even attack a threat stimulus, while boldness in feeding contexts is characterized by shorter latencies to feed, more feeding attempts over longer distances and faster feeding rates in the presence of an observer, whom squid treat as a perceived risk. Shyer squid in threat tests move away from the threat stimulus after fewer touches and in the feeding test, take longer to feed and perform fewer feeding attempts over longer periods of time. Differences along the activity index in squid are described by differences in frequency and time spent swimming and ambling (a type of squid 'crawling') in tests. Reactivity in squid is defined by the magnitude of fleeing responses. Highly reactive squid ink and jet more, regardless of the test context (i.e. threat or feeding). Phenotypically, expression for all three traits is context-specific, that is, squid that are bold in threatening situations are not bold in the feeding one and this pattern of context-specific trait expression is age-independent (D.L. Sinn, unpublished data). Trait repeatabilities are also high in juveniles and adults (repeatability for threat traits = 0.5–0.8, for feed traits = 0.4–0.5: Sinn & Moltschaniwskyj, 2005), indicating the potential for substantial genetic components underlying these traits.

Southern dumpling squid are small (5–8 cm), oviparous, semelparous, solitary benthic squid found on sand flats in shallow continental shelf areas surrounding southern Australia. While nothing is known concerning the mating systems in *Euprymna*, no obvious sexual dimorphism occurs; in general, cephalopod mating systems are characterized by internal fertilization and high promiscuity by both sexes (Shaw & Sauer, 2004). Our observations under laboratory conditions indicate that mating in dumpling squid may be coercive; males readily attack females under almost any conditions, females then enter a catatonic state while copulation occurs for 4–8 h. Female squid readily lay discrete batches of small eggs in the laboratory within days after matings. *Euprymna* eggs are small and cylindrical (~5-mm diameter) and laid singly, attached to substrate, in batches of up to 200 individuals. Reproduction occurs over a limited period of time, usually 2–3 weeks for both males and females previous to death (Boyle & Boletzky, 1996). Given the tractability of measuring reproductive output in females and the strong consistency of behavioural types, we chose to examine the evolutionary basis of animal personality traits by examining the heritability of traits in squid and what impact, if any, a female's personality type had on her reproductive output. During experiments many squid pairs mated but did not produce fertilized eggs; therefore, we also report patterns of

personality types in male and female squid which contributed to successful reproduction.

Methods

Subjects

Adult *E. tasmanica* were collected from two wild populations [Kelso (41°06′S × 146°47′E) and Margate (43°1S × 147°16′E), Tasmania, Australia] on multiple dives between June 2002 and January 2004. Squid were transported to a 2500 L closed-seawater system at the University of Tasmania, Launceston, Australia, where they were housed individually, subjected to behavioural tests (see below) and then mated 2 weeks after capture. Egg deposition on PVC pipe occurred within 2–3 days of mating; eggs were incubated at 18 °C for 35–40 days, after which they began to hatch. Environmental conditions in the laboratory during experiments were maintained at constant temperature (18 °C), salinity (33–35 ppt) and light cycle (14 : 10 h day/night cycle). Sixty-two females [mean mantle length (ML) = 25.0 mm; mean wet weight (WW) = 7.48 g] were mated with 21 males (mean ML = 27.3 mm; mean WW = 8.67 g). Mate pairings between squid were performed blind with respect to behavioural phenotype; instead mates were chosen roughly on similarity in body size and availability. Each male was mated to two to three females and five to six subjects from each resulting egg brood were used in testing. Twenty-seven crosses were successful and 147 total offspring were tested. Twenty-three broods contributed four to six siblings for testing and four broods contributed three or less squid. Behavioural results from progeny from successful matings were used for genetic analyses. Reproductive parameters were measured for successful mate pairings and were used in reproductive analyses (see below).

Unlike loliginid squid, dumpling squid do not school *per se*, but are loosely aggregated in field populations in Tasmania. Thus, squid were housed individually in test containers from the time of capture and all experiments occurred in the same containers that squid were housed. Offspring were housed individually from 2–3 days post-hatching. For adults, containers were blue opaque rectangular plastic tubs (34 cm long × 29 cm wide × 13 cm deep) while for offspring plastic containers were circular, opaque and black (14 cm diameter; 13 cm deep). Both types of containers contained a thin layer of sand (1–3 cm) which allowed squid to bury and were housed behind black cloth to minimize disturbance during nontest periods. All containers were floated in the larger 2500 L system and were continuously illuminated with low levels of red light (0.22×10^{14} quanta s^{-1} cm^{-2}) to allow visual observation during night-time hours when squid were most active.

Female squid can store sperm (Hanlon *et al.*, 1997), however, this mechanism has not been documented in *Euprymna* and the length of time sperm can be stored is unknown. Wild-caught females in our experiments had up to 2 weeks prior to mating in which to lay eggs. Normally, egg batches are laid in the laboratory within 11 days of copulation. Furthermore, over 3 years of observations, no female squid captured in the wild laid eggs in our laboratory prior to observed mating. While our genetic analyses assume that no females were carrying sperm when captured from the wild, this does not exclude the chance that females which successfully reproduced were those that carried sperm from previous matings in the wild but required additional matings in the laboratory to stimulate egg laying (e.g. Chevrier & Bressac, 2002). However, multiple paternity would have a dilution effect on our heritability estimates, therefore making our genetic results more conservative. In order to reduce the effect of mating history on reproductive analyses, female squid were mated multiple times (up to three) with the same male.

Behavioural testing

Methods of behavioural testing for adults and juveniles were the same and identical to that described in Sinn & Moltschaniwskyj (2005). Briefly, squid were subjected to two behavioural tests given on two separate days within a week of capture, with test days separated by at least 48 h. After capture, adult squid were allowed to acclimate for 48 h, fed to excess once and then given the first behavioural tests 48 h later. Offspring hatched in the laboratory were given the same tests during their third week of life. Two tests, a threat and a feeding test were given to each individual squid and 12 discrete, observable behaviours from both tests were measured using an audiocassette recorder and handheld timer. Detailed definitions of the 12 behaviours recorded in each test are given in Sinn & Moltschaniwskyj (2005). The threat test consisted of the experimenter (DLS) touching the squid with an eyedropper on one of its arms, up to 10 times, or until the squid moved away; feeding tests were conducted 30–90 min after threat tests and consisted of a live food (mysid shrimp) presentation in the presence of the experimenter. Each test was for 5 min, and frequency and duration of behaviours were recorded. The same two tests were given on each test day and results were summed for individuals within each test across the two test days, in order to account for within-test within-individual variability (Fleeson, 2004). Individual housing allowed for individual identification and experimental methods involved testing while not disturbing neighbouring subjects. For the duration of all experiments, squid were fed mysid shrimp 2–3 times per week to excess, except during test weeks, when squid were fed only during the two feeding tests. To minimize behavioural differences because of hunger levels, all squid were starved 48 h prior to testing and tests were given to squid in a different order on each test day. Tests

were given only during a 1 week period because consistency of boldness, activity and reactivity indices are high for both juveniles and adults (Sinn, unpublished data; Sinn & Moltschaniwskyj, 2005).

Personality trait scores

Previously, using a large sample of adult wild-caught squid, we used principal components analysis (PCA) to reduce a large number of behavioural variables from threat and feeding tests into three components, reflecting shy–bold (PC1), activity (PC2) and reactivity (PC3) indices (Sinn & Moltschaniwskyj, 2005). PCA analysis by age and by test situation using the current subjects resulted in behaviours from juveniles and adults loading onto the same PCA components at approximately the same magnitude and sign in both test situations (Table 1). Thus, the same observable behaviours can be used to describe the same three PC components regardless of age and sample. Therefore, in order to facilitate comparisons between adults and juveniles and also with a reference population, PCA scores were generated for all squid in the current study by computing regression scores based on the two PCA solutions (one for each test situation) reported in Sinn & Moltschaniwskyj (2005). This method resulted in six unique PCA scores for each squid (e.g. threat activity, threat reactivity, feed activity, feed reactivity, etc.) (for further discussion of this method, see: Aspey & Blankenship, 1977; Tabachnick & Fidell, 1996).

Because the PCA solution matrix from Sinn & Moltschaniwskyj (2005) is stable regardless of age or population of origin (Sinn, 2005), this method has the biological advantage in that it refers to species-specific personality traits and avoids generating study-specific indices/scores, which lack comparative power across studies. From a statistical standpoint, this also means that regression scores (PCA scores) based on a separate matrix result in PCA scores which are not constrained mathematically to be orthogonal to one another (which is biologically advantageous as well, because it is unlikely that personality traits are completely unrelated to one another psychologically or biologically within a given individual). Thus, these methods can result in PCA scores that can be correlated both within and across tests. This method is akin to techniques used in primate studies, where researchers often devote large amounts of time and resources obtaining large sample sizes to develop scales to describe personality traits, and then go on to use these scales on a number of smaller, separate samples to test hypotheses (for an example of this, see Fairbanks, 2001 where the PCA matrix is generated, then Fairbanks *et al.*, 2004a,b where this PCA scale is used on separate samples of individuals to estimate genetic parameters and developmental trajectories).

This method characterizes individual tendencies in the following manner: lower PCA scores indicate 'shyer' squid that tend to retreat when subjected to a threat; their bolder counterparts (higher PCA scores) are ambivalent, or even attack the threat stimulus. In feeding tests, bolder feeders (higher scores) feed quicker, travel longer distances to do so, and feed more times than shyer squid (lower scores). More active squid (higher PCA scores) spend more time moving, i.e. ambling (a type of crawling) and fin swimming (hovering in the water column); these behavioural 'definitions' of activity are the same for both tests. Reactive squid (high scores) in both tests ink and jet more than their less reactive counterparts (low scores) (Tables 2 and 3). All further analyses were performed on PCA scores.

Data analysis: Genetic analysis of squid personality traits

Phenotypically, squid personality traits are expressed in a sex-independent manner in adults and at 3 weeks of age (Sinn, unpublished data). The significance of sex on personality scores for adults in the current sample was assessed using a one-way ANOVA (Sokal & Rohlf, 1995), with sex as a fixed factor and the six personality traits as dependent variables ($n = 81$). The phenotypic effect was not significant for adults and offspring sex was unknown, so sex was not included in genetic analyses.

We used a derivative of a mixed-linear model, an 'animal model,' which uses restricted maximum likelihood algorithms to estimate genetic variance components (Kruuk, 2004). This model also fit additive genetic variance directly (rather than fitting it through a family component) and took into account the relatedness between individuals using a numerator relationship matrix, which was based on a pedigree file (Lynch &

Table 1 Pearson correlations (two-tailed) between behavioural loadings from principal components analysis (PCA) on the current subjects [3-week old ($n = 147$) and adult squid ($n = 81$)] and a previous study [Sinn & Moltschaniwskyj (2005), $n = 97$]. Loadings from each PCA solution matrix were first normalized with Fisher's *r*-to-*z* ratio (Snedecor & Cochran, 1980). $n = 12$ for the threat test, $n = 11$ for the feeding test.

Behaviours	Squid from Sinn & Moltschaniwskyj (2005)	
	Threat test	Feeding test
PC1: shy–bold		
Three-week-old squid	0.698	0.897*
Adult squid	0.952*	0.928*
PC2: activity		
Three-week-old squid	0.744*	0.756*
Adult squid	0.875*	0.862*
PC3: reactivity		
Three-week-old squid	0.449	0.438
Adult squid	0.659	0.919*

*Statistically significant after a Bonferroni adjusted $\alpha = 0.004$.

Table 2 Principal component loadings used to generate shy–bold (PCA1), activity (PCA2) and reactivity (PCA3) scores in threat tests. Original loadings were derived from PCA analysis on a large sample ($n = 97$) of adult squid (Sinn & Moltschaniwskyj, 2005).

	Principal component		
Behaviour	Shy–bold	Activity	Reactivity
Number of touches	**0.802**	−0.201	**−0.338**
First behaviour after touch	**0.864**	0.111	−0.282
Jet	**−0.638**	0.150	**0.562**
Grab	**0.845**	0.000	0.140
Arm flower posture	**0.424**	**0.683**	0.264
Log time spent moving	**−0.434**	**0.684**	0.019
Amble	−0.128	**0.569**	−0.326
Colour change	−0.032	**0.561**	−0.089
Fin swim	−0.037	**0.863**	−0.051
Log time to first bury	−0.023	−0.035	−0.171
Bury	−0.038	0.057	−0.017
Ink	−0.210	−0.140	**0.823**
Percentage variance explained in the current subjects			
Adults	40.4	18.4	11.0
Offspring	26.7	19.9	15.2

Boldface type indicates the highest component loading(s) for each behaviour.

Table 3 Principal component loadings used to generate shy–bold (PCA1), activity (PCA2) and reactivity (PCA3) scores in feeding tests. Original loadings were derived from PCA analysis on a large sample ($n = 97$) of adult squid (Sinn & Moltschaniwskyj, 2005).

	Principal component		
Behaviour	Shy–bold	Activity	Reactivity
Time to first feeding strike	**−0.932**	−0.115	−0.022
Number of feeding strikes	**0.963**	0.071	0.043
Feeding rate (time/mysid)	**−0.937**	−0.070	0.006
Amble	0.044	**0.809**	−0.324
Fin swim	0.026	**0.661**	0.335
Log time spent moving	0.069	**0.911**	0.228
Bury	−0.095	0.016	0.136
Log time to first bury	−0.104	0.018	0.051
Jet	0.194	0.149	**0.794**
Ink	−0.150	0.030	**0.708**
Percentage variance explained in the current subjects			
Adults	35.3	22.0	14.0
Offspring	25.0	19.6	15.9

Boldface type indicates the highest component loading(s) for each behaviour.

Walsh, 1998). Given our small sample size, we chose to retain a number of squid from the Margate site ($n = 22$), even though they were collected from a separate population. Potential differences between population means were accounted in the model through a fixed site factor. We estimated additive genetic (σ_a^2), maternal (σ_m^2) and residual (σ_e^2) variances in the six squid personality traits using the following mixed linear model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1a} + \mathbf{Z_2m} + \mathbf{e}$$

where **y** is the vector of phenotypic observations, **b** is the vector of fixed effects (overall mean and site), **a** is the vector of random additive genetic effects, **m** is the vector of random maternal effects (both genetic and environmental, see Kruuk, 2004) and **e** is the vector of random residuals (environmental and nonadditive effects). **X**, $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are design matrices linking the phenotypic observations with the fixed and random effects. Random effects are assumed to follow a multivariate normal distribution:

$$\begin{bmatrix} \mathbf{a} \\ \mathbf{m} \\ \mathbf{e} \end{bmatrix} \overset{iid}{\sim} N \left(\begin{bmatrix} \mathbf{0} \\ \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \begin{bmatrix} \mathbf{G} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{M} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{R} \end{bmatrix} \right)$$

where $\mathbf{G} = \mathbf{A}\sigma_a^2$, $\mathbf{M} = \mathbf{I}\sigma_m^2$, $\mathbf{R} = \mathbf{I}\sigma_e^2$, **A** is the numerator relationship (Lynch & Walsh, 1998), and **I** and **0** represent identity and null matrices of appropriate sizes, respectively. Narrow-sense heritability (h^2) estimates were then calculated for each trait by:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$$

As heritability and genetic correlation estimates are sensitive to age-related effects (Mazer & Damuth, 2001b), the genetic models described above were run for each PCA index for progeny only ($n = 147$). The statistical genetic analyses were conducted using ASREML (Gilmour *et al.*, 2002). The significance of random (additive genetic and maternal) components were tested using a log-likelihood ratio test comparing the full model with its appropriate restricted version (i.e. dropping the effect and comparing the fits of the models). Critical values of chi-square used to test significance of model log likelihood differences were $\chi^2_{1,0.05} = 3.84$ and $\chi^2_{1,0.01} = 6.63$ (Self & Liang, 1987). The significance of fixed site effects was tested using a Wald test. For all models, a maternal and site effect was included in the overall model first and then removed if it did not make a significant contribution. All variables were screened for univariate normality prior to genetic analyses and no transformations were deemed necessary. We do not report genetic correlations between traits because estimates of additive genetic variation in feeding traits were not different from zero (see Results).

Data analysis: reproductive consequences of squid personality traits

Two factors of female reproduction, fecundity and hatching success, were used to examine fitness consequences of female personality traits. Fecundity was measured by counting the number of eggs that were laid by a female after successful mating by a male. Females normally laid eggs within 2 weeks of mating (mean gestation: 11 days, SD = 9.1) and at that time, individual

eggs were counted either by eye or using a low-powered (10×) dissecting microscope. Hatching success, expressed as percentage of eggs hatched to those laid, was measured by counting all unhatched eggs 6 days after the first squid hatched from a given egg batch. In most cases only 6 days were necessary in order to determine which eggs had been unfertilized or had ceased development. Two separate stepwise regression analyses (DV1 = fecundity, $n = 27$; DV2 = hatching success, n = 22) were used with body size and six female personality trait scores as independent predictors to examine the contribution of personality type and body size to female reproductive output (Tabachnick & Fidell, 1996). After controlling for body condition (and therefore hunger), 'shy' female squid in feeding tests from the Kelso population also tend to be larger (Sinn & Moltschaniwskyj, 2005); foraging shyness in the current sample of females was also associated with larger size (Spearman's rank = −0.45, $P = 0.01$). Because we had no *a priori* reason to suspect a causal relationship between body size and shy/bold tendencies, both regression models were run twice: once with body size in a first step followed by six personality scores in a second step and once with feeding boldness in a first step followed by body size and the remaining five personality scores in a second step. Female personality traits and WW had nonnormal distributions and were log-transformed prior to analyses.

During the study a large number of mate pairs copulated (85%) but did not produce fertilized eggs (53%). Therefore, successful or unsuccessful pairings between adult squid were characterized to understand patterns in personality scores which contributed to resulting reproductive success. First, logistic regression was used with mating success/nonsuccess as a dependent variable and WW and six female personality trait scores as independent predictors ($n = 58$). Logistic analysis was not performed with males because some males were both successful and unsuccessful. Second, Spearman-rank correlations between body size and the six personality trait scores were used to characterize successful ($n = 29$) pairings vs. those pairings that were unsuccessful ($n = 34$). Nonparametric correlations were used because adult

trait scores were nonnormal. Because of the large number of comparisons (36) and the exploratory nature of this analysis, we report *P*-values for correlations but used effect sizes of 0.50 as a cut-off to indicate important relationships between variables (García, 2004). Third, one-way ANOVA models were used to examine mean differences in personality trait scores and body size between successful ($n = 29$) and unsuccessful ($n = 33$) females and between males and females of successful ($n = 14$ males; $n = 29$ females) and unsuccessful ($n = 15$ males; $n = 33$ females) reproducers. PCA scores and WW were log transformed prior to using ANOVA. SPSS 12.0 was used for all reproductive analyses.

Results

Genetic analyses of squid personality

The observed group means and variation in trait scores used in genetic models are shown in Table 4. In no case did a significant improvement in fit occur when modelling either the maternal component or site (i.e. Margate and Kelso) as a fixed factor. Thus, each genetic model used to estimate variance components and heritability was considered only with a grand mean, additive genetic and residual effect. Heritabilities in threat tests were moderate to high (0.2–0.9); shyness–boldness, activity and reactivity indices each had significant additive genetic components (shyness–boldness: $\chi^2_{(1)} = 5.59$, $P < 0.05$; activity: $\chi^2_{(1)} = 33.76$, $P < 0.01$; reactivity: $\chi^2_{(1)} = 18.97$, $P < 0.01$). Genetic analyses on feeding behaviours indicated that trait indices had lower heritabilities (0.05–0.08), none of which reached statistical significance (shyness–boldness: $\chi^2_{(1)} = 1.14$, $P = 0.29$; activity: $\chi^2_{(1)} = 0.356$, $P = 0.55$; reactivity: $\chi^2_{(1)} = 1.06$, $P = 0.30$). For shy–bold traits, the magnitude of additive components did not differ between test contexts, but residual components were 2.5 times larger in feeding tests than in threat ones. For activity traits, additive genetic components were three times smaller in feeding tests, combined with a 14-fold increase in residual components in feeding tests contributing to phenotypic

Table 4 Estimates of additive genetic components of variation and heritability and their associated standard errors for six squid personality traits.

Trait	Threat			Feed		
	Shy–bold	Activity	Reactivity	Shy–bold	Activity	Reactivity
Progeny only ($n = 147$)						
Mean; SD	−1.45; 0.58	0.83; 1.32	−0.33; 0.70	0.04; 0.88	5.18; 2.88	1.24; 2.75
Additive genetic	0.072 ± 0.052*	1.173 ± 0.488**	0.556 ± 0.192**	0.062 ± 0.092	0.389 ± 0.794	Φ
Residual	0.267 ± 0.049	0.567 ± 0.288	0.068 ± 0.100	0.716 ± 0.114	7.927 ± 1.166	7.565 ± 0.886
Heritability	0.213 ± 0.143	0.674 ± 0.197	0.891 ± 0.174	0.079 ± 0.117	0.047 ± 0.095	Φ

Residual term includes both nonadditive genetic effects and environmental effects. The probabilities of the estimates refer to significant log-likelihood ratio tests.
Φ, estimate does not differ from zero. *$P < 0.05$, **$P < 0.01$.
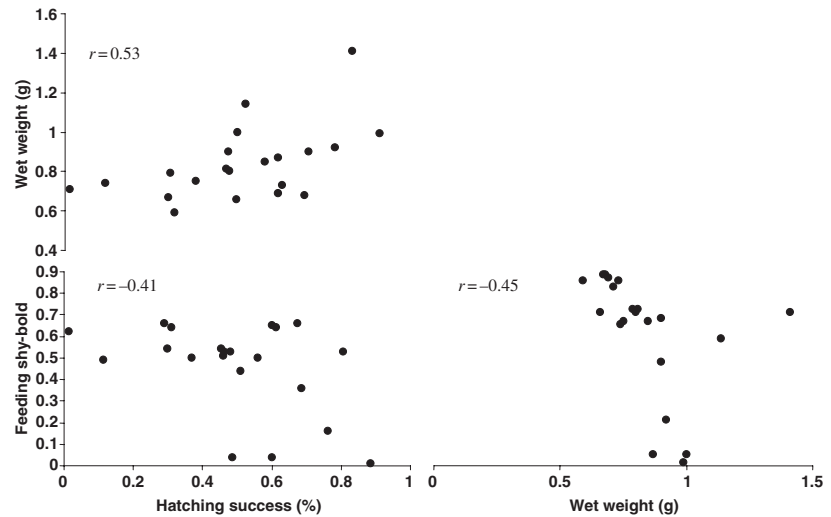
Fig. 1 Relationship between feeding shy–bold scores, hatching success of egg broods (number eggs laid – number eggs unhatched/ number eggs laid) and wet weight (WW) of females. Feeding shy–bold scores and wet weights were log-transformed because both variables were non-normally distributed.

Reproductive consequences of squid personality traits

There was wide variation in the number of eggs laid by each female (mean brood size = 95.4, SD = 40.1), however, no personality traits or body size measures in females explained significant variation in fecundity (WW only: F[1, 25] = 0.33, P = 0.57; foraging shy–bold only: F[1, 25] = 0.23, P = 0.63; WW and personality traits: F[7, 19] = 0.55, P = 0.79). Egg broods had a hatching success rate of approximately 50% and displayed high variation between broods (mean percentage hatched successfully = 51.4; SD = 21.4). Wet weight (R^2 = 0.28, F[1, 20] = 7.91, P < 0.05) and foraging shy–bold scores (R^2 = 0.21, F[1, 20] = 5.37, P < 0.05) alone explained small but significant levels of variation in hatching success, with larger and shyer females producing broods with greater hatching success rates. In second steps, the six personality trait scores (following WW: R^2 = 0.33, F[6, 14] = 0.991, P = 0.48) and WW and five personality trait scores (following foraging shy–bold: R^2 = 0.36, F[6, 14] = 1.11, P = 0.41) did not explain additional variation in hatching success. Shyer females tended to be larger and this resulted in increases in brood hatching success (Fig. 1).

Success or nonsuccess of mate pairs was not because of population of origin; only three of 34 unsuccessful mate pairs were between squid from the two separate populations (Margate and Kelso). For females, there was no relationship between the odds of successful mating, body size and personality trait phenotype ($\chi^2_{(6)}$ = 7.86, P =

0.25). Only one of 36 behavioural correlations reached an effect size of 0.50. Successful mate pairs had similar levels of boldness in feeding tests (Spearman's rank = 0.47, P = 0.01). Intermediate and bold females tended to successfully reproduce when paired with intermediate or bold males, but not shy ones. Shy females tended to reproduce with all three types of males (shy, bold or intermediate; Fig. 2). In unsuccessful mate pairings, this relationship between foraging shy–bold phenotypes was absent (Spearman's rank = 0.28, P = 0.10). For all other comparisons between behaviours of mate pairs, no other correlation exceeded 0.35 (feeding activity, Spearman's rank = 0.34, P = 0.07). Unsuccessful mate pairs of squid were roughly size assorted (Spearman's rank = 0.51, P = 0.004), while successful pairs were not (Spearman's rank = 0.30, P = 0.13). There were no mean differences in WW or personality scores between successful and unsuccessful females, or between males and females within either set of mating pairs (successful and unsuccessful). In summary, the only phenotypic relationship which characterized successful vs. unsuccessful reproduction in our sample appeared to be that successful reproduction occurred between squid which shared similarities in phenotypic values for shyness–boldness in feeding tests.

Discussion

This study is one of the first reports of heritability and fitness-related consequences of 'personality' traits within the same wild animal population and contributes to our understanding of the evolutionary basis for these trait categories in nonhuman animals. Personality trait variation in squid was not a function of sex-specific expression, nor did maternal effects make significant contributions to genetic models. There were significant additive genetic components underlying phenotypic
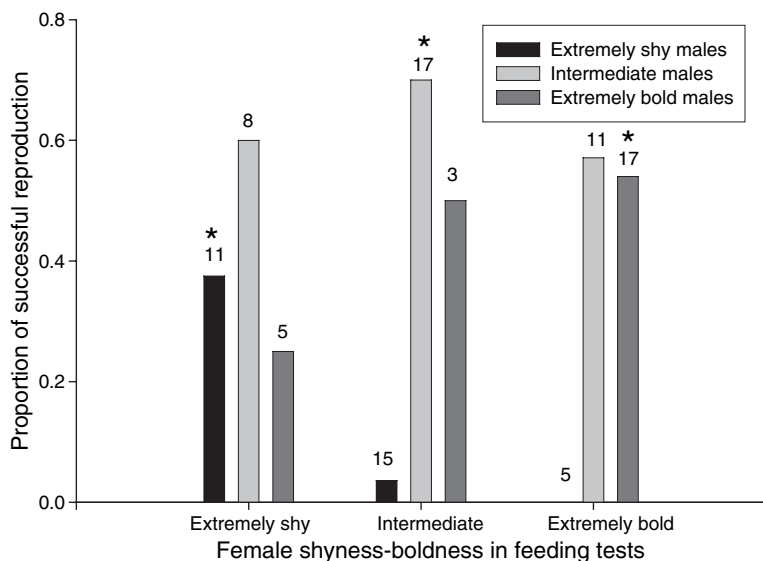
Fig. 2 Proportion of pairings between shy–bold phenotypes, which resulted in successful reproduction. Categories of 'shy', 'intermediate' and 'bold' were based on the 33rd and 66th percentiles of the frequency distribution of shy–bold principal components analysis scores (shy = <33rd percentile, intermediate = 33–66th percentile, bold = >66th percentile). Numbers over bars represent total mating attempts within each pair category. Stars represent combinations of mate pairs, which would have the highest proportion of successful mating under complete assortative mating.

variation in boldness, activity and reactivity behaviours expressed during antipredator contexts in squid. Substantial heritabilities in feeding traits were not detectable in the current sample resulting from the large residual (nonadditive genetic and environmental) components of phenotypic variation. We found two links between personality types and an individual's fitness; females who were classified as 'shy' in feeding tests, who were also larger, were more likely to produce egg batches with higher hatching rates. Successful fertilization between mate pairs in our study also occurred when males and females with similar foraging shy–bold phenotypes were paired together. Bold and intermediate female phenotypes tended to reproduce successfully with bold and intermediate males only; shyer females reproduced equally well with shy, bold or intermediate males.

Heritability estimates from traits expressed in threat tests ranged from 0.21 to 0.89, and these results are broadly consistent with studies on heritability of personality traits in humans (range = 0.34–0.42: McGue & Bouchard, 1998) as well as nonhuman animals (range = 0–0.63: van Oers *et al.*, 2005). For traits in threatening situations, there is substantial additive genetic variation to respond to selection in a predictable manner and genetic variation is clearly responsible for some of the observed phenotypic variation observed in squid antipredator behaviour. On the other hand, additive genetic components for traits expressed in feeding tests was small (0.05–0.08). Levels of residual components for feed traits, which included nonadditive genetic components and environmental effects, were also substantially greater for feed traits than their threat trait counterparts.

There are a number of possibilities that may explain these results for foraging related traits. One explanation is that strong selection has depleted genetic variation in feeding traits (the elimination hypothesis: Jones, 1987; Merilä & Sheldon, 1999). Traits closely related to fitness

are often characterized by little additive genetic variation but large dominance components (Merilä & Sheldon, 1999) and this trend has also been reported for exploratory behaviour in great tits (van Oers *et al.*, 2004c). Another scenario is that foraging traits are more labile and sensitive to environmental influences relative to antipredator behaviours. Mistakes when encountering predators are costly, while optimal foraging theory predicts that behaviours associated with foraging should be highly flexible and subject to learning to cope with changing metabolic requirements and environments as animals grow (Stephens & Krebs, 1986). These learning effects, unless they are heritable, can confound estimates of heritability of foraging behaviours (Gibbons *et al.*, 2005). Third, foraging behaviours integrate a number of physiological and morphological traits and a high degree of integration of traits across biological levels can lower measured heritability for behavioural traits (Stirling *et al.*, 2002). Thus, while certain components of foraging behaviour, such as sensory and chemical prey preferences may be highly heritable (e.g. Luthardt-Laimer, 1983), measuring an aggregate foraging 'personality' trait which is subject to learning effects may obscure estimates of heritability, even at 3 weeks of age. Unfortunately, our data does not allow us to discriminate between these possibilities. Further work is clearly needed in understanding the role of environments in inducing expression of additive genetic variation in foraging traits (Falconer & Mackay, 1996), as well as determining the influence of nonadditive genetic components of variation (i.e. dominance and epistasis) in animal personality traits (e.g. van Oers *et al.*, 2004c).

While we were unable to detect any relationship between female squid personality traits and subsequent fecundity, two other links between female squid behaviour and fitness were found. The first of these was between a female's shy–bold foraging strategy and her

subsequent brood's offspring hatching success. Larger female squid, who were also the shyest foragers in our sample, produced broods of eggs with higher hatching success rates. Biologically, larger body size in female squid can result in increased nutritional resources being partitioned to offspring eggs, which result in higher hatching success rates (Steer *et al.*, 2004). Unfortunately, we do not have data to suggest which trait (behaviour or body size) drives this relationship in squid, but foraging shy–bold strategies which co-vary with body size are common in many fishes (Sundström *et al.*, 2004; Ward *et al.*, 2004; Brown *et al.*, 2005). These results reinforce the notion that there are energetic costs associated with foraging strategies (Werner & Anholt, 1993) and suggest animal personality traits related to foraging may have trait-linkages and fitness consequences with regards to an individual's body size. It is worth noting that this relationship between behaviour and body size is most likely dependent on current environments, such as variation in prey availability (Clark & Ehlinger, 1987; Dingemanse *et al.*, 2004).

The second relationship between fitness and personality variation was found in the relationship between pairings of shy–bold phenotypes and successful fertilization. Females classified as 'bold' or 'intermediate' along the shy–bold axis in feeding tests tended to produce viable eggs when mated with only bold or intermediate males. 'Shy' females, on the other hand, produced viable offspring with males in all three trait categories. Mating in *Euprymna* has not been described previously, but observations in the laboratory and from other cephalopods in the field suggest that many males may force copulations (i.e. Hanlon *et al.*, 1997). In systems where forced copulations occur, competition between the sexes can result in strong selection for female cryptic choice (Clutton-Brock & Parker, 1995). Female squid are able to store sperm and can bias paternity by selectively using sperm from different males, although the mechanisms for these choices are unknown (Shaw & Boyle, 1997). Our results suggest that female squid post-copulatory sperm choice may be partly based on cues related to her own and her mate's shy–bold phenotype, either through behavioural mate preference (e.g. Persaud & Galef, 2005) or genetic compatibility between mate pairs which is linked to behavioural shy–bold strategies (Tregenza & Wedell, 2000; Puurtinen *et al.*, 2005).

From an evolutionary standpoint, the current study is, at first glance, paradoxical given that antipredator behaviours, with significant levels of additive genetic variation, were heritable, but not related to our fitness measures. Foraging behaviours, on the other hand, appeared to be influenced to a greater extent by nonadditive genetic and environmental components of variation, but were related to our measures of fitness. Clearly, our study was limited to measures of fitness related to reproduction in females; intuitively, fitness consequences of antipredator behaviours in both sexes may be better measured through an individual's survival and competitive abilities (Sih *et al.*,

2004a). For foraging traits, it is difficult to predict the evolutionary significance of a personality trait heavily influenced by environmental factors and in this case, the significance of assortive mating in maintaining genetic variation. However, in most animals we would expect behaviours involved in foraging to have substantial survival consequences; thus, we would expect them also to be heritable to some extent (Turkheimer, 1998; Stirling *et al.*, 2002). Our results highlight the fact that behaviours are also substantially influenced by nongenetic factors (West *et al.*, 2003). The relative influence of genetic factors (i.e. additive and nonadditive components), the level of morphological and physiological integration and learning mechanisms will affect researchers ability to detect substantial heritability estimates in personality traits of animals from wild populations, even though these traits may be heritable and undergoing evolutionary change (Mazer & Damuth, 2001a). The phenotypic links between foraging behaviours and body size, as well as mating success, suggest further work is necessary to understand the genetic and environmental mechanisms resulting in phenotypic variation in personality traits related to foraging. One obvious starting point is to examine the development of trait-linkages between a number of behavioural, morphological and life history characters (Sih *et al.*, 2004a,b).

A number of studies have begun to document heritable personality variation in a variety of vertebrate taxa (e.g. Bakker, 1986; Reale *et al.*, 2000; Drent *et al.*, 2003; van Oers *et al.*, 2004a), but combined estimates of heritable behavioural traits along with their fitness-related consequences in wild populations of animals are rare. The current results begin to provide some evidence of an evolutionary basis for shyness–boldness, activity and reactivity in wild populations of animals, while also highlighting a number of issues with regards to the measurement of heritability in personality traits associated with foraging. It is likely that in squid, as in other animals, fitness-related consequences for personality traits will fluctuate through time and be mediated by current environmental conditions (Dingemanse *et al.*, 2004; Dingemanse & Reale, 2005). Thus, the salient issue will be to identify the mechanistic bases through which environmental heterogeneity maintains behavioural diversity (Kassen, 2002). In order to accomplish this task, there is still a basic need to document the genetics and fitness-related consequences of 'personality' traits in wild populations of animals. Through doing so, we stand to significantly contribute to our knowledge of the processes responsible for the maintenance of genetic and phenotypic variability in wild populations, a major issue in evolutionary biology (Dall *et al.*, 2004).

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