The Evolutionary Genetics of Behavioural Variation: multivariate perspectives on personality in the Trinidadian guppy.

Submitted by Stephen John White to the University of Exeter
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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……..

Abstract

Animal personality is found in a wide range of taxa, yet our knowledge of what maintains consistent among-individual variation in behaviour is still incomplete. Many personality traits are associated with fitness, leading to the expectation that, under selection, genetic (and among-individual) variation will be eroded over time. Several adaptive models have been developed in order to explain this maintenance of variation. These include state-dependence, state-behaviour feedback loops, life-history and behavioural coadaptation and the Pace of Life syndrome. These models represent good starting points for thinking about what drives and maintains among-individual variation in behaviour, and while empirical support for these models is mixed, one thing they do have in common is the assumption of a significant genetic basis underpinning personality traits. Significant heritability is required for an evolutionary response to selection and for among-individual variation to be adaptive. The univariate estimates of heritability for personality traits that are growing in the literature, while useful, are likely insufficient to predict how personality traits will respond to selection. This thesis uses the Trinidadian guppy, *Poecilia reticulata*, and other species to explore patterns of among-individual and genetic variation in personality traits, advocating the benefits of using multivariate perspectives throughout. Firstly, the among-individual covariance structure between measures of boldness, growth and metabolic rate are estimated in a test of the Pace of Life syndrome. Secondly, an appraisal of the relative strength of maternal and genetic effects on offspring personality and how it changes over ontogeny. Next, a comprehensive treatment of sexual dimorphism in behaviour and size followed by analysis of genotype-by-sex interactions using both univariate and multivariate methods. Finally, a comparative analysis of personality in 7 species of small fish, identifying the main

axis of among-individual variation from a single assay in each and quantifying the phylogenetic signal.

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Author's declaration

The work described in this thesis was carried out by S White, unless otherwise stated below.

Chapters 2, 3 and 4 are based on work designed by S White and A Wilson. Data were collected by S White at the University of Exeter, Penryn campus with assistance from A Grimmer and T Kells where necessary. A Wilson and T Houslay assisted in statistical models. All manuscripts were analysed and written by S White.

In **Chapter 2**, advice on aquatic respirometry was received by L Hawkes and S Killen. **Chapters 2 and 3** have undergone peer review.

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Chapter 1

General Introduction

1.1 Among-individual variation in behaviour

Considering the fitness advantages of being behaviourally plastic, the apparent widespread existence of consistent variation among individuals in a wide variety of behaviours presents an evolutionary conundrum. Behaviour is largely reversible and can respond over the order of seconds or minutes, thus it has traditionally been considered the most labile of traits. From an optimality perspective, we would therefore expect individuals to respond to changes in the environment with the appropriate behavioural phenotype in order to maximise fitness across a heterogeneous environment (Dall et al. 2004; Mathot and Dingemanse 2012). While on average, behaviour often does change plastically in a way that enhances fitness (Day and McPhail 1996; Chapman et al. 2009; Bretman et al. 2012; O'Rourke and Mendelson 2013) there is considerable variation around these 'adaptive' mean effects that in the past has been treated as irrelevant noise (Dall et al. 2004). In recent years, however, there has been a surge of interest in consistent and stable among-individual variation in behaviour, otherwise known as 'animal personality' (Réale et al. 2007; Wolf and Weissing 2012). While individuals alter their behaviour in response to external cues, it is the rank order differences among individuals that are quite often stable over time (Dingemanse et al. 2010; Mathot and Dingemanse 2012). This results in individuals responding in different ways or to different degrees to the same cue, with some responding in what appears to be a maladaptive way.

Animal personality can have far reaching effects on the ecology and dynamics of a population through dispersal (Dingemanse et al. 2003; Cote and Clobert 2007; Bókony et al. 2012), social interactions (Pike et al. 2008; Krause et al. 2010; Aplin et al. 2013), reproductive success (Reaney and Backwell 2007; Schuett et al. 2011; Ariyomo and Watt 2012; Martin-Wintle et al. 2017) and competitive ability/resource defense (Smith and Blumstein 2008; Amy et al. 2010; Wilson et al. 2013; Briffa et al. 2015). In addition, consistent differences in the movement or social propensity of inividuals can influence the transmission of parasites (Barber and Dingemanse 2010; Boyer et al. 2010; Aalvik et al. 2015) and disease (Koprivnikar et al. 2011; Araujo et al. 2016) as well as facilitate the invasion of new habitats (Rehage and Sih 2004; Wright et al. 2010; Fogarty et al. 2011; Chapple et al. 2012). Behavioural variation also facilitates adaptation to urban environments (Bókony et al. 2012; Lowry et al. 2013; Miranda et al. 2013) and human induced rapid environmental change (Sih et al. 2011; Lapiedra et al. 2017). In order to fully understand the effect of personality on population level processes we first need to understand why among-individual variation in behaviour is maintained. Why are individuals consistent in their behaviour and why, despite the apparent advantages of being endlessly plastic, do individuals differ from each other rather than all expressing the optimum phenotype for all conditions?

A great deal of progress has been made in uncovering the potential drivers of personality in recent years, particularly with the development of statistical methodologies and theoretical frameworks. There are two aspects of animal personality that require explaining: the maintenance of consistency in behaviour, despite the benefits of plasticity, and the maintenance of multipe behvioural types

within a population. Some hypotheses posit non-adaptive explanations for these questions. For instance, the costs and limitations of being behaviourally plastic could result in consistency in behaviour (Hazlett 1995; Dewitt et al. 1998; Auld et al. 2010). If the cost of making a mistake in the phenotype is high, the costs associated with having the ability to be behaviourally plastic are high or if the environment is highly unpredictable then consistency in phenotype is likely to be favoured. Multiple behavioural types within a population could simply arise from environmental hetergeneity during important developmental windows (Stamps and Groothuis 2010). Individuals that develop under even slight differences in physical or social conditions could have large differences in adult behaviour as a result of altered developmental trajectories. This has elements of the constraint and/or costs of plasticity argument as once the 'developmental window' has closed it is likely difficult or expensive to alter physiological or neurological pathways underlying the behaviour (Stamps and Groothuis 2010).

This concept of long term developmental conditioning can be expanded to maternal effects - the effect of a mothers phenotype on the offsprings phenotype above and beyond the effect of directly inherited genes (Mousseau and Fox 2008). If mothers are spread over an environment that is variable in resources, for example, then differential maternal effects may result in different behavioural types in the offspring (Tobler and Sandell 2007; Reddon 2011; Mainwaring and Hartley 2013; Hinde et al. 2015). Costs and constraints of plasticity and early life environment thus appear to be an intuitive way for personality to be maintained (Gracceva et al. 2014; Guenther et al. 2014; Urszán et al. 2015; DiRienzo et al. 2016). The empirical support is not universal (Relyea 2002; Buskirk and Steiner

2009; Favati et al. 2015; Carter et al. 2016), however, indicating there are other mechanisms at play in maintaining personality.

Personality traits are often linked to survival and reproductive success and, therefore, are likely to be under direct selection (Réale et al. 2007; Smith and Blumstein 2008; Ariyomo and Watt 2012; Niemelä et al. 2015). This has led many to hypothesise adaptive mechanisms through which variation in behaviour can be maintained through selection on the behaviours themselves. An implicit assumption here is that behavioural variation arises, at least in part, from genetic effects (a point returned to in section 1.3).

1.2 Brief overview of adaptive models for maintenance of animal personality

Under stabilising or directional selection, all else being equal, we would expect suboptimal phenotypes to be removed from a population over time, until only the fittest remains. As among-individual variation is the raw material upon which selection acts, we should see it decrease as selection is applied (Réale et al. 2007). How then is among-individual variation maintained when we would expect erosion by selection?

Considering the type of selection acting on particular behaviours could allow the maintenance of multiple behavioural types within a population to be explained. Negative frequency-dependent selection, a relatively common regime in the wild, causes rare behavioural types to have high fitness. This initially results in the rapid spread of the behavioural type through the population. As this behavioural type becomes more frequent, however, its fitness declines, resulting in the stable

coexistence of multiple behavioural types within a population (Wolf et al. 2008; Wolf and McNamara 2012; Lichtenstein and Pruitt 2015; but see Kurvers et al. 2012). Directional selection that fluctuates over time or space can favour different behavioural types at different times or in different parts of a populations range (Dingemanse et al. 2004; Quinn et al. 2009; Le Coeur et al. 2015). In addition, coexisting behavioural types can occur when disruptive selection generates multiple fitness peaks (Bergeron et al. 2013) or when certain combinations of behavioural types in mating pairs leads to higher reproductive success (Both et al. 2005; Martin-Wintle et al. 2017).

These patterns of selection could maintain behavioural variation, however, but do not by themselves fully explain the consistency of individuals over time (Wolf et al. 2008). In this instance, behavioural consistency may be imposed by strict developmental pathways, invoking the costs and limitations of plasticity argument as outlined above. An alternative is to include other aspects of an individual's phenotype that may alter the benefits and costs of a particular behaviour. Numerous mathematical and verbal frameworks have been developed to explain the consistency of individual behaviour as a consequence of 'state-dependence' (Dall et al. 2004; Dingemanse and Wolf 2010; Wolf and Weissing 2010). These models state that consistency in behavioural traits can be caused by associations with more stable aspects of phenotypic state such as size/growth (Stamps 2007), life history (Wolf et al. 2007; Biro and Stamps 2008; Nicolaus et al. 2012) or underlying physiological (Biro and Stamps 2008, 2010; Millidine et al. 2009; Careau and Garland 2012) or neurological (Coppens et al. 2010) pathways. These states are presumed to be costly to alter once an individual is on a particular developmental trajectory and so are expected to be stable over long

periods of time. If state variables are indeed consistent and vary among-individuals then so will state-dependent behaviour (Wolf and Weissing 2010). This passes the buck, however, as we then have to explain the origin of among-individual variation in state. These models appeal to mechanisms previously outlined in the context of explaining variation in behaviour, such as random variation in environment (Rands et al. 2003; Stamps and Groothuis 2010), frequency dependent selection (Wolf et al. 2007) or fluctuating selection over time/space (Stamps 2007).

The adaptive state-dependent framework does not require the state to be inherently stable, however. More labile state variables, such as energy reserves or body condition, can also be drivers of behavioural consistency, at least over the short term. Taking the example of energy reserves, the asset protection principle (Clark 1994) predicts that with low initial reserves, and therefore low expectation for survival, individuals should behave “boldly”. If the risk of starvation is serious then the cost of foraging in the presence of predators will, in a relative sense, be lowered to an acceptable level. Individuals with a higher initial energy reserves have a high expectation of survival and future reproduction and so ‘protect’ this asset by behaving more cautiously. This is a negative feedback, however, as those bolder individuals that successfully increase their energy reserves will subsequently become more cautious. Conversely, the initially cautious individuals become bolder as their energy reserves are depleted. Ultimately, negative state feedbacks should eventually lead to similarity of average behaviour across individuals. Positive feedbacks between state and behaviour, however, can lead to increased among-individual differences in behaviour. Take experience, as an example of state. If experience in a particular

behavioural response reduces the cost or increases the benefit of that response, then this behaviour should be favoured, leading to consistency (Dall et al. 2004; Wolf et al. 2008; Dingemanse and Wolf 2010).

Wolf et al. (2007) outlined a model where life-history trade-offs and asset protection lead to consistent differences in behaviour. If individuals differed in the emphasis they place on current vs. future reproduction/fitness, then this could lead to individuals varying in behaviour. In addition, they argue that asset protection need not result in negative feedback as some payoffs of a behaviour don't necessarily contribute to the 'asset', such as future reproduction. Instead it is utilised for current reproduction or survival. This is particularly relevant if risky payoffs offer immediate benefit whereas less risky payoffs contribute to future reproductive success (asset) or if some payoffs benefit relatives rather than the focal individual, such as parental care. Luttbeg and Sih (2010) expanded on this idea by simulating variation in predation risk and resource abundance and assuming 'state-dependent safety'. This is where individuals with 'high state' are better able to escape or repel the attack of predators than individuals with 'low state'. Their simulations found that it is positive feedbacks that promote consistency in behaviour and state and the relative importance of positive and negative feedbacks varies across high predation and high resource environments (McElreath et al. 2007; Luttbeg and Sih 2010).

It is important to note that while state-dependent models are appealing, a challenge for empiricists is that the term 'state' can cover a very wide variety of phenotypes and variables (up to and including the weather, Wolf and Weissing 2010). Not all states will contribute to the consistency of behaviour and those that

do are not necessarily adaptive. Sometimes state variables that, *a priori*, are predicted to be linked with behaviour may turn out not to be when investigated further (Dosmann et al. 2015; DiRienzo et al. 2016). Conversely, just because a measured state variable does not appear to influence a behaviour does not mean that state-dependence is not a useful concept in that system.

The state-dependent framework ultimately assumes that behaviour is altered adaptively in line with the state, but this can be expanded to consider the co-evolution of both behaviour and multiple state variables (Sih et al. 2015). The Pace of Life syndrome (POLS) is a more integrated and holistic approach to the study of animal personality (Réale et al. 2010) that can be seen as one attempt to do just this. It predicts that, among-individuals, behaviour will covary with life-history and physiological traits on a slow-fast axis (Ricklefs and Wikelski 2002; Réale et al. 2010). Individuals with a fast pace of life are expected to grow faster and have higher resting metabolic rates, have more reactive immune response and produce more offspring per reproductive bout. However, they will also have reduced longevity (Figure 1). Trade-offs among fitness components mean that different points along this slow-fast axis have the same expected fitness and the lack of a single optimum results in the maintenance of variation in all traits (Réale et al. 2000, 2010; Biro and Stamps 2008; Careau et al. 2008). While there is no clear prediction of a single directional causal relationship between state variables and behaviour (indeed bidirectional causality and feedback loops are likely), selection will act on the entire multivariate phenotype, driving co-evolution of behaviour with life-history and physiology (Sih et al. 2015).

Overall, concepts like frequency dependent selection and state-dependent models present useful starting points for thinking about the drivers of among-individual variation in behaviour. There is, however, mixed support for these adaptive models as explanations for the behavioural makeup of a population. What they do have in common, however, is an implicit assumption of a significant genetic basis to behavioural variation.

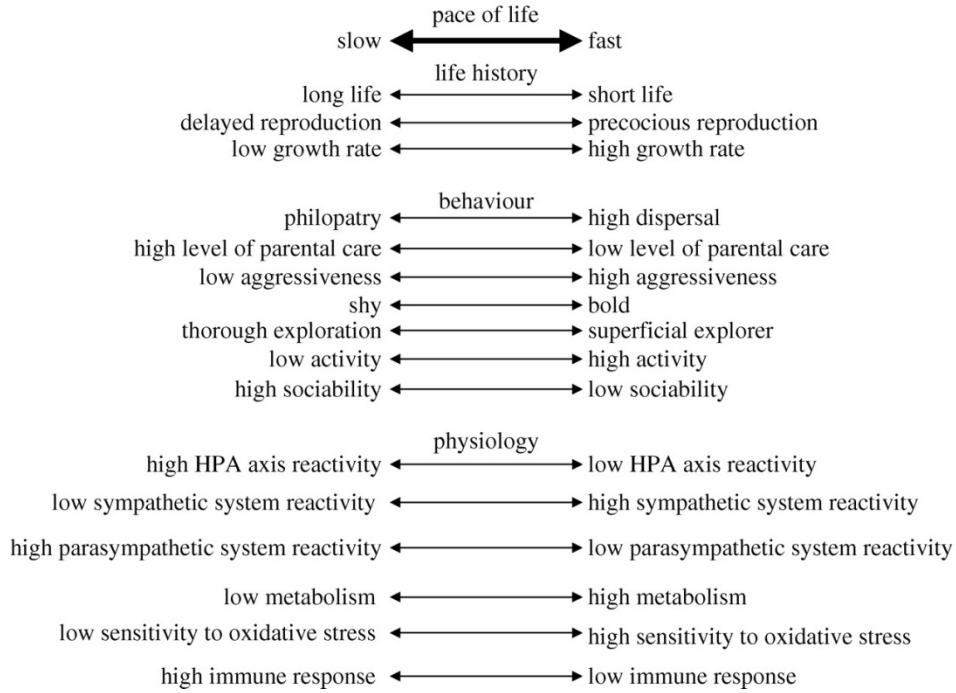

Figure 1: The Pace of Life syndrome. Life history, physiological and behavioural traits all covary across a slow-fast axis. After (Réale et al. 2010).

1.3 A quantitative genetics approach to animal personality

Many traits that are of interest to behavioural ecologists and evolutionary biologists vary continuously between individuals. This among-individual variation has its origins in both the underlying genetic architecture and the environment. The genetic portion of variation for such continuous, or “quantitative” traits is typically not explained by a single (or few) genes, but rather by many genes all

with small effects (Falconer and Mackay 1996; Lynch and Walsh 1998). While the genes themselves follow Mendelian inheritance patterns, evolution of these quantitative traits is more complex. How much of the observed phenotypic variation is determined by underlying additive genetics vs environment and how changes in the distribution of phenotypes are passed on to the next generation are common questions in quantitative genetics. This field employs statistical approaches originally developed for the animal breeding industry to estimate the genetic variance and covariance of traits and predict how they will respond to selection without the need for specific knowledge of the genes active in the focal trait.

Quantitative genetic modelling relies on the premise that related individuals tend to share more genes than unrelated individuals. If the variance of a trait of interest has a high degree of genetic determination, we would expect more closely related individuals to have a more similar phenotype compared to distantly related individuals. Conversely, if trait variation is not underpinned by genetics, there should be little or no relationship between individual relatedness and phenotypic similarity (Falconer and Mackay 1996). The breeder's equation predicts the average phenotypic change after one generation (R) given knowledge of the genetic basis of the trait and strength and direction of selection:

$$R = h^2 S \quad (1)$$

where h^2 is the narrow sense heritability, defined as the proportion of phenotypic variance attributable to additive genetic effects, and S is the selection differential (a measure of the relationship between trait and fitness).

The “animal model” is one commonly used statistical method to partition the total phenotypic variance (V_P) of a trait into additive genetic (V_A) and residual (unexplained, V_R) components:

$$y_i = \mu + a_i + \varepsilon_i \quad (2)$$

where *y* is the phenotypic measure of a trait in individual *i*, μ is the population mean, *a* is the additive genetic merit or breeding value of individual *i*, defined as the effect of an individual’s genes on the trait, with a mean value of zero and variance to be estimated. ε_i is the residual term, also with a mean of zero and variance to be estimated (V_R). It is not possible to directly estimate the breeding value of each individual, so individual identity is fitted as a random effect and the additive genetic variance (V_A) is estimated using relatedness information (Kruuk 2004; Wilson et al. 2010). h^2 is then calculated as $V_A/(V_A+V_R)$. Unlike other methods for obtaining heritability estimates, such as parent-offspring regression, the animal model utilises multiple forms of relatedness, can deal with unbalanced data sets and can control for other non-genetic sources of similarity between relatives (Lynch and Walsh 1998; Akesson et al. 2008; Charmantier et al. 2013). Using this approach, personality traits have been found to have low to moderate heritabilities (van Oers et al. 2005; Dingemanse et al. 2009; Niemelä et al. 2013; Petelle et al. 2015), indicating an evolutionary response to selection is possible. While this suggests that the adaptive models outlined above have the potential to explain the presence of animal personality, it is important to note that estimates of heritability alone are likely insufficient to predict how personality traits will respond to natural selection (discussed further below).

Failure to model non-genetic sources of similarity between relatives can cause bias when estimating the heritability of a trait. For instance, common environment and maternal effects can be additional sources of phenotypic similarity between relatives. Therefore, neglecting to control for them can upwardly bias heritability estimates and lead to erroneous conclusions about the evolutionary potential of personality (or other) traits (Falconer and Mackay 1996; Kruuk 2004; Wilson et al. 2010). If maternal effects themselves have a genetic component, this can lead to offspring traits responding to selection pressure on the current and previous generations (Kirkpatrick and Lande 1989; Räsänen and Kruuk 2007) resulting in complex responses of behaviour to selection on both mothers and offspring. Correlations between maternal genetic and direct (additive) genetic effects can facilitate response to selection on offspring traits (if positive), or constrain a response (if negative) and thus maintain additive genetic variation in both maternal and offspring traits (Kirkpatrick and Lande 1989; Wolf et al. 1998; Wilson and Réale 2005). Very little work has been done linking maternal genetic effects models with the adaptive frameworks for personality research outlined above, so little is known how these two important concepts will interact. Furthermore, maternal genetic effects could be an adaptive way for among-individual variation in behaviour to be maintained in their own right (Reddon 2011).

Why traits known to be under selection hold significant genetic variation is a long standing question in quantitative genetics. Interestingly, this question is exactly where the fields of animal personality and quantitative genetics overlap - if among-individual variation in behaviour has a genetic basis (and is therefore a reasonable proxy for additive genetic variation) what maintains it in the face of selection? So far, I have briefly outlined a univariate formulation of the

quantitative genetic approach. However, natural selection rarely operates on single traits in isolation. It is the multivariate phenotype as a whole that determines fitness and undergoes adaptive change. Furthermore, it is the genetic correlations between traits under selection that ultimately shapes the phenotypic response to selection. Considering a multivariate phenotype gives a much broader and realistic view of how traits are likely to respond to selection and may show ways through which genetic and phenotypic variation in behaviour can be maintained (Wolf and Weissing 2012). Equation 1 can be expanded to the multivariate breeder's equation:

$$\boldsymbol{Z} = \boldsymbol{G\beta} \tag{3}$$

where $\boldsymbol{Z}$ is a vector of mean trait responses to selection, **G** is the additive genetic (co)variance matrix and $\boldsymbol{\beta}$ is the vector of selection gradients. Here, the symmetrical **G** matrix contains not only the genetic variances for each trait measured, but also the genetic covariances between them:

$$\boldsymbol{G} = \begin{bmatrix} V_{A\,1,1} & COV_{A\,1,2} & COV_{A\,1,3} \\ COV_{A\,1,2} & V_{A\,2,2} & COV_{A\,2,3} \\ COV_{A\,1,3} & COV_{A\,2,3} & V_{A\,3,3} \end{bmatrix} \tag{4}$$

where genetic variances for each trait (V_A) are found on the matrix diagonal and genetic covariances between each trait pair (COV_A) are found on the off-diagonal. **G** can be estimated using a multivariate version of the animal model (equation 2). Traits are often not genetically independent from each other (Walsh and Blows 2009), either because they are influenced by shared genes (pleiotropy), or because of non-random associations of alleles at different loci (linkage

disequilibrium) (Falconer and Mackay 1996; Charmantier et al. 2013). The strength and direction of genetic correlations between traits can have a major influence on how traits respond to selection (Lande 1979; Lande and Arnold 1983). For instance, if two traits are positively genetically correlated then selection acting on one trait will elicit a positively correlated response in the other. Moreover, if selection operates in opposite directions for two positively genetically correlated traits, neither are likely to reach their respective fitness optima, meaning additive genetic variance will be maintained in these traits (Walsh and Blows 2009).

Another way of viewing the genetic (co)variance structure **G** is as the outcome of co-adaptation of traits through correlational selection (Sinervo and Svensson 2002), in which multiple trait combinations have similar fitness, maintaining a continuum of multivariate phenotypes based on trade-offs (Roff and Fairbairn 2007). Genetic correlation structure is therefore not only an important determinant of response to contemporary selection, it is also itself a result of past selection (Walsh and Blows 2009). In multivariate trait space, instead of a single fitness peak, there may be a fitness 'ridge' along which combinations of different traits yield high fitness (Roff and Fairbairn 2012). This can result in genetic and among-individual correlation structure among different traits, for example, between behaviour, physiology and life history (Careau et al. 2010, 2011; Niemelä et al. 2013). This quantitative genetic view relates back to the expansion of state-dependent models into the Pace of Life syndrome, with consistent among-individual variation in behaviour, life-history and physiology being the result of correlational selection and coadaptation (Wolf et al. 2007; Biro and Stamps 2008; Réale et al. 2010).

It is important, then, to assess the genetic basis of personality and its relationship with other traits types if we are to truly understand its development and maintenance. Such quantitative genetic analyses require phenotypic data on large numbers of individuals and accurate relatedness information from a pedigree obtained from either a planned breeding program or molecular/genomic techniques. In many systems, particularly wild populations, it is difficult to meet such data requirements, especially for behavioural traits that are commonly hard to measure. This means that estimation of quantitative genetic parameters may not be possible. Some have suggested that phenotypic patterns of variance and covariance could be suitable proxies for the underlying genetic architecture (Cheverud 1988; Roff 1996), an assumption termed the 'phenotypic gambit'. If this assumption holds true, then evolutionary inferences can be made without the need for large, long-term data sets that are common in the quantitative genetics literature. A number of studies have sought to test the phenotypic gambit, some have found that phenotypic and genetic parameters are highly correlated, so phenotypic information provides a useful proxy for genetics (Roff 1995; Reusch and Blanckenhorn 1998; Dochtermann 2011; Brommer and Kluen 2012). This is not the case in other systems, however (Reusch and Blanckenhorn 1998; Hadfield et al. 2007; Kruuk et al. 2008; Brommer and Kluen 2012), suggesting the suitability of phenotypic measurements as a genetic proxy are dependent on the species, population and traits under consideration.

Furthermore, additional attributes of the underlying genetic architecture may be obscured when only viewing variation through a phenotypic lense. For instance, genetic correlations can be present between traits expressed by individuals from different generations (e.g. in the case of maternal genetic effects). This means

that offspring traits will respond to selection on mothers (and vice versa). Genetic correlations are also possible between homologous (or different) traits expressed in different sexes. Because the sexes are quite often under antagonistic selection, but share much of their genetic architecture, it is likely that sexual conflict will occur (Lewis et al. 2011; Gosden et al. 2012; McPherson and Chenoweth 2012). Neither sex will be able to reach the fitness optimal which results in genetic variation being maintained for the focal traits (Kruuk et al. 2008). We therefore need a solid understanding of the complexities of the underlying genetic architecture if we are to draw sensible conclusions about the evolutionary potential of animal personality.

1.4 Study species – the Trinidadian guppy, *Poecilia reticulata*

In this thesis I take a largely quantitative genetics approach to understanding behavioural variation in a number of fish species. The majority of this thesis (chapters 2, 3 and 4) is based on work with the Trinidadian guppy, *Poecilia reticulata*. This is a small, shoaling species from the family *Poeciliidae* that generally inhabits freshwater streams found along the coastal fringes of mainland South America (Magurran 2005). While its natural range encompasses Trinidad and Tobago, Venezuela, Guyana and Surinam (Magurran 2005), in the last 100 years there have been numerous introductions of the guppy both intentionally for mosquito control (FAO 1997) and accidentally via release of aquarium fish. As a result, the guppy now thrives in over 70 countries across 5 continents and is one of the most widespread tropical fish in the world (fishbase.org).

This species is sexually dimorphic for size and growth, with female guppies exhibiting indeterminate growth after maturity in order to maximise fecundity.

Male growth plateaus once mature, with priority switching to reproduction. In addition, males are brightly coloured and ornamented (figure 2), relative to the cryptic females. In order to attract females, males perform a sigmoid swimming behaviour to display the orange, black and iridescent body markings, with both the intensity of colour and frequency of display being important factors in mating success (Liley 1966; Magurran and Seghers 1990; Nicoletto 1993; Endler and Houde 1995). Females mate with multiple males in a promiscuous mating system, with multiple paternity in most broods (Evans and Magurran 2001).

Like most Poeciliids, guppies are livebearers, with mature males using a modified anal fin (the gonopodium) for insemination and internal fertilisation (Wourms 1981). Females provision the eggs prior to fertilisation and retain them in the ovary cavity until the hatching and ‘birth’ of offspring (Magurran 2005, figure 3). Broods range in size from 1 to 25 fry, with the average brood at around 15 fry (Figure 4). Once released, fry are fully independent and capable of feeding with no active parental care exhibited by either parent.

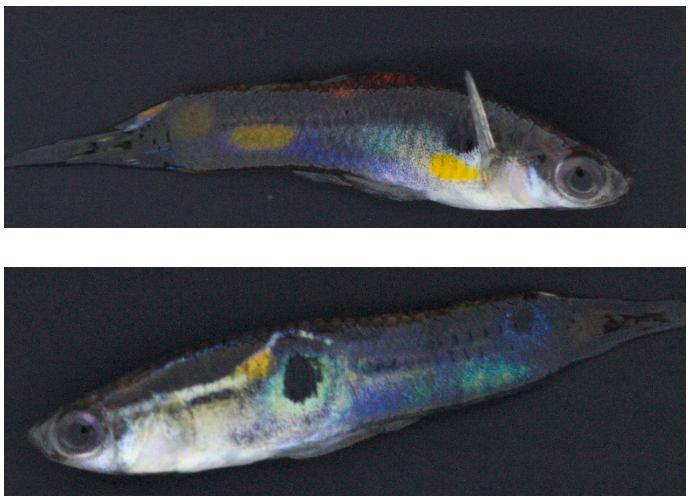

Figure 2: Examples of male ornamentation. Photos by Stephen White

Guppies live in shoals primarily to reduce predation risk (and potentially increases foraging efficiency). There is a high frequency of fission-fusion events, with males being the more mobile sex (Croft et al. 2003a), resulting in a dynamic social environment. Males maximise fitness by moving between multiple shoals of females, increasing potential mating encounters (Griffiths & Magurran, 1998, Kelley *et al.*, 1999, Croft *et al.*, 2003a, b). In females, fitness depends on longevity and fecundity rather than mating opportunities (Magurran and Seghers 1994). Therefore, females tend to exhibit stronger shoaling tendencies and higher shoal fidelity to reduce mortality from predation (Griffiths & Magurran, 1998, Magurran & Garcia, 2000, Magurran, 2005, Richards *et al.*, 2010).

Figure 3: Upper photo shows a highly gravid female approximately one month after male exposure and ready to birth the brood. Lower photo shows a female shortly after release of a brood. Photos by Stephen White.

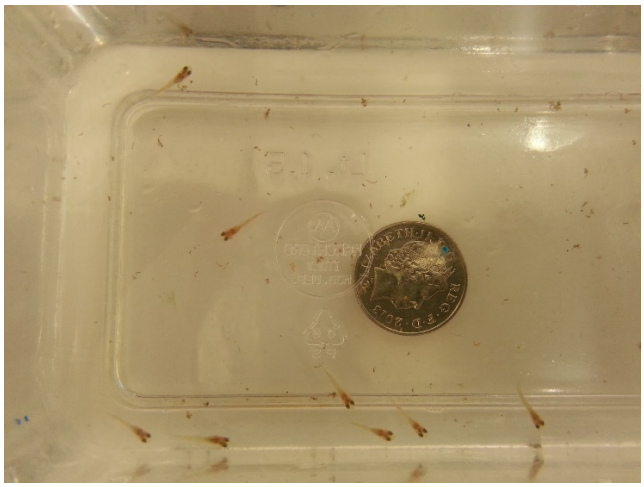

Figure 4: Guppy fry at approximately 10 days old. 10 pence piece for scale. Photo by Stephen White.

The guppy is a popular choice for aquarium keepers owing to their varied colours and ease of breeding and husbandry. It is the ability of this species to adapt to new environments in introduced areas as well as across multiple communities in its native South America that make it an ideal model organism for testing evolutionary theory (Magurran 2005). For instance, in Trinidad, guppy populations are spread over numerous streams that vary in predator/prey assemblages (as well as primary productivity, canopy cover etc.) over a relatively small spatial scale. These environments have typically been categorised into 'high predation' and 'low predation', with both observational and experimental studies assessing the evolution of life-history (Reznick 1982; Reznick and Endler 1982; Reznick and Heather 1987), male colouration (Endler 1983) and behaviour (Fraser and Gilliam 1987; Botham et al. 2008; Smith and Blumstein 2010; Elvidge et al. 2016) in relation to varying amounts/types of predation on guppies. While this simple dichotomous categorisation of the environments has dominated the guppy literature in the last 40 years, more recent work has looked at other aspects of the environment, such as canopy cover and resource availability (Grether et al. 2001), that differ among these communities and their effects on guppy trait evolution. The promiscuous mating style and prominent male display also makes the guppy an ideal model for studying sexual selection (Kodric-Brown 1989; Magurran and Seghers 1994; Godin and Dugatkin 1996; Brooks and Endler 2001; Karino and Shinjo 2004; Pélabon et al. 2014).

Finally, the guppy is easily maintained in a laboratory environment and it is possible to breed large numbers over a relatively short space of time (females produce broods monthly). This, coupled with the ease with which behavioural and

life-history data can be collected means this species is an ideal model for studying the quantitative genetics of animal personality.

One of the most common testing paradigms for quantifying personality is the open field trial, which provides a measure of exploratory behaviour and/or boldness. This has been used successfully in several fish species, including the guppy (Burns 2008; Oswald et al. 2013; Boulton et al. 2014; Diaz Pauli et al. 2015). While the open field trial can vary with respect to objects or shelters in the test arena, it generally measures the behaviour of a focal individual in a novel environment (Burns, 2008). In the context of guppies, the open field arena can also represent a risky environment - guppies are a shoaling species where being alone leaves an individual open to increased predation risk. The behaviour of guppies in this environment can be considered a response to a risky situation, with the prediction that a shy-bold axis of variation is captured by the open field trial.

The benefit of the open field trial is that it is quick to perform, allowing large numbers of individuals to be phenotyped quickly. In addition, it is ecologically relevant for guppies where, in the wild, individuals are often swept from shoals by currents to new areas (Magurran, 2005). One drawback of the OFT paradigm is that there is little agreement on which functional personality axis (e.g. exploratory behaviour, shy-bold axis) is represented by traits observed in the trial (Carter et al. 2013). This makes it difficult for cross study comparisons and drawing general conclusions of the maintenance of variation in these behavioural traits. Also, very little work has been done on how the idea of a shy-bold axis from

the OFT holds up across different populations or among-species, making evolutionary inferences difficult.

1.5 Thesis overview

This thesis aims to investigate the cause of among-individual variation in behaviour and to study, in greater detail, the genetic basis of variation in personality traits. It will initially be focussed on a single species in chapters 2-4 before taking a wider, multi-species approach in chapter 5. Throughout this thesis I advocate the use of quantitative genetic style modelling, and especially multivariate modelling, as a valuable tool for quantifying animal personality and testing hypotheses about the mechanisms that drive it.

In chapter 2, I evaluate the Pace of Life model by estimating among-individual (co)variation in metabolic rate, growth and four behaviours thought to represent a “shy-bold” axis of personality. The Pace of Life hypothesis predicts coadaptation between behaviour, physiology and life-history traits, where individuals fall on a slow-fast axis. In this context bolder individuals should exhibit fast pace of life. They are likely to acquire more resources, grow faster and mature sooner in order to maximise current reproduction at the expense of future reproduction due to increased predation risk. These individuals therefore have a low expectation of future fitness compared to shy individuals. Shy individuals on the other hand fall on the slow side of the continuum, maximising future reproduction by avoiding contact with predators, growing slowly and having a lower metabolic rate. As these strategies are two ends of a continuum, along which equal fitness returns are expected, we would expect among-individual variation in behaviour to be maintained. While several studies have tested this

framework, few have done so at the among-individual level. In this chapter I utilise a repeated measures, multivariate mixed model approach to test this framework at the among-individual level.

In chapter 3, I scrutinise the contribution of additive genetic and maternal effects to personality variation. I utilise a 2 generation guppy pedigree to quantify maternal effects on offspring personality traits in juveniles and mature adults. In life-history and morphological traits, maternal effects have been shown to diminish as individuals mature and as time since the offspring last received maternal care (e.g. last maternal provisioning before becoming independent) increases. Additionally, as offspring mature, additive genetic effects often explain a greater proportion of trait variance. While maternal effects on offspring behaviour have been studied previously, little work has been done on how they change over offspring ontogeny. I use the animal model to estimate the maternal and additive genetic variances of five personality traits and compare them between juvenile and adult guppies. I further investigate whether any maternal effect on offspring behaviour is mediated through offspring size at birth and whether any maternal effects present have a significant genetic basis.

Chapter 4 considers the possible role of genotype-by-sex interactions in the evolution of personality. This chapter is split into two parts. In the first, I investigate sexual dimorphism in personality traits, also relating this to known size dimorphism. Dimorphism has been found in various personality traits in a number of species, with males tending to be bolder than females, for instance. However, these studies rarely extend beyond reporting average differences in behaviour between the sexes. Here I consider dimorphism in trait averages and in the

behavioural variance-covariance structure. The first part of this chapter therefore consists of a comprehensive treatment of dimorphism in a set of personality behaviours indicative of a shy-bold axis. The second part of this chapter concerns the genetic architecture underpinning sexual dimorphism in behaviour. Behaviours expressed in both sexes are likely to share a common genetic architecture, but if the sexes are under different selection pressure then intra-locus sexual conflict can arise. This ultimately has the potential to maintain genetic variation in traits, with neither sex able to reach its phenotypic optimum. The widespread presence of sexual dimorphism in homologous traits suggests that this conflict can, at least in part, be resolved. Further to this, we would expect that constant and strong sexually antagonistic selection should favour mechanisms that reduce this conflict. This ultimately results in genotype-by-sex (GxS) interactions that allow the sexes to diverge. In this part of the chapter, I quantify sex-specific genetic variances and cross-sex genetic correlations for personality traits using bivariate animal models to assess the presence of GxS interactions. I also compare sex-specific genetic covariance between behaviour and length and growth, traits known to be sexually divergent. I then move on to a multivariate view of GxS interactions, first comparing the sex-specific genetic (co)variance matrices ($\mathbf{G_m}$ and $\mathbf{G_f}$) followed by the estimation of the **B** matrix, the cross-sex, cross-trait additive genetic covariance matrix. While this latter matrix has been estimated for a handful of morphological traits, it has never, to my knowledge, been estimated in the context of personality. While this is unsurprising given the amount of data required for convergence of such a large multivariate animal model, this matrix gives us a much fuller picture of how the sexes will respond to selection and reveal avenues of divergence or sources of constraint not apparent in single trait approaches.

In chapter 5, I expand my focus to a multi-species comparison of personality using traits from the open field trial (OFT), a widespread method of personality testing in fishes (and rodents). This assay is thought to capture a shy-bold axis, with shy individuals exhibiting low activity and high thigmotaxis and generally visiting only a small portion of the arena. Bolder individuals, on the other hand are expected to be more active and visit a greater proportion of the arena. In the past, arguments for observed OFT traits actually representing boldness have largely been verbal, and while there have been attempts at validation of the OFT there are still disagreements among studies on what constitutes boldness. Furthermore, assuming that we know what axis of variation the OFT captures (whether this really is boldness or something else) few studies have compared a single assay across species and assessed the generality of personality assays across taxa. In this chapter, I fill this gap by conducting OFTs on 7 species of small tropical fish and compare both the mean multivariate phenotype and its (co)variance structure. By identifying the major axis of among-individual variation through multiple OFT traits, I seek to identify the underlying personality variable captured by the OFT and test whether this differs across different species. In addition, I ask whether any difference in covariance structure between the species is associated with the phylogenetic relatedness between the species.

Finally, in chapter 6 I summarise my findings from each chapter and end with some final thoughts on improvements and directions for future personality research.

Chapter 2

Metabolism, personality and pace of life in the Trinidadian guppy, *Poecilia reticulata*

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2.1 Abstract

While among-individual variation in behaviour, or personality, is common across taxa, its mechanistic underpinnings are poorly understood. The Pace of Life syndrome (POLS) provides one possible explanation for maintenance of personality differences. POLS predicts that metabolic differences will covary with behavioural variation, with high metabolic rate associated with risk prone behaviour and 'faster' life-histories (e.g., high growth, early maturation). We used a repeated measures approach, assaying metabolic traits (rate and scope), behaviour and growth to test these predictions in the Trinidadian guppy, *Poecilia reticulata*. We found that while individuals varied significantly in their behaviour and growth rate, more risk prone individuals did not grow significantly faster. Furthermore, after accounting for body size there was no support for among-individual variation in metabolic traits. Thus, while personality differences are clearly present in this population, they do not covary with metabolism and the POLS framework is not supported.

2.2 Introduction

Among individual variation in behaviour, or personality, is widespread across taxa, yet our knowledge of the mechanisms driving and maintaining this variation is limited. The Pace of Life Syndrome (POLS) predicts that behaviour and life-history covary with physiology along a slow-fast axis (Ricklefs and Wikelski 2002; Réale et al. 2010). Individuals with higher metabolic rates are predicted to grow more quickly on average, mature earlier, invest in less responsive immune machinery, have more offspring per reproductive bout, and have a reduced longevity. POLS also predicts that a fast pace of life will be associated with more 'risk prone' behavioural types (Briffa et al. 2015) typically defined by greater boldness, exploratory tendency, and/or aggressiveness (Réale et al. 2010).

These patterns are relatively well supported by studies comparing suites of traits at among-species and among-population levels. For instance, tropical bird species that live longer have, on average, lower metabolic rates than temperate species (Wiersma et al. 2007; Williams et al. 2010). In addition, species of wild rodent with a faster pace of life rely more on innate immune responses than more expensive adaptive machinery (Previtali et al. 2012), a pattern also seen among populations of house sparrows (*Passer domesticus*) (Martin et al. 2006). Empirical studies of behavioural traits have also found correlations as predicted by POLS. For instance, Careau et al. (2010) found that domesticated dog breeds that were more trainable and obedient lived longer than more aggressive breeds that had higher metabolisable energy intakes. Bird species exhibiting riskier flight behaviour have higher metabolic rates (Moller 2009) and populations of Trinidadian guppies, *Poecilia reticulata,* exposed to higher levels of predation

tend to exhibit fast growth, early maturation and more risk-prone behaviours (Reznick et al. 1996b; Bronikowski et al. 2002; Harris et al. 2010).

With behaviour, life-history and physiology seemingly well integrated at the among species/population level, it is intuitive to ask whether the POLS framework might also explain among-individual variation within populations, including the widespread presence of animal personality (Careau et al. 2008; Réale et al. 2010). If different combinations of metabolic rate, growth and behaviour confer similar lifetime fitness, among-individual variation in these traits may be maintained and significant correlations between traits should persist (Biro and Stamps 2010; Réale et al. 2010). Individuals exhibiting more risk-prone tendencies (e.g. being bolder, more exploratory or more aggressive) are likely to encounter or acquire more resources at the expense of increased mortality risk from predation, whereas risk-averse individuals may acquire fewer resources but experience less mortality risk. Thus, if optimal growth rate varies among-individuals, perhaps because of underlying metabolic variation, risky behaviours should correlate positively with growth (Ward et al. 2004; Stamps 2007; Mas-Muñoz et al. 2011). This can be expanded further by considering trade-offs between current and future reproductive success: if future reproduction is unlikely, then it pays to employ risky behaviours to gain the resources to fuel a high growth rate. All else being equal, in juveniles, rapid growth facilitates earlier reproduction, while in organisms with indeterminate growth, fast adult growth typically delivers increased fecundity. Conversely, future reproductive prospects may be enhanced by being risk-averse, thus decreasing mortality risk (e.g. from predation), but also resulting in delayed maturation and slower growth (Wolf et al. 2007; Biro and Stamps 2008).

Applied within populations, the POLS framework predicts a positive relationship between metabolic rate and risky personalities, although causality is potentially bidirectional. For instance, if risk-prone individuals have higher food intake they may develop larger food processing organs (liver, intestines etc.) that have high mass specific metabolic rate (Biro and Stamps 2010; Careau and Garland 2012; Wiersma et al. 2012; but see Russell and Chappell 2007). Alternatively, individuals with high metabolism and therefore high base energetic requirements may be compelled to take risks (e.g. by needing to feed sooner after a disturbance than those with lower metabolic costs), resulting in a risk-prone behavioural phenotype (Finstad et al. 2007; Careau et al. 2008). Despite this uncertainty over causation, positive relationships between behaviour and metabolic rate consistent with the POLS framework have been found among-individuals in a range of species, including several fish species (McCarthy 2001; Cutts et al. 2002; Huntingford et al. 2010; Robertsen et al. 2013). The evidence, however, is far from conclusive since Bouwhuis et al. (2014) actually found a weak negative correlation between exploratory behaviour and basal metabolic rate in female (but not male) great tits (*Parus major*). In the same species, Mathot et al. (2014) found that the sign of the correlation between basal metabolic rate and post-disturbance time to resume feeding depended on the type of disturbance. Context dependent correlations between metabolic traits and risk related behaviours have been reported in juvenile sea bass (*Dicentrarchus labrax*) (Killen et al. 2011, 2012), while several have reported no relationship at all in salamanders (*Desmognathus brimleyorum*), root voles (*Microtus oeconomus*) and common lizards (*Zootocai vipara*) (Lantová et al. 2011; Le Galliard et al. 2013; Gifford et al. 2014).

A possible reason for the mixed support for the predictions of POLS is that, while most studies to date have focused on basal, resting or standard metabolic rate, metabolic scope may be a more important determinant of the link between individual physiology and behaviour (Careau and Garland 2012; Mathot and Dingemanse 2015; Metcalfe et al. 2015). Metabolic scope (MS) can be viewed as the energetic capacity, after base metabolic demands are met, available for processes such as exhibiting behaviours. If individuals vary in MS this could potentially drive and maintain among-individual variation in behaviour. Importantly, relationships between resting metabolic rate and MS vary across species (Cutts et al. 2002; Speakman et al. 2003; Hansen and Hunt Von Herbing 2009; Careau et al. 2013, 2015), potentially limiting the generality of resting metabolic rate-based investigations of POLS (Mathot and Dingemanse 2015). In addition, assessing among-individual (co)variation requires repeated measures of all traits concerned (Nakagawa and Schielzeth 2010). While recent years have seen an increase in the use of repeated measures approaches to the study of behaviour and physiology, more studies taking an integrated approach with multiple measures of each individual are required to fully understand POLS within populations.

The aim of this study is to evaluate the POLS framework in Trinidadian guppies (henceforth guppies). We use a captive population of guppies and a multivariate repeated measures approach to assess the (co)variance structure between metabolic rate and scope, risk related personality traits and growth rate. If POLS is present in this population we predict that i) individuals will differ consistently in metabolic traits (metabolic rate and scope), ii) individuals will show personality differences consistent with a shy-bold continuum of behavioural variation and iii)

metabolic and behavioural traits will be correlated at the among-individual level, with fast paced individuals (high metabolic rate, bold) also showing faster growth rates than slower paced conspecifics.

2.3 Materials and methods

2.3.1 Study Species

Guppies used in this experiment were from a captive population housed at the University of Exeter's Penryn campus fish facility. The population is descended from wild individuals caught in 2008 from a high predation site in the lower Aripo River, Trinidad (c. 18-20 generations ago) and has been maintained at an effective population size of several thousand (with no deliberate selection or inbreeding).

Thirty-two adult females were sampled from the stock population and tagged using visible implant elastomer tags (VIE). Sampling was haphazard but we tried to limit size variation by selecting fish of similar size. The tagging process consisted of submersion in an 80mg.L^{-1} MS222 solution buffered with sodium bicarbonate for several minutes, until fish stopped swimming and rested on the tank floor. Sedated fish were then tagged and placed immediately into a large, well-aerated tank and monitored for 5 minutes, during which all fish recovered from anaesthesia. VIE tags have been shown to have no significant effect on growth or behaviour in zebrafish (*Danio* rario) and guppies (Croft et al. 2004; Hohn and Petrie-Hanson 2013) and there was no tagging related mortality in this experiment.

As isolation can cause unnecessary stress, each fish was randomly allocated to one of 4 groups (8 individuals per group). Groups were housed in separate home tanks (15L, 18.5cm x 37cm x 22cm) but shared a common recirculating sump water supply, maintained at 23-24^{0}C and on a 12:12 light:dark cycle. The tank stack used was a well aerated closed system subject to a 25% water change once per week with weekly tests for ammonia, nitrite and nitrate levels. All fish were fed to satiation twice daily on commercial flake food and live brine shrimp (*Artemia salina*) nauplii. Female guppies are indeterminate growers, continuing to exhibit significant growth well after maturity, making them ideal to test the predictions of POLS. Males were excluded from this study as growth rate is much lower post maturity.

The experiment was conducted under the auspices of the Animals (Scientific Procedures Act) under licence from the Home Office (UK) and with local ethical approval from the University of Exeter. All periods of handling and emersion were kept as short as possible. At the end of the experiment, fish were moved to a "retirement" stock tank (containing males and other females) and allowed to reproduce to contribute to the stock population. These fish were not subject to any further licensed procedures.

2.3.2 Experimental design

We used a repeated measures approach to test for among-individual (co)variation in metabolic rate, personality and growth. Metabolic rate was assessed from intermittent flow respirometry while personality was assessed using two behavioural testing paradigms (open field trials, OFT and emergence trials, ET). Individuals from all groups experienced a sequence of phenotypic

assays comprising: day 1 - OFT, day 2 – routine metabolic rate (RMR), day 4 - ET and day 5 - active metabolic rate (*AMR*). We repeated this week one sequence for a second week. Fish were then subject to two additional OFT and ET each. These were conducted in weeks 7 and 9 for groups 1 and 2 (with one trial per type per week per fish). However, due to space and equipment constraints, we conducted these additional trials in weeks 4 and 6 for groups 3 and 4. This difference is controlled for statistically in the analysis. *Standard length* (measured from tip of snout to end of caudal peduncle, in mm) and mass (g) were measured at every behavioural and metabolic trial and 1 month after the final behavioural trial experienced by each fish to allow calculation of growth rate. Emersion time to conduct these measures (which were not conducted under anaesthetic) was typically less than 10 seconds and fish were fully recovered several minutes after being returned to the tank. In total, each fish had a maximum of 4 metabolic measures, 4 OFT, 4 ET and 13 size measures with total data collection spanning 13 (groups 1 and 2) or 10 (groups 3 and 4) weeks. At each sampling, the order (i.e. 1-8) in which each fish was haphazardly captured from its group tank was also recorded.

Our experimental design should have led to 128 metabolic trials (64 *RMR*, 64 *AMR*) and 256 behavioural trials (128 OFT, 128 ET). However, we experienced some mortality late in the data collection period and incomplete data were thus obtained for 9 individuals (with 120 metabolic and 215 behavioural trials completed). Based on the absence of adverse effects attributable to the protocols a general water quality problem in the facility was the suspected cause, although age may also be a factor (fish were sampled from a stock tank containing larger and, since female guppies exhibit indeterminate growth, putatively older than

average fish). In the following analyses we used all available data, however, including individuals with incomplete data collection since the mixed model analyses used are robust to unbalanced data sets. We also account for cumulative trial number and group size in all statistical models (see statistical methods below) to avoid any potential for bias.

2.3.3 Metabolic measures

An automated intermittent flow respirometer from Qubit biological systems (http://qubitsystems.com) was used to measure metabolic rate. The respiration chamber (1.6cm x 4.5cm, 9ml) was submerged in a 2.5L water bath with water temperature maintained at 24^{o}C (23.9 – 24.1) using a submersible heater (Visi-therm 25W, www.aquariumsolutions.eu) and a UV steriliser to minimise bacterial growth. *RMR* is here defined as the metabolic rate of a post-absorptive non-reproductive fish at rest while including random movement required to maintain position in the water column (Killen et al. 2011). Guppies, even at rest, still exhibit some tail and fin movement to maintain position in the water. We were unable to account for this movement and therefore we define our measures as *RMR* rather than standard metabolic rate (SMR). One could argue that such random movements are a necessary part of the metabolic expenditure when an aquatic organism is at rest and should not be removed at all.

To measure *RMR*, the focal fish was placed in the respiration chamber following 24 hours of fasting. Oxygen consumption was then measured over four 10 minute 'closed' periods (i.e. chamber and pump closed off from the water bath) separated by 4 minute 'flush' periods. *Standard length* and *mass* were measured immediately after every metabolic trial to be used to calculate mass-specific

metabolic traits (see below). *RMR* was estimated as the average of the last three oxygen consumption rates (each determined as the slope over the most stable part of the corresponding 10-minute period in mg O_2 L^{-1} s^{-1}). The first metabolic rate measure of each trial was excluded as pilot trials suggested it was significantly higher, likely reflecting a response to the physical stressor of being moved into the respirometer. *AMR* was measured similarly, but immediately following 2 minutes of being chased by a hand net. The aim of the net chasing was to provoke a 'burst and glide' swimming technique that has been found to be aerobically demanding (Cutts et al. 2002; Norin and Malte 2011). Due to ethical considerations we did not measure true maximal metabolic rate (MMR) as this requires exercising the fish to complete exhaustion, which in guppies may have resulted in mortality. *AMR* was estimated as the rate of oxygen consumption from the first 2 minutes of being in the respiration following the chasing. See Appendix 1 for further details on respirometer use and setup.

2.3.4 Behavioural trials

Open Field Trial

Our OFT followed a protocol very similar to that described by Boulton et al. (2014). The focal fish was placed into an empty tank (30cm x 20cm x 20cm) with 5cm water depth, and lit from below using a light box. A video camera fixed above the tank allowed the movement of the fish to be tracked using Viewer software (www.biobserve.com), removing observer bias and minimising measurement error. Placing a cardboard screen around the tank during the trials prevented disturbance by researcher activity. Following 30 seconds of acclimation, a 4 min 30 sec tracking period was used to determine total *tracklength* swum (*Tl*, cm), *activity* (*Act*, percent time swimming above 4cm s^{-1}) and percent of tank *area*

covered (*AC*). We also recorded the amount of time spent in an outer 'safe' zone near to the side of the tank and an inner 'risky' zone (*TIM*, seconds*)*, the zones being defined as equal in size following Boulton et al. (2014). These behaviours have been shown to predict risky or 'bold' personality effectively in other poeciliid fishes (Burns 2008; Boulton et al. 2014), with bolder individuals expected to have a longer track length, be more active, cover more tank area, and spend more time in the 'risky' middle tank zone. The water in the OFT tank was changed between each group of fish. We controlled for any effects of order of testing (within group) that might arise due to, for instance, release of hormones or other chemicals into the tank by including *order caught* as a fixed effect in models for all traits in our statistical analysis (see below).

Emergence trial

The focal fish was placed in a shelter area within a larger tank (40cm x 20cm x 20 cm) filled to 8cm depth and screened as described above with a video camera placed overhead. It was allowed to acclimate for 30 seconds before a sliding door in the shelter wall was opened, allowing access to the rest of the tank. Time to emergence (henceforth *ET*) was then recorded and trials were ended at emergence or at 15 minutes if the fish had not emerged by this time (6 out of 106 trials).

2.3.5 Statistical methods

We used a series of univariate and multivariate linear mixed effect models to test among-individual (co)variation in metabolic traits, personality and growth as predicted by POLS. Random regression methods were used to characterise variation in *MS* and growth as described fully below. We applied a log

transformation to metabolic rate data to help control for size effects (since the relationship between metabolic rate and *weight* appeared linear on a log-log scale) and to *ET* to reduce slight positive skew. We also mean-centred all (transformed) traits and scaled them to standard deviation units. This was to facilitate multivariate model convergence and prevent different trait scales from driving conclusions. Linear mixed effects models were then fitted with restricted maximum likelihood (REML) using ASReml 4.0 (www.vsni.com). Conditional F statistics were used to determine significance of all fixed effects while inference on random effects used likelihood ratio tests (LRT). Twice the difference in log-likelihood between full and reduced models was assumed to be distributed as χ^2 with degrees of freedom (df) equal to the number of additional parameters in the full model. For testing a single variance component only, we assumed a 50:50 mix of χ^2_0 and χ^2_1 (subsequently denoted $\chi^2_{0,1}$) following the recommendations of Visscher (2006).

Metabolic traits

Univariate models containing individual as a random effect were fitted to the metabolic rate data. Repeatability (conditional on fixed effects) was then calculated as the intraclass correlation, $R = V_I/(V_I+V_R)$, where V_I is the among-individual variance and V_R is the residual variance (Nakagawa and Schielzeth 2010). We included fixed effects of *group*, *trial* number (the cumulative number of trials of any type previously experienced), *order caught* (1-8 within each group, factor) and measure type (*RMR* or *AMR*, factor). The *group* effect controls for differences in physical and social environments among tanks. *Order caught* refers to the order in which each fish in a group was assayed on a trial day and is intended to account for any cumulative disturbance effect of removing fish

sequentially from the home tank. The *measure type* fixed effect accounts for any differences between mean *RMR* and mean *AMR* measures, allowing all 4 measures per individual to be included in the calculation of repeatability.

This model tests for among-individual variance in metabolic rate (across routine and active contexts) as well as for the expected increase in average oxygen consumption with activity (resting vs recently active). We then characterised variation in *MS* by adding a random interaction of *fish identity* with measure type to the above model. Measure type was treated as a continuous variable indicative of activity level and arbitrarily scaled (such that at *RMR* activity= -0.5, and at *AMR* activity = 0.5). Note that, conventionally, MS is measured as the difference between standard (SMR) and maximal metabolic rates (MMR), neither of which were formally assayed in our experiment. However, MS can equally be represented as the slope of an individual's reaction norm between two activity states on an arbitrarily scaled axis (i.e., 'standard' and 'maximal'; Figure 1). Since a slope is defined by any two points on the reaction norm, we are able to characterise rank order variation in MS using assays of metabolic rate at intermediate 'routine' and 'active' levels instead (Figure 1). Although complete correspondence is strictly contingent upon a linear reaction norm through all four activity states (Figure 1), in practice the *RMR-AMR* reaction norm slope will be strongly correlated to, and thus a suitable proxy for, MS as standardly defined over a much wider range of scenarios. The reaction norm framework, using random regression, allows the value of a random effect to vary with an additional covariate. This technique has been used extensively to model among-individual variation in morphological and life-history traits as well as genotype-by-environment interactions (Nussey et al. 2007; Dingemanse et al. 2010; Roff and

Wilson 2014). Both models were first fitted using log metabolic rate data uncorrected for mass. We then refitted with log body mass as an additional fixed covariate such that V_I is interpretable as variance in mass-specific metabolic rate while (in the reaction norm formulation) among-individual variance in slope (V_S) is interpretable as variance in mass-specific MS.

Behavioural traits

Behavioural traits were similarly modelled with a random effect of individual and fixed effects of *temperature*, *group*, *order caught*, *trial number* and *weight*. Interestingly, pilot analysis indicated that *order caught* was itself repeatable, and so this was modelled as an additional behaviour potentially indicative of risk-taking (note *order caught* was necessarily not fitted as a fixed effect in this case). Following Boulton et al. (2014), we then fitted a multivariate mixed model with all 6 behavioural traits (i.e., *TI*, *Act*, *AC*, *TIM* from OFT; *ET* from the emergence trial; and, *order caught* from both OFT and emergence trial). This allowed us to test the prediction that all OFT behaviours would be positively correlated with each other at the individual level and negatively correlated with *ET* and *order caught*, consistent with an underlying axis of shyness-boldness. The resulting variance/covariance matrix was subject to eigen vector decomposition, allowing us to identify the major axes of variation and see how the behavioural traits load on to these axes. Eigen vector decomposition is analogous to principle component analysis (PCA), but used here to describe only the among-individual component of phenotypic (co)variation (Wilson et al. 2011a; Boulton et al. 2014).

Growth

Among-individual variation in growth was also characterised using random regression mixed models of *standard length* that included random effects of *fish identity* and a fish by '*time since start of experiment'* interaction (zero centred from an actual mean across all size measurements of 25 days). Fixed effects included were *group*, *last day seen* (to account for mortality effects on average growth) and *time since start of experiment* (days) as a linear covariate to account for average growth. We chose a simple linear function because actual age of fish was unknown (though all females were mature) and growth was observed over a short period only. In this model V_I is interpretable as the among-fish variation in *standard length* at the intercept (i.e. 25 days from the start of the experiment) while the variance in individual regression slopes (V_S) is among-fish variance in growth rate. Finally, *standard length* was added to the above multivariate model to assess the among-individual (co)variance structure of size and growth with behaviour.

2.4 Results

While whole animal metabolic rate shows significant among-individual variation (R=0.27 (0.11), $\chi^2_{0,1}$=8.031, P=0.002), inclusion of *log weight* as a fixed effect results in the estimate of V_I being bound to zero to stay in allowable parameter space. We thus estimate a repeatability of zero for mass-specific metabolic rate (across the two activity levels). Furthermore, comparison of the random regression model to this simple formulation provide no evidence that individuals vary significantly in either whole animal MS (χ^2_2 = 0.277, P=0.871) or mass-specific MS (χ^2_2 =0.702, P=0.704) (note 2 DF for the model comparisons as the random regression formulation includes intercept-slope covariance as well as the

two variance terms). A significant positive effect of measure type (*AMR* relative to *RMR*) was found confirming the expectation that *AMR* should be significantly higher on average (coefficient = 0.758 (0.062), $F_{1,106}$=150.66, P=<0.001). Other fixed effect results are not directly relevant to current hypotheses but can be found in supplemental table 1.1 for completeness.

Thus we conclude that while whole animal metabolic rate varies significantly among-individuals, this can be explained by body size alone, and there is no evidence of among-individual variation in mass-specific metabolic rate (reaction norm height) or scope (reaction norm slope; Figure 2a). This study applies the POLS framework at the among-individual level, and among-individual variance in metabolic traits is a prerequisite for among-individual covariance between metabolism and other traits. Consequently, metabolic traits are not included in subsequent multivariate models (We note of course that while within-individual covariance between metabolism and behaviour is still expected, our data are not informative for this as metabolic rate and behaviour were not measured simultaneously).

In contrast to metabolic traits, univariate models show moderate repeatabilities (SE in parentheses) for behavioural traits, ranging from 0.31 (0.12) for *TI* to 0.46 (0.11) for *AC*, and statistically significant in all cases (Table 1). Of the OFT traits, only *TI* and *AC* changed significantly over the trials with both increasing with increasing trial number (see Supplemental table 1.1). Our univariate model of *standard length* confirms that fish vary significantly in size, as was obvious *a priori* (comparison of models with and without random *fish identity* effect; $\chi^2_{0,1}$=387, P=<0.001), but also growth rate (comparison of the random regression

formulation including *fish identity* by *time* to a model with just *fish identity*; χ^2_2= 18.5, P=<0.001). Thus, while there is a modestly positive average rate of growth (of 0.013 (0.003) sdu day^{-1} or 0.265 mm day^{-1}) there is also significant variation around this (Figure 2b).

Multivariate models of the behavioural traits confirm significant covariance structure between behaviours at the among-individual level (comparison of full model to a reduced multivariate model in which all among-individual covariance terms are fixed to zero; χ^2_{15}=34.5, P=0.003). Post hoc testing of pairwise covariances with a series of bivariate mixed models (see Supplemental table 1.2) suggests significant among-individual covariance structure is largely driven by a strong positive relationship between *TI* and *Act*, and strong negative relationships between these two traits and *TIM* (Table 2). We note that not all pairwise correlations among behavioural traits are as expected *a priori* (Table 2; see discussion for full interpretation). Eigen vector decomposition of the (co)variance matrix (see Supplemental table 1.3) does not clearly support our *a priori* expectation that among-individual (co)variance in behavioural traits would be consistent with a single shy-bold axis. Finally, extending the multivariate model to include *standard length* as an additional response variable shows that, while some moderate among-individual correlations between behaviours and size were estimated, only the correlation between *AC* and growth is significant (χ^2_2= 6.05, P= 0.048**)** (tested using bivariate models; Supplemental table 1.4).

2.5 Discussion

Using a repeated measures design we tested the prediction of POLS that among-individual differences in metabolic traits (rate and scope) covary with behaviour

and growth variation, with the additional prediction that it is among-individual variation in MS that drives behaviour variation. All observed behaviours tested were repeatable, consistent with the presence of underlying personality variation, and growth rate also varied significantly among-fish over the experimental period. However, after accounting for the expected increase of oxygen consumption with body size, we found no support for repeatable variation in mass specific metabolic rate or MS. Furthermore, there was little evidence of the predicted among-individual correlation between risky behaviour and growth rate. Thus our data are not consistent with our assertion that metabolic processes is a potential driver personality variation and we also conclude that the POLS is not supported in this population.

The lack of among-individual repeatability in metabolic traits in this study contrasts notably with other work on wild caught fish species held under laboratory conditions. For instance, mass-specific SMR has generally been reported to have moderate to high repeatabilities (e.g., R ranging from 0.50-0.74) in most fish species tested under highly controlled conditions (McCarthy 2000; Maciak and Konarzewski 2010; Seppänen et al. 2010; Boldsen et al. 2013; Svendsen et al. 2014). Mass-specific RMR is sometimes expected to exhibit greater variation within individuals than SMR (due to uncontrolled activity during measurement of the latter), but nonetheless is often characterised by moderate (R= 0.30-0.60) repeatability (Marras et al. 2010; Killen et al. 2011, 2014). Furthermore, variable, but significant, repeatability estimates have also been reported for mass-specific MMR (e.g., R from 0.27-0.76; McCarthy 2000; Marras et al. 2010; Norin and Malte 2011; Svendsen et al. 2014; Norin et al. 2015) and MS (e.g., R from 0.39-0.43; Norin and Malte 2011; Norin et al. 2015).

We note of course that measurement error could be a non-trivial source of within-fish variation, and inadequate precision of respirometers can cause low repeatability of metabolic traits (Nespolo and Franco 2007; Careau et al. 2008). Nonetheless, we feel this is unlikely to explain the complete absence of detectable V_I here. Firstly, within each RMR sampling assay, we averaged the three oxygen consumption slopes estimated over the 50-minute period to reduce error as described above. However, scrutiny of these measures shows significant repeatability among slopes within-assay, even without being able to control for changing level of fish activity ($R=0.56$, $\chi^2_1=52.47$, $P=<0.001$) indicating stable performance of the instrumentation at least over the short term. Secondly, our repeated measures sampling was designed to detect repeatabilities as low as 20% (i.e below published estimates) with high (>75%) power (following Wilson et al. 2011b). Thirdly, we note that the experiment did in fact successfully detect among-individual variation in whole organism metabolic rate ($R=0.27$), but that our results show this can be totally explained by differences in individual weight.

Previous studies have shown the potential role of early life conditions, including the maternal nutritional environment, in generating variation in, and correlations between putative components of POLS. For instance, food restriction during juvenile stages can increase the repeatability of metabolic rate later in life, with individuals varying in response to nutritional stress experienced as juveniles (O'Connor et al. 2000; Careau et al. 2014a,b). The environment experienced by parents, particularly the mother, can also lead to variation between individuals in a range of traits, including adult metabolic rate (Tobler et al. 2007; Régnier et al. 2010; Burton et al. 2011; Van Leeuwen et al. 2015). In our study, the laboratory conditions experienced by fish during these important developmental windows

were likely relatively homogeneous by comparison to field environments. This could have resulted in a reduction of among-individual variance in metabolic rate and scope, relative to wild caught fish used in other studies that have experienced greater patchiness of resources (Grether et al. 2001; Magurran 2005).

Since we found no support for among-individual variation in metabolic traits, our data do not support the hypothesis that metabolism is an important determinant of individual differences in behaviour. Nonetheless, such differences are clearly present among the guppies tested, with significant repeatability found for *ET* and all OFT traits. In general, repeatabilities were of similar magnitude to those reported in the literature for behaviours generally, and in poeciliid fishes specifically (Bell et al. 2009; Cote et al. 2011; Boulton et al. 2014). We also found that, within each housing group, the order in which fish were caught was repeatable. The tendency for some individuals to be trapped or caught more easily than others has been used as a measure of boldness or risk taking behaviour. In general, bolder/risk-prone individuals are more easily caught than the shy/risk-averse (Réale et al. 2000; Biro and Sampson 2015; Le Coeur et al. 2015; Petelle et al. 2015), consistent with the predicted consequences of this personality trait for predation risk (but see Diaz Pauli et al. 2015). Since fish in this study were actively collected (albeit haphazardly), there is an obvious possibility that some form of researcher bias that would not be exhibited by a natural predator in the field contributes to the repeatability of order caught. We note that fish tags are only clearly visible after capture, and researchers were blind to the behavioural profile data of each fish. Regardless, this finding also suggests initial sampling of experimental fish from stock tanks could itself have been selective with respect to behaviours to be studied. The possibility of

samples not being fully representative of behavioural variation in a studied population has wider implications for personality studies (as discussed by Carter et al. 2012a).

The individual traits observed in OFT and emergence trials have been widely used to assay risky or bold behaviour in fishes, including guppies (Budaev 1997; Burns 2008; Diaz Pauli et al. 2015). However, our analysis provided somewhat mixed support for our second prediction, that individuals would show (multivariate) personality variation consistent with a simple axis of variation along a shy-bold continuum. Under this model, we expected that all OFT traits would be positively correlated with each other and negatively correlated with *ET* and capture order at the individual level. In fact, significant among-individual correlations were found only between *TI* and *Act* (positive as predicted) and between these two traits and *TIM*. Surprisingly, TIM was actually negatively correlated among-individuals with the former two traits. Eigen vector decomposition of the among-individual variance-covariance matrix (**I**) estimate identifies two major vectors that, together, explain 74% of the variation. The first vector, accounting for 47% of the variation, is dominated by *TI*, *Act* and *TIM*. The second vector, accounting for 27% of the variation, is more characterised by *ET* and *AC*.

Thus the among-individual covariance structure of behavioural traits suggests that the simple model of a shy-bold continuum is not valid in this population, and/or that it is being masked by other aspects of personality being expressed in our trials. This result differs from a study on a different poeciliid, *Xiphophorus birchmanni* conducted by Boulton et al. (2014) in which strong positive

correlations between the same OFT traits were found, with the **I** matrix dominated by a single-vector interpretable as a shy-bold axis. Thus an important conclusion emerging from the current behavioural data is that a particular assay or observed trait(s) may not be informative for the same personality trait in different species, even if closely related. Indeed, this may also be the case for different populations of a single species. For instance, while we know that mean boldness differs among natural populations of guppies according to predation regime (Reznick et al. 1996b), among-population comparisons of **I** matrices would add considerable resolution to our understanding of where among-individual variation is maintained and how it is structured by genetic and ecological factors. In this instance, differences in the behavioural ecology between guppies and swordtails could contribute to differences in OFT patterns, with swordtails being more territorial relative to the shoaling, social guppy. Regardless, by measuring multiple behaviours from different tests, measures of personality can be validated rather than relying on *a priori* definitions of personality that may not be appropriate for a given species.

More speculatively, we consider it likely that OFT traits in this case are capturing elements of behavioural stress response or coping style (Koolhaas et al. 1999; Boulton et al. 2015), particularly as this was a 'forced' rather than voluntary trial (Huntingford 1976; Walsh and Cummins 1976; Carter et al. 2013). Behavioural responses to stress in fish have been described as ranging from reactive (often characterised by freezing behaviour) to proactive (e.g., highly active fight or flight behaviour). This axis is sometimes, but not always, viewed as synonymous with variation in risky behaviour (van Raaij et al. 1996; Koolhaas et al. 1999; Brelin et al. 2005; Øverli et al. 2007; Silva et al. 2010). Here we note that video

observations revealed a relatively common behavioural pattern of swimming rapidly back and forth along one side of the tank (generating high *TI* and *Act*, but low *TIM* and *AC*). This was more consistent with expectations for a proactive coping style (i.e. active attempt to escape) rather than risky or bold behaviour as normally defined (e.g., reduced thigmotaxis, higher exploration).

A final prediction made under the POLS was that individuals with more risk-prone personalities would have higher growth rates. Even in the absence of metabolic variation as a driver, the prediction of a risky personality trait being positively associated with resource acquisition is unchanged (Stamps 2007; Biro and Stamps 2008). While several studies of fish species to date have found this relationship (Ward et al. 2004; Huntingford et al. 2010; Mas-Muñoz et al. 2011), it is not supported by our data. Individuals did vary significantly in growth rate over the short term study, but only *AC* showed a significant correlation with growth rate, and it was negative not positive as predicted. Given the lack of a clear shy-bold behavioural axis it may be misleading to over-interpret this finding from a single behavioural trait (i.e. we do not conclude that shy fish grow faster).

More generally we note that while a degree of social competition is expected, fish were all fed to satiation in the study. Social environments can certainly contribute to development of personality traits (Webster and Ward 2011) and could also influence wider patterns of trait correlation. Thus if personality-growth correlations found elsewhere are generated by competitive advantage of, for instance, bold over shy individuals (Biro and Stamps 2010; Niemelä et al. 2012), then these are expected to be stronger under conditions of resource limitation (Wilson 2014). In contrast, relationships should be weaker under conditions that tend to equalise

food intake levels between shy and bold individuals, such as under high resource environments.

2.6 Conclusion

In conclusion this study found no support for POLS in the guppy population tested. Once the dependence on body size was accounted for, we found no support for variation among-individuals in metabolic rate or scope. Thus we conclude that metabolism is not always a plausible driver of among-individual variation in behaviour. All behavioural traits chosen as putative indicators of a shy-bold behavioural axis were repeatable. However, the among-individual covariance structure was not actually consistent with the presence of a single underlying latent personality trait, and there was no support for the predicted association of risky behaviour with faster growth. Although we note that patterns of among-individual trait (co)variation are certainly expected to show environmental sensitivity, our behavioural results highlight the value of multivariate (i.e., multiple trait and multiple trial type) repeated measures data. In seeking to test mechanistic explanations for the maintenance of animal personality, it is important that we have an understanding of how behavioural variation is actually structured among-individuals in the focal population (i.e. to what extent do individual behaviours provide information about personality axes that are generalizable over population or species). This is particularly important in POLS research where the expectation of positive correlations between behaviour, physiology and growth may be dependent on access to resources, territory or mates.

Finally, we stress that while among-individual (co)variation provides the raw material upon which selection can act, it is the structure of genetic (co)variation that will determine how traits such as personality evolve, and coevolve, under selection. Others have found abundant evidence for heritable variation underpinning personality (van Oers et al. 2005; Dingemanse et al. 2012; Oswald et al. 2013), though tests of genetic (co)variance structures remain limited. While we found no support here for POLS at the level of the individual phenotype, we suggest that quantitative genetic studies to test for and characterise genetic integration of behaviour, physiology and life-history traits would provide a useful route to understanding the evolution of personality.

Table 1: Estimated repeatabilities of behavioural traits (conditional on fixed effects) assayed in open field and emergence trials. Estimates are from univariate models with standard errors in parentheses.

Trait	Repeatability	$\chi^2_{0,1}$	P
Emergence Time	0.33 (0.12)	9.37	0.001
Track Length	0.31 (0.12)	6.84	0.005
Activity	0.37 (0.12)	9.32	0.001
Order Caught	0.27 (0.07)	66.4	<0.001
Area Covered	0.46 (0.11)	21.8	<0.001
Time in Middle	0.42 (0.12)	14.4	<0.001

Table 2: Among individual variance-covariance-correlation matrix from the final multivariate model incorporating all behavioural traits, size and growth showing variances (V_I, diagonal), covariances (COV_I, lower triangle) and correlations (r_I, upper diagonal) with standard errors in parentheses. Note since (transformed) data were scaled to standard deviation units V_I for behavioural traits (but not Length and Growth) can be interpreted as a repeatability (but not conditioned on fixed effects). * denotes statistical significance at α=0.05 based on likelihood ratio tests of parameter in univariate (for variances) or bivariate (for covariances) mixed models (see supplemental table 3).

	Emergence time	Track Length	Activity	Order Caught	Area Covered	Time in Middle	Length	Growth
Em	0.328*	0.157	0.181	0.197	-0.296	-0.327	0.436	0.205
	(0.152)	(0.320)	(0.307)	(0.287)	(0.278)	(0.297)	(0.231)	(0.313)
TI	0.052	0.337*	0.967*	0.070	0.216	-0.756*	0.315	0.225
	(0.108)	(0.161)	(0.022)	(0.281)	(0.280)	(0.158)	(0.238)	(0.330)
Act	0.067	0.363*	0.418*	0.282	0.253	-0.772*	0.324	0.145
	(0.116)	(0.168)	(0.182)	(0.250)	(0.263)	(0.143)	(0.224)	(0.330)
Order Caught	0.059	0.021	0.096	0.277*	0.073	-0.176	0.383	-0.026
	(0.090)	(0.086)	(0.094)	(0.091)	(0.254)	(0.262)	(0.187)	(0.289)
AC	-0.107	0.079	0.104	0.024	0.402*	0.384	0.200	-0.508*
	(0.107)	(0.115)	(0.123)	(0.087)	(0.151)	(0.261)	(0.225)	(0.240)
TIM	-0.114	-0.267*	-0.303*	-0.056	0.148	0.370*	-0.091	-0.294
	(0.111)	(0.133)	(0.142)	(0.085)	(0.110)	(0.153)	(0.242)	(0.316)
Length	0.208	0.152	0.174	0.168	0.106	-0.046	0.692*	0.223
	(0.132)	(0.127)	(0.135)	(0.099)	(0.124)	(0.124)	(0.197)	(0.248)
Growth	0.024	0.027	0.019	-0.003	-0.067*	-0.037	0.039	0.043*
	(0.039)	(0.041)	(0.045)	(0.031)	(0.039)	(0.043)	(0.045)	(0.017)

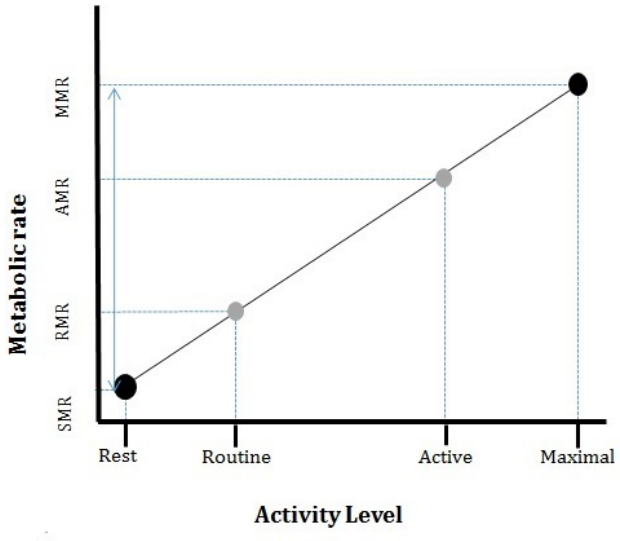


Figure 1: Metabolic scope (MS) is defined as the difference between standard metabolic rate (SMR) and maximal metabolic rate (MMR) (blue arrow) but can equally be determined as the slope of a reaction norm (black line) between resting and maximal activity states (black circles). Here we use observations of routine metabolic rate (RMR) and active metabolic rate (AMR) made at intermediate activity levels (grey circles) to infer the reaction norm slope.

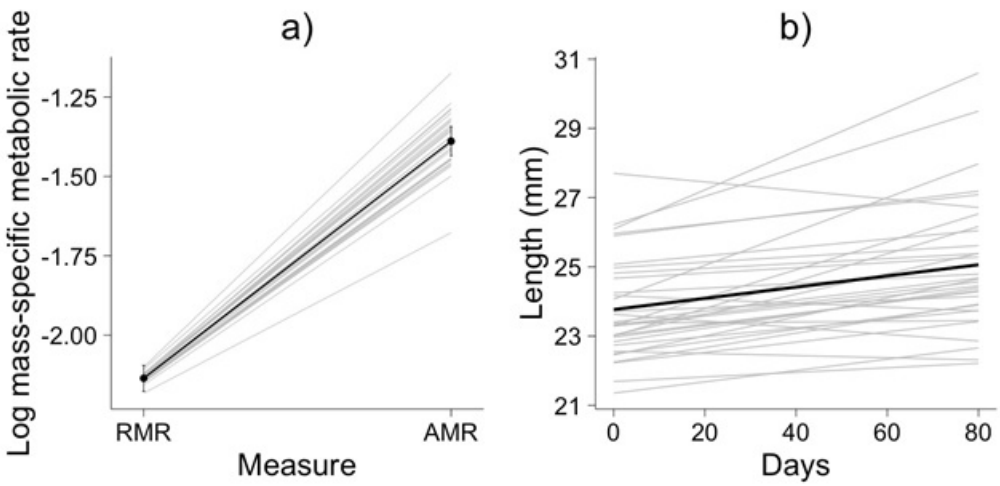


Figure 2: Metabolic traits (a) and *standard length* as a function days since the start of the experiment (b). Black lines show (a) the predicted mean metabolic reaction norm between activity state specific means (± SE) and (b) mean growth trajectory. Grey lines indicate reaction norms and growth lines for each individual as predicted by the mixed model analysis.

Chapter 3

Maternal and genetic effects on personality over ontogeny in the Trinidadian guppy, *Poecilia reticulata*.

3.1 Abstract

Among-individual variation in behaviour is a widespread phenomenon, with several frameworks developed to explain its existence. One under-studied source of behavioural variation is maternal effects, which can have significant influence over evolutionary processes. Maternal effects are not necessarily static however, since their importance can change over offspring ontogeny, typically declining with age relative to additive genetic effects. Here, using a quantitative genetic approach, we test the prediction that maternal effects will influence age-specific risk-taking behaviour in Trinidadian guppies, *Poecilia reticulata*. Individuals were subject to a single open field trial as juveniles and up to 4 repeat trials as adults, with 5 traits indicative of risk-taking behaviour measured in each trial. We then partitioned phenotypic variance into additive genetic (V_A) and maternal identity (V_M) components, in addition to testing brood size and maternal weight as specific sources of maternal traits. We found that V_M had significant influence over juvenile traits, with very low V_A estimates. Whereas all adult traits were significantly heritable, with little support for V_M. We also found a strong influence of maternal traits on juvenile behaviours as predicted, with significant, albeit smaller, effects found in adults. Maternal weight was heritable and itself subject to maternal effects, thus, maternal weight is a likely source of maternal genetic effects that are expected to alter response to selection on personality in this system. More generally our study highlights that while maternal effects can be an important source of personality variation, this varies over ontogeny of offspring.

3.2 Introduction

Among-individual variation in behaviour, or personality, has been well documented in a large number of animal species. No longer considered as simply noise around the mean, there have been multiple adaptive frameworks developed to try to explain the maintenance of personality variation. These frameworks include frequency dependent selection (Wolf et al. 2008), fluctuating selection (Dingemanse et al. 2004; Le Coeur et al. 2015), pace of life syndrome (Biro and Stamps 2008; Réale et al. 2010) and state dependent feedback loops (Luttbeg and Sih 2010; Sih et al. 2015). Although there is some empirical support for each of these, it is not clear that a single explanation will apply to all cases. Furthermore, since linear and/or stabilising selection is expected to erode genetic, but not necessarily environmentally induced variance, adaptive explanations for behavioural variation require a significant genetic basis to this variation in the first instance. While evidence for additive genetic variation underpinning personality is now growing, few studies have considered the potential role of maternal effects in driving among-individual differences. Here we seek to address this gap, by evaluating maternal effects as both a potential cause of bias and a further source of evolutionarily significant variation in a study of age-specific personality in the Trinidadian guppy, *Poecilia reticulata*.

Personality traits such as boldness and aggression have been linked to survival and reproductive success (Smith and Blumstein 2008; Ariyomo and Watt 2012). Given this association with fitness-related traits, if personality traits exhibit sufficient additive genetic variation then they have the potential for evolution. However, we might predict that – at least where selection is linear and/or stabilising – genetic variance for personality should diminish over time (Falconer

and Mackay 1996; Kruuk et al. 2008). Despite this expectation of reduced variation due to selection, genetic variation in personality traits has been quantified in a range of taxa including fish (Dingemanse et al. 2012; Ariyomo et al. 2013), birds (Drent et al. 2003; Brommer and Kluen 2012) and mammals (Brent et al. 2014; Johnson et al. 2015; Petelle et al. 2015). A recent review of published studies concluded that the average heritability of personality traits was as high as 0.52 (Dochtermann et al. 2015). This estimate is perhaps potentially misleading as additive genetic variance estimates were scaled by among-individual phenotypic variance only (which logically follows the definition of personality variation as being among-individuals, but means within-individual behavioural variation from plasticity and/or measurement error is excluded). Nonetheless, evidence of genetic variance underpinning personality traits is certainly growing, and it is in this context that explanations have been sought for the maintenance of consistent among-individual differences in behaviour.

While quantitative genetic studies have largely sought to test the additive genetic basis of variation, additional factors are known to influence development and/or expression of personality, including aspects of the social environment (Moretz et al. 2007; Piyapong et al. 2010; King et al. 2015), abiotic variables such as temperature (Biro et al. 2010; Briffa et al. 2013) and availability of food or other resources (Dingemanse et al. 2004; Le Coeur et al. 2015). Here we consider maternal effects as a potential source of variation in behaviour. Maternal effects occur when the maternal phenotype influences the offspring phenotype, above and beyond the normal inheritance of genes (Mousseau and Fox 2008). This can occur through a range of pathways, such as provisioning of food and types of parental care (Reznick et al. 1996a; Hunt and Simmons 2002; D'Amore et al.

2015), or exposure to maternal hormones during development (Tobler and Sandell 2007; Groothuis et al. 2008; Rokka et al. 2014; Hinde et al. 2015). Although some maternal effects on offspring behaviour are known (Storm and Lima 2010; Taylor et al. 2012), most studies have focussed on physiology (Bacigalupe et al. 2007; Tobler et al. 2007), life-history (Hunt and Simmons 2002; Bashey 2006) and growth (Wilson et al. 2005).

Despite maternal effects having thus far remained an understudied source of among-individual variation in behaviour, they can be important for our understanding of the evolution of personality traits for two major reasons. First, failing to consider maternal effects can result in upwardly biased estimates of heritability (h^2) and so to over-estimate responses to selection (Falconer and Mackay 1996; Kruuk 2004; Wilson et al. 2010). Secondly, maternal effects can themselves have a significant genetic (among-mother) basis of variation, with important consequences for the evolutionary dynamics of offspring traits. For instance, maternal genetic effects can cause time-lagged responses to selection, even if the offspring trait itself has little or no additive genetic basis (Räsänen and Kruuk 2007). Furthermore, correlations between maternal genetic and additive genetic effects can either constrain or facilitate the response of offspring traits to selection (Kirkpatrick and Lande 1989; Räsänen and Kruuk 2007; Charmantier et al. 2013). Although maternal genetic effects on personality have received little attention to date, their presence is actually implicit in ideas such as ‘adaptive priming’, in which maternal effects are viewed as having evolved to increase offspring fitness by priming their behaviour for an anticipated local environment (Reddon 2011; Mainwaring and Hartley 2013; Rokka et al. 2014).

Maternal effects can thus be a source of offspring behavioural variation and can act to alter their evolutionary trajectories, yet the strength of these effects can change over the ontogeny of offspring (Arriero et al. 2013; Andree et al. 2015; Houde et al. 2015; Van Leeuwen et al. 2015). Previous studies have shown that as individuals grow and mature, the relative importance of environmental and additive genetic variance components often tends to increase at the expense of maternal effects (Wilson and Réale 2005; Lindholm et al. 2006; Dibattista et al. 2009). In light of this, a more complete picture of how maternal effects influence personality traits requires such effects to be measured at multiple points in the offspring's life.

Here, we test the importance of maternal and additive genetic effects on risk-taking behaviours expressed during an open field trial (OFT) and whether this changes over ontogeny in *P. reticulata.* This species provides an ideal model as it is easily bred in captivity (facilitating a quantitative genetic approach), while differential yolk provisioning of eggs is a known source of maternal effects on offspring size/growth (Reznick et al. 1996a; Bashey 2006). Here, we ask whether maternal effects might contribute to among-individual variation in juvenile risk-taking behaviour. If so, we go on to ask how such effects change as offspring reach maturity. In addition, we test whether this maternal effect on offspring personality is mediated by offspring size, given the prevalence of maternal effects on size in this and other fish species (Einum and Fleming 1999; Bashey 2006; Leblanc et al. 2014; Murphy et al. 2014) and the link between size and boldness traits (Brown and Braithwaite 2004). In doing so, we build on the results of our previous study which demonstrated that risk-taking behaviours, putatively indicative of shy-bold type personality variation and behavioural stress 'coping

style', are repeatable in this population and can be classed as personality traits (White et al. 2016).

Using an animal model framework, we test for maternal effects arising specifically from maternal weight (at offspring birth) and brood size. These traits are tested because we expect them to provide insight into likely among-female variation in resource allocation. We also estimate non-specific maternal effects (i.e. arising from unknown aspects of maternal phenotype) and additive genetic effects using a standard variance partitioning approach. We predict, firstly, that maternal effects on risk-taking behaviour will be present (such that failure to model them will lead to inflated h^2 estimates). Secondly, that the relative importance of maternal and additive genetic effects will change across ontogeny, with the former being less important for determining adult offspring personality. And thirdly, these maternal effects will be mediated, in part, through direct impacts on offspring size that in turn have consequences for behaviour. Finally, we test for genetic variance in two suspected sources of maternal effects, female weight and brood size. If these traits are both heritable and a source of maternal effects, it follows that they are a source of maternal genetic effects expected to have important consequences for the evolutionary dynamics of personality.

3.3 Materials and methods

3.3.1 Fish husbandry and breeding

Fish used were from a captive population of *P. reticulata* maintained at the University of Exeter, Penryn campus fish facility. The population is descended from wild fish caught in 2008 from the lower Aripo River, Trinidad (ca. 18-24 generations ago) and has been maintained at an effective population size of

several thousand, with no deliberate selection or inbreeding. Data was obtained for 653 juvenile and 831 adult guppies, spread across a 3 generation pedigree (Parental, F1 and F2) using a paternal half-sib breeding design. See Appendix 2 for details of the breeding methodology, associated husbandry and visualisation of the pedigree structure.

Juvenile fish were initially kept in full-sib family groups, with each family housed in a 2.8l tank. These fish were untagged, so identification of individuals was not possible. All juvenile family groups were kept on a single water supply to prevent tank effects arising from water chemistry differences. Note however that family sizes were not reduced to a common standard such that maternal brood size directly determines early life density. To the extent that early rearing density influences individual behaviours, our estimation of maternal brood size effects (see below) will therefore integrate across pre-natal and post-natal effects. One week after the juvenile open field trial (see below), all juveniles were moved to 15L “grow on tanks”, still in family groups.

At an average age of 132 days (range 59-226), the now mature fish were tagged with visible implant elastomer (under anaesthetic, using a buffered solution of MS222) for individual identification, and transferred to mixed family groups of size 16 - 8 males and 8 females. Variation in age is controlled for in all models of behaviours (see statistical methods below) and arose because groups were necessarily established sequentially as sufficient fish from multiple families reached a size at which tagging was deemed a safe procedure for the animals. Thus each adult group comprised a mix of mature fish available from all broods in which individuals are sufficiently large enough to tag. By mixing fish among

families in this way we reduce the potential for common environment effects to upwardly bias the maternal and/or genetic parameters estimated.

3.3.2 Phenotyping of fish

At an average age of 49.8 days (range 35-55) each untagged individual from each brood was subject to a single Open Field Trial (OFT; described further below) in what constitutes the juvenile measure. One week after tagging, all F1 adult fish experienced 4 repeat OFTs over a two-week period (with at least 48 hours between trials). For F2 fish, 4 behavioural trials were also conducted over a two-week period but we performed only 2 OFT per individual. These were alternated with two 'emergence trials' similar to those described in (White et al. 2016), the data from which are not included in the present study. F1 fish therefore had one juvenile OFT measure and 4 adult OFT measures. F2 individuals had one juvenile measure and 2 adult measures.

OFT data were also collected on the parental generation of fish prior to beginning the breeding program (again, four repeats separated by a minimum of 48 hours over a two-week period). Note that the age of the parental generation fish was unknown (but all were mature adults as inferred from external morphology). The temperature of the OFT tank water was measured at the end of each behavioural trial allowing subsequent statistical control for variation around the mean of 23.7°C. Additionally, standard length (measured from snout to caudal peduncle, mm) and weight of each fish was recorded after each trial before fish were returned to their group housing.

Open field trials

We followed the OFT methodology described by the previous chapter (White et al. 2016). Briefly, an individual fish was introduced to an empty arena (30cm x 20cm x 20cm tank filled to a depth of 5cm and lit from below). Using a digital camera and Viewer software (www.biobserve.com), fish were then tracked over a 4 minute 30 second period (after 30 seconds acclimation period). From the tracking data we extracted the total distance swum (cm) by the focal fish (henceforth *TI*), the percentage of time spent active, which we defined as moving at >4 cm s^{-1} (henceforth *Act*), the percentage of the tank floor area that was explored during the trial (henceforth *AC*), the number of times velocity dropped below 4 cm s^{-1} for more than 2.5 seconds (henceforth *Fr*) and the amount of time spent in the inner, putatively 'risky', zone of the tank (henceforth *TIM*). For the last of these the floor area of the tank was partitioned into middle and outer zones of equal size using the Viewer software. Water in the OFT tank was replaced between each group, and any effect of chemical cue build up is controlled for statistically (see statistical methods). Note, the OFT is a standard approach for quantifying among-individual behavioural variation (or personality), in small fishes (Oswald et al. 2013; Boulton et al. 2014), including guppies (Burns 2008; Diaz Pauli et al. 2015). The traits measured in the present study have been found to all effectively assay a shy/bold type axis of behavioural variation in the sheepshead swordtail *Xiphophorus birchmanni*, a species closely related to the guppy (Boulton et al. 2014). A somewhat different pattern was found in a previous study of this population. All traits are repeatable (a prerequisite for heritability) and putatively bolder (or risk-prone) fish tend to be more exploratory and spend more time in the inner zone. However, *TI* and *Act* also appear to capture variation in behavioural stress response (or "coping style") that does not quite conform to

predictions made under a simple shy-bold continuum (White et al. 2016). We therefore refer to the assayed traits collectively as risk-taking behaviours here.

3.3.3 Statistical methods

Univariate mixed models for each of the 5 OFT traits were fitted to both juvenile and adult data sets using a restricted maximum likelihood (REML) framework in ASReml-R (Butler et al. 2009). *Fr* and *TIM* in both adult and juvenile data were square root transformed to better meet assumptions of homoscedasticity and normality of residuals. All traits were then mean centred and rescaled to standard deviation units prior to analysis to allow direct comparison of variance components for each trait. Conditional F statistics were used for ascertaining significance of fixed effects. For variance components, we assumed a χ^2 statistic to be equivalent to twice the difference in log-likelihood between full and reduced models with degrees of freedom equivalent of the number of parameters being tested. A 50:50 mix of χ^2_0 and χ^2_1 (henceforth $\chi^2_{0,1}$) is also assumed when testing a single variance component, as recommended by (Visscher 2006).

Estimating additive genetic and maternal effects over ontogeny

For each age-specific trait we partitioned the phenotypic variance (V_P) into components attributable to maternal effects and additive genetics. Maternal effects were estimated using the "hybrid" strategy suggested in (McAdam et al. 2013) in which we: i) fitted the maternal traits of *brood size* and *maternal weight* at offspring birth (and their interaction) as fixed effects to test the hypothesis that these maternal traits affect personality (in addition to known effects on growth and life-history; Shikano and Taniguchi 2005, Bashey 2006); and, ii) included a random effect of *maternal identity* to capture variance in maternal 'performance'

for offspring behaviour (V_M). Both *maternal weight* and *brood size* were mean centred and transformed into standard deviation units (*maternal weight*, mean= 0.45g, sd=0.13; *brood size* mean=17.21, sd=6.65). Additive genetic variance (V_A) was estimated by including a random effect of individual identity linked to the pedigree following a standard maternal effect animal model formulation (Wilson et al. 2010), with a permanent environment effect (V_{PE}) included for adult traits to account for repeat measures on individuals. A housing group effect (V_{GROUP}) was also included in the adult models representing the social and 1 environment experienced by each individual.

In both juvenile and adult models, *temperature*, *age, order caught* and *generation* were fitted as fixed effects to control for sources of variance not relevant to our hypotheses. *Temperature* and *age* were modelled as continuous linear effects. *Order caught* is the order in which fish were caught from their home tank prior to the OFT and controls for among-individual variation in disturbance and any build-up of chemical cues in the OFT tank over the course of measuring a brood/group. Differences between the breeding protocol and housing between the parental, F1 and F2 generations are controlled for with the categorical *generation* fixed effect.

The adult models had an additional fixed effect of *repeat*, to control for potential habituation to the OFT procedure over the repeat measures. Note that while sexual dimorphism in behaviour is likely, sex was known in adults only, so in order to allow direct comparison between juvenile and adult results we present results from models that do not to include a fixed effect of sex at the adult life stage. This is appropriate to hypotheses being tested, with model parameter estimates thus interpretable as averaged across sexes in both juveniles and adults. We refitted

adult models with a fixed effect of sex to confirm there was no qualitative difference in conclusions (results not shown).

Narrow sense heritabilities ($h^2=V_A/V_p$) were calculated for juveniles and adults, and maternal identity effects were similarly standardised to a proportion of total phenotypic variance ($m^2 = V_M / V_P$). In all cases phenotypic variance was defined conditional on fixed effects and calculated as the sum of the estimated variance components. For each trait we estimated h^2 and m^2 under the 'full' model (including fixed effects as described below), but also compared the fit of this model to a 'null' that included neither additive nor maternal identity effects, and two intermediate models containing additive or maternal identity effects only. Model comparisons were based on likelihood ratio tests where models were nested and AIC where they were not.

Does offspring length mediate maternal effects on offspring behaviour?

In order to test whether maternal effects influence offspring risk-taking behaviour through offspring size, we refitted the above full models for juveniles and adults with an additional fixed effect of offspring standard length. If maternal effects on offspring behaviour are present and mediated by impacts on offspring size or growth, then we expect a) significant effects of standard length (SL) on behaviour and b) reduced support for maternal trait effects with inclusion of length as a predictor in the model.

Estimating maternal genetic effects

Finally, given our hypothesis that maternal effects on offspring behaviour could arise through causal dependence on maternal weight and/or brood size we tested

these traits for both (among-female) heritable variation and maternal effects. The former is of interest since, if these traits do causally influence offspring behaviour, then heritable variation in them will be a source of maternal genetic effects. The latter is potentially important because cascading maternal effects (*sensu* Mcglothlin and Galloway 2013) are expected if maternal effects on offspring are mediated by traits that themselves have a maternal influence. We fitted an animal model of *female weight* using all available measures of adult females and a fixed effect of age (as a cubic function to allow for non-linear growth) in addition to the mean. Random effects as described above were used to partition variance into V_A, $V_{M,}$ V_{PE} and V_R. The *Brood size* model was similar but we included *female weight* as a fixed covariate, enabling us to condition our estimates on the known increase in fecundity with female size (Reznick 1983). This model therefore tests for genetic variance in *Brood size* after accounting for female body size.

3.4 Results

3.4.1 Additive genetic and maternal effects on offspring behaviour over ontogeny

Model comparisons provided strong evidence for among-family variance consistent with additive genetic and/or maternal identity effects across all traits in juveniles and adults. Comparison of model likelihoods (shown in Table 1) indicates that the full ($V_A + V_M$) model is a significantly better fit than the null model in every case (χ^2_2 ranges from 13.6 to 69.9, all P=<0.001; Supplemental table 2.1). In juveniles, the V_M effect is significant for *Tl*, *Act*, *AC* and *Fr* (Tl χ^2_1=8.17 P=0.002, Act χ^2_1=7.78 P=0.003, AC χ^2_1=4.04 P=0.022, TIM χ^2_1=2.62 P=0.053, Fr χ^2_1=4.31 P=0.019). While neither the V_A nor V_M effects were significant in *TIM,*

AIC scores indicate the preferred model (i.e. lowest AIC) is V_M-only for this and all traits. The estimate of V_A is bound to zero in all full models and there is no change in log likelihood by dropping the V_A effect for any trait (Table 1). While unclear for *TIM*, this does suggest that maternal effects are the main driver of variation in the other traits at the juvenile stage.

For adult traits, the V_A-only model is clearly the preferred model for all but one trait. For *TI*, the V_M-only model is preferred to the V_A-only model (ΔAIC = 5.2) but is only marginally better than the full model (ΔAIC = 0.2). We thus conclude maternal identity effects are important for *TI* in adults. For *AC*, TIM and *Fr*, the estimate of V_M is bound to zero in the full model (resulting in no improvement of log-likelihood). For these traits it is therefore clear that the among-family variance is largely driven by additive genetic effects, the preference for the V_A-only model being reflected by ΔAIC $\geq$ 2 for all other models (Table 1).

Given the expectation that dropping either V_A or V_M could lead to upward bias of the retained component, we elected to estimate h^2 and m^2 from the full model for all traits (while acknowledging this necessarily means greater uncertainty on all parameter estimates; Table 2). Indeed, omitting V_M leads to much higher (and statistically significant) heritability estimates for juvenile traits (range from 0.173-0.615; Supplemental table 2.2) when compared to the full model (zero for all juvenile behaviours). In adults, V_M was bound to zero in 3 of the 5 traits in the full model (Table 2) and there is a pattern of m^2 being higher in juveniles (range 0.081-0.254, median=0.170) than in adults (range 0.00-0.10, median=0.00). Where V_M=0, dropping the maternal identity has no impact on estimated heritability. In adult *TI* and *Act*, heritability is increased by dropping the maternal

identity effects (as in the juvenile traits, though to a much lesser extent; supplemental table 2.2).

Although not directly relevant to our primary hypothesis we also note that *post hoc* testing of adult traits indicated that among-group variance was significant for all adult traits (potentially indicative of social effects on behaviour). Additionally, permanent environment effects accounted for 10-26% of phenotypic variance in adult traits (Table 2), highlighting the importance of further (but currently unknown) sources of among-individual behavioural differences.

We find support for significant maternal effects mediated by *maternal weight*, *brood size* and/or their interaction on all juvenile behaviours (Fig. 1, Table 3). Juvenile offspring born to heavier mothers, on average, have a significantly shorter *TI* and a non-significant trend towards lower activity (Table 3). Juveniles from larger broods covered more tank area. For *TIM*, there was a significant interaction between brood size and maternal weight. Visualising the predictions from this model shows that while *maternal weight* has no effect on juvenile *TIM* at an average brood size, the predicted relationship is negative for small *brood sizes* and weakly positive for large ones (Fig. 1).

In adults, there was a significant positive effect of *maternal weight* on *area covered*, while *brood size* negatively predicted *TI* and *Act* (Table 3). Adult *activity* is subject to a significant interaction between *maternal weight* and *brood size* (with maternal weight positively predicting activity for small broods but negatively for the largest ones; Fig. 1). Overall, these maternal effects show a clear tendency of being stronger in juveniles compared to adults (i.e. tendency for smaller effect

size estimates in adult traits; Table 3). Moreover, in a qualitative sense the maternal trait(s) that significantly influence each observed behaviour differs between juveniles and adults (Table 3). For completeness, estimates of all other fixed effects from the full models can be found in Supplemental table 2.3.

3.4.2 Offspring length mediates maternal effects on offspring behaviour

In additional models, length had a positive effect on *Tl* and *Act* and a negative effect on *TlM* and *Fr* in juveniles. Similarly, in adults, *Tl* and *Act* were positively influenced while both *AC* and *TlM* were negatively influenced by offspring length (see Table 3). However, while this suggests relationships between risk-taking behaviour and size and/or growth, for juvenile behaviours, the inclusion of length as a predictor did not notably reduce the estimated effects of *maternal weight* or *brood size* (in fact, effect sizes estimates increased in a number of cases; Table 3). For adult *Tl* and *Act*, however, the addition of length to the model resulted in a large drop in the effects size of *brood size*. This suggests that maternal brood size effects on adult behaviour may well be mediated by intermediate effects on size.

3.4.3 Maternal genetic and grand-maternal effects

Meaningful testing for heritable variation and/or maternal identity effects for the *brood size* maternal trait was not possible due to insufficient numbers of broods from females with known parentage themselves. However, the animal model analysis of *maternal weight* indicated that both additive genetic and maternal identity effects are major components of variance in this trait (h^2=0.62 (0.06), $\chi^2_{0,1}$=107.26, P=<0.001; m^2= 0.30 (0.07), $\chi^2_{0,1}$=74.36, P=<0.001), while the permanent environment effect was bound to zero.

3.5 Discussion

Here we estimated maternal and additive genetic effects on offspring risk-taking behaviour in the guppy, and asked whether the importance of these two sources of among-individual variation changes over ontogeny. We found that both additive genetic and maternal effects were present, and while the latter did persist into adulthood, they were more important determinants of juvenile behaviour. Our analysis suggests that maternal effects on offspring behaviour arise, at least in part, from variation in maternal weight and brood size, and are in some instances mediated by offspring size. In addition, we show that maternal weight is both heritable and subject to maternal effects itself. Below we discuss the ontogenetic patterns in maternal and additive genetic effects in more detail, before further considering the consequences of genetic variance in maternal weight. We place our results in the context of the wider quantitative genetics literature, and discuss their implications for understanding the evolutionary dynamics of personality in this species.

3.5.1 Maternal and additive genetic effects both contribute to variation in risk-taking behaviour

We found that maternal effects for offspring risk-taking behaviour are present in this population of guppies. This was evidenced by estimates of the maternal identity variance component and by the estimated effects on offspring behaviour of maternal weight and brood size. Heritabilities were estimated at zero for juvenile behaviours and were low to moderate for adult OFT traits, relative to those published in the personality literature (van Oers et al. 2005; Dingemanse et al. 2009; Niemelä et al. 2013; Petelle et al. 2015). We highlight that, for juvenile traits, heritability estimates made in the assumed absence of maternal identity

effects were much higher than those from the full models since almost all among-family variance was partitioned as additive. For adult traits, V_M accounted for a much smaller proportion of total phenotypic variance in the full models (discussed further below). Accordingly, h^2 estimates were not increased as much by assuming an absence of maternal identity effects. More generally, these results demonstrate the point that failing to account for maternal effects in animal models can upwardly bias estimates of additive genetic variance(Falconer and Mackay 1996; Kruuk 2004; Wilson et al. 2010; Mcglothlin and Galloway 2013). To date, few studies of personality have explicitly tested for maternal effects, and the possibility exists that our emerging view of additive genetic contributions to behavioural variation is biased.

Changing importance of maternal and additive genetic effects over ontogeny

Our results are consistent with the prediction made that maternal effects on offspring traits will decrease with offspring age. While acknowledging that separation of V_M and V_A can be problematic in some data structures, under the full model m^2 estimates for each trait were higher than for the corresponding adult behaviours (for which the V_M explained very little variance in all but *TI*). A clear pattern of declining maternal effects with age is also seen in the effects of maternal weight and brood size on offspring behaviour, which are consistently stronger in juveniles than adults. This matches the general pattern of age-related declines in maternal effects in the literature. For instance, Houde et al. (2013) found that maternal effects on survival declined during development from egg to fry stages in Atlantic salmon (*Salmo salar*). Similarly, maternal effects decline with age for body size in *Poecilia parae* (a close relative of the Trinidadian guppy; Lindholm et al. 2006) and the lemon shark (*Negaprion brevirostris*; (Dibattista et

al. 2009), while maternal identity explains more variation in pathogen resistance in younger than in older whitefish (*Coregonus palaea*) (Clark et al. 2014).

Despite this general pattern, some maternal effects were detected on adult behaviours. Interestingly, there was little qualitative correspondence between the specific maternal traits that significantly influenced behaviour in juveniles vs. adults. For example, maternal weight significantly affected juvenile but not adult *TI*, while *AC* was affected by *brood size* in juveniles but *maternal weight* in adults. This suggests that not only does the overall maternal influence on offspring behaviour wane over ontogeny, but that age-specific maternal effects could arise through different pathways.

As well as declining maternal effects, we predicted that additive genetic contributions to behavioural variation would increase with age. This pattern is well documented for a range of trait types in the literature (Atchley and Zhu 1997; Houle 1998; Wilson and Réale 2005; Lindholm et al. 2006) and is also supported in our study. More specifically, our estimates of h^2 clearly uphold the quantitative prediction and we do note that statistical support for additive genetic variance is only present in adult behaviours. More generally, and while not directly relevant to current hypotheses, our analysis also shows that a lot of among-individual variance described previously by us and others in these OFT traits is explained by neither additive nor maternal effects. The source of this behavioural variation is unknown, and we have controlled as much as possible for shared environment using common water supplies and identical tanks for each family/group. Nonetheless, among-individual variance can arise from uncontrolled (and unmodelled) aspects of the physical environment or potentially from the social

environment (Lindholm et al. 2006; Moretz et al. 2007; Krause et al. 2010; Piyapong et al. 2010). In fact, the *Group* random effect is significant for all traits in adults, consistent with the latter being an important determinant of behaviour here.

3.5.2 Offspring length as a mediator of maternal effects

Given known maternal effects on offspring size and growth in guppies (Reznick et al. 1996a; Bashey 2006) and the widely reported size-dependence of personality (Brown and Braithwaite 2004; Rödel and Meyer 2011; Biro and Sampson 2015), size provides a plausible link between maternal traits and the offspring behaviours they influence. Somewhat consistent with this hypothesis, we did find that adding length as a fixed predictor led to large decreases in the estimated effect of brood size on *TI* and *Act* in adults. We also note that, in accordance with earlier studies (Reznick et al. 1996a; Bashey 2006), offspring born into larger broods are on average smaller at birth and when measured as juveniles (results not shown). However, while length significantly predicted four of the five juvenile behaviours and all of the adult traits, its inclusion as a covariate did not, with the two exceptions noted above, result in a decrease to maternal effect estimates. This indicates that maternal effects on behaviour may be mediated through offspring growth in some cases, but that additional pathways (for instance hormonal transfer – Rokka et al. 2014, Hinde et al. 2015) are also involved.

3.5.3 Maternal genetic and grand-maternal effects on risk-taking behaviour

As discussed above, our analyses indicate maternal weight and brood size to be significant sources of maternal effects on offspring behaviour. Furthermore, we

found that maternal weight has a significant additive genetic component of variance, and is thus expected to generate maternal genetic effects (McAdam et al. 2013). In the presence of maternal genetic effects, offspring personality traits will respond not just to direct selection on them, but also to any selection on the maternal trait (in this case weight) in the previous generation (Kirkpatrick and Lande 1989). Covariance between additive and maternal genetic effects can also occur, potentially constraining phenotypic evolution and maintaining genetic (and therefore phenotypic) variation in both maternal and offspring traits (Kirkpatrick and Lande 1989; Wilson et al. 2005; Räsänen and Kruuk 2007). Thus the presence of maternal genetic effects alters expectations for evolutionary change relative to those based on direct selection alone. For instance, McAdam and Boutin (2004) showed that failing to account for selection on litter size (the maternal trait) in the red squirrel (*Tamiasciurus hudsonicus*) led to a predicted change in offspring size that was five times lower than the observed rate.

In the present case, the relationship between risk-taking behaviour and fitness is unknown so it is difficult to comment on the extent of direct selection on them in wild populations. However, selection on female (maternal) weight is expected. Like many fish species, female guppies exhibit indeterminate growth, with fecundity increasing as a function of size (Bronikowski et al. 2002). Also, when given the choice, male guppies will choose to mate with larger females (Dosen and Montgomerie 2004; Herdman et al. 2004). Thus, we can at least speculate that the evolution of personality traits in guppies will depend on selection on size through maternal fitness, highlighting another putative mechanism by which morphological and behavioural traits may co-evolve.

Finally, not only is maternal weight heritable, but we found evidence that it is itself subject to maternal effects, manifest as a significant estimate of V_M. Accepting that maternal weight does causally influence offspring behaviour, this actually implies the possibility of grandmaternal effects on personality (Mcglothlin and Galloway 2013). This implies that patterns of variation and selection in the grandmaternal generation could have knock on effects on current generation behaviours via the maternal generation. In *Drosophila*, both maternal and grand-maternal age influenced offspring viability and in the spider mite (*Tetranychus urticae*), offspring dispersal distance is affected by the density that both maternal and grand-maternal generations experienced (Hercus and Hoffmann 2000; Bitume et al. 2014). Very few studies outside of domestic animal breeding have looked into grand-maternal effects, however, owing to the difficulty in collecting multigenerational pedigree data and none to our knowledge have looked at personality in this regard.

3.6 Conclusion

In conclusion, we found that both additive genetic and maternal effects are important determinants of risk-taking behaviour traits in guppies, although the former are only evident in adult fish. Not accounting for the maternal effects resulted in much higher h^2 estimates in some cases, raising the possibility that current estimates for personality traits are upwardly biased. Robust evidence of additive genetic variance was found for adult traits but maternal effects are also present, though with generally smaller effect sizes than in juveniles. In contrast our models did not provide statistical support for additive variance on juvenile behaviours. Rather our results indicate among family variance arises principally from maternal identity effects, as well as maternal effects occurring via variation

in maternal weight and brood size. Moreover, the specific maternal traits influencing offspring behaviour differed between juveniles and adults, suggestive of a shift in the mechanism through which maternal effects influence behaviour over ontogeny. Offspring size is a plausible candidate trait for mediating maternal effects on behaviour in some cases but not all. Our study highlights the benefit of employing the hybrid approach for estimating maternal effects at different stages over offspring ontogeny, and of using animal models to estimate both the additive genetic structure and maternal effects for personality traits. We suggest that wider efforts to characterise maternal effects, and especially to test their genetic basis, could greatly benefit our understanding of the evolutionary dynamics of animal personality.

Table 1: Comparison of null, V_A only, V_M only, and full (V_A+V_M) models for all juvenile and adult traits. Shading denotes the preferred model in each case as determined by minimum AIC score. ΔAIC is the difference in AIC between every model with the preferred model.

Trait	Juvenile				Adult			
	Model	AIC	ΔAIC	Loglik	Model	AIC	ΔAIC	Loglik
TI	null	357.99	45.4	-178.00	null	1485.6	36.4	-739.8
	V_A	320.77	8.2	-158.38	V_A	1454.4	5.2	-723.2
	V_M	312.60	0.0	-154.30	V_M	1449.2	0.0	-720.6
	V_A+V_M	314.60	2.00	-154.30	V_A+V_M	1449.4	0.2	-719.7
Act	null	380.73	52.4	-189.37	null	1885.7	39	-939.8
	V_A	336.07	7.8	-166.04	V_A	1846.7	0.0	-919.4
	V_M	328.29	0.0	-162.15	V_M	1859.8	13.1	-925.9
	V_A+V_M	330.29	2.0	-162.15	V_A+V_M	1847.6	0.9	-918.8
AC	null	691.96	67.9	-344.98	null	2096.3	19.4	-1045.1
	V_A	628.10	4.0	-312.05	V_A	2076.9	0.0	-1034.4
	V_M	624.06	0.0	-310.03	V_M	2095.4	18.5	-1043.7
	V_A+V_M	626.06	2.0	-310.03	V_A+V_M	2078.9	2.0	-1034.4
TIM	null	720.80	14.6	-359.40	null	2048.5	11.6	-1021.2
	V_A	707.44	1.2	-351.72	V_A	2036.9	0.0	-1014.5
	V_M	706.23	0.0	-351.12	V_M	2050.2	13.3	-1021.1
	V_A+V_M	708.23	2.0	-351.12	V_A+V_M	2038.9	2.0	-1014.5
Fr	null	529.82	33.9	-263.91	null	2317.9	25.1	-1155.9
	V_A	500.19	4.3	-248.10	V_A	2292.8	0.0	-1142.4
	V_M	495.88	0.0	-245.94	V_M	2314.5	21.7	-1153.3
	V_A+V_M	497.88	2.0	-245.94	V_A+V_M	2294.8	2.0	-1142.4

Table 2: Estimated variance components and their corresponding ratios to phenotypic variance (conditional on fixed effects). Estimates were made under the full model for each juvenile and adult behaviour and standard errors are shown in parentheses (but note where parameters were bound to zero no SE is estimatable).

Trait	V_A	V_M	V_{PE}	V_{Group}	V_R	h^2	m^2	pe^2	$Group^2$
Juvenile									
Tl	0.000	0.096	-	-	0.469	0.000	0.170	-	-
	(-)	(0.033)			(0.028)	(-)	(0.049)		
Act	0.000	0.134	-	-	0.474	0.000	0.220	-	-
	(-)	(0.043)			(0.028)	(-)	(0.057)		
AC	0.000	0.257	-	-	0.756	0.000	0.254	-	-
	(-)	(0.077)			(0.045)	(-)	(0.059)		
TIM	0.000	0.080	-	-	0.910	0.000	0.097	-	-
	(-)	(0.037)			(0.053)	(-)	(0.039)		
Fr	0.000	0.113	-	-	0.634	0.000	0.151	-	-
	(-)	(0.040)			(0.037)	(-)	(0.047)		
Adult									
Tl	0.056	0.079	0.215	0.043	0.423	0.068	0.097	0.263	0.053
	(0.045)	(0.037)	(0.034)	(0.019)	(0.014)	(0.055)	(0.042)	(0.042)	(0.023)
Act	0.164	0.021	0.182	0.023	0.504	0.184	0.023	0.204	0.026
	(0.055)	(0.023)	(0.040)	(0.014)	(0.017)	(0.058)	(0.026)	(0.046)	(0.015)
AC	0.167	0.000	0.114	0.155	0.587	0.163	0.000	0.111	0.151
	(0.050)	(-)	(0.037)	(0.045)	(0.020)	(0.046)	(-)	(0.038)	(0.038)
TIM	0.158	0.000	0.237	0.026	0.534	0.165	0.000	0.248	0.027
	(0.056)	(-)	(0.044)	(0.015)	(0.018)	(0.055)	(-)	(0.048)	(0.016)
Fr	0.202	0.000	0.093	0.021	0.662	0.206	0.000	0.096	0.022
	(0.054)	(-)	(0.039)	(0.013)	(0.022)	(0.051)	(-)	(0.041)	(0.013)

Table 3: Estimated effects of brood size (BS, number of fish) and maternal weight (MW, g) and their interaction on offspring behaviours at juvenile and adult stages. All estimates come from full (i.e. V_A+V_M) models. * denotes significant effect (P=<0.05), boldness indicates maternal fixed effect that differed in significance between the full model and model extended with offspring length (SL).

			Full model				Full model plus offspring standard length			
	Trait	Fixed effect	Effect size	DF	F	P	Effect size	DF	F	P
Juv	Tl	**BS**	**0.062 (0.052)**	**1, 188.7**	**0.92**	**0.338**	**0.231 (0.057)**	**1, 257.8**	**14.68**	**<0.001***
		MW	-0.118 (0.052)	1, 57.3	4.79	0.033*	-0.161 (0.051)	1, 55.1	9.11	0.004*
		BS-MW	-0.032 (0.042)	1, 110.3	0.58	0.447	-0.050 (0.041)	1, 104.9	1.53	0.219
		SL	-	-	-	-	0.236 (0.039)	1, 603.7	37.70	<0.001*
	Act	**BS**	**0.035 (0.055)**	**1, 208.0**	**0.08**	**0.779**	**0.239 (0.060)**	**1, 279.3**	**13.86**	**<0.001***
		MW	**-0.114 (0.057)**	**1, 57.9**	**3.63**	**0.062**	**-0.168 (0.055)**	**1, 55.6**	**8.31**	**0.006***
		BS-MW	-0.042 (0.045)	1, 122.8	0.88	0.351	-0.066 (0.043)	1, 116.6	2.34	0.129
		SL	-	-	-	-	0.286 (0.039)	1, 612.1	54.75	<0.001*
	AC	BS	0.198 (0.072)	1, 237.1	11.08	0.001*	0.204 (0.081)	1, 320.5	9.25	0.003*
		MW	0.020 (0.076)	1, 64.6	0.04	0.834	0.019 (0.077)	1, 65.0	0.03	0.855
		BS-MW	0.035 (0.058)	1, 141.4	0.369	0.545	0.035 (0.059)	1, 140.6	0.35	0.555

		SL	-	-	-	-	0.008 (0.051)	1, 616.6	0.03	0.869
	TIM	**BS**	**-0.057 (0.064)**	**1, 141.8**	**0.01**	**0.917**	**-0.226 (0.073)**	**1, 199.7**	**5.56**	**0.019***
		MW	-0.025 (0.059)	1, 51.7	0.54	0.466	0.015 (0.058)	1, 49.9	0.02	0.901
		BS-MW	0.103 (0.049)	1, 72.6	4.37	0.040*	0.119 (0.048)	1, 68.1	6.08	0.016*
		SL	-	-	-	-	-0.237 (0.053)	1, 564.2	20.22	<0.001*
	Fr	**BS**	**-0.075 (0.059)**	**1, 177.5**	**1.90**	**0.170**	**-0.156 (0.067)**	**1, 243.1**	**5.96**	**0.015***
		MW	0.077 (0.058)	1, 55.6	1.76	0.190	0.096 (0.057)	1, 54.7	2.73	0.104
		BS-MW	0.001 (0.047)	1, 102.1	<0.01	0.982	0.010 (0.046)	1, 95.7	0.05	0.831
		SL	-	-	-	-	-0.120 (0.046)	1, 596.0	6.89	0.009*
Adult	TI	**BS**	**-0.070 (0.050)**	**1, 217**	**4.31**	**0.039***	**-0.008 (0.050)**	**1, 229.4**	**0.617**	**0.433**
		MW	0.057 (0.49)	1, 64.6	1.53	0.220	0.060 (0.049)	1, 65.9	1.707	0.196
		BS-MW	-0.042 (0.038)	1, 166	1.24	0.268	-0.048 (0.037)	1, 173.6	1.664	0.199
		SL	-	-	-	-	0.173 (0.026)	1, 1028.8	43.160	<0.001*
	Act	**BS**	**-0.055 (0.048)**	**1, 194.5**	**5.46**	**0.021***	**0.004 (0.049)**	**1, 202.9**	**1.104**	**0.295**
		MW	0.023 (0.044)	1, 65.2	0.35	0.555	0.030 (0.044)	1, 65.6	0.559	0.457
		BS-MW	-0.079 (0.036)	1, 130.9	4.69	0.032*	-0.084 (0.036)	1, 135.9	5.489	0.021*
		SL	-	-	-	-	0.170 (0.028)	1, 992.4	36.500	<0.001*

AC	**BS**	**-0.091 (0.046)**	**1, 616.1**	**2.04**	**0.150**	**-0.127 (0.047)**	**1, 576.2**	**4.915**	**0.027***
	MW	0.085 (0.041)	1, 454.0	4.23	0.040*	0.078 (0.040)	1, 413.9	3.633	0.057
	BS-MW	0.053 (0.034)	1, 576.6	2.48	0.116	0.055 (0.033)	1, 538.8	2.801	0.095
	SL	-	-	-	-	-0.108 (0.028)	1, 939.1	15.080	<0.001*
TIM	**BS**	**-0.038 (0.048)**	**1, 436.7**	**0.12**	**0.732**	**-0.131 (0.046)**	**1, 351.2**	**6.447**	**0.012***
	MW	0.005 (0.042)	1, 300.0	0.02	0.897	-0.025 (0.039)	1, 222.6	0.414	0.520
	BS-MW	0.039 (0.036)	1, 425.5	1.23	0.269	0.043 (0.033)	1, 304.0	1.728	0.190
	SL	-	-	-	-	-0.253 (0.029)	1 1028.7	74.360	<0.001*
Fr	BS	0.013 (0.046)	1, 563.6	1.66	0.198	-0.001 (0.046)	1, 476.6	0.660	0.417
	MW	0.045 (0.041)	1, 529.0	1.21	0.272	-0.029 (0.040)	1, 493.5	0.500	0.480
	BS-MW	0.065 (0.034)	1, 637.0	3.75	0.053	0.055 (0.034)	1, 603.2	2.719	0.100
	SL	-	-	-	-	-0.037 (0.029)	1, 892.8	1.610	0.205

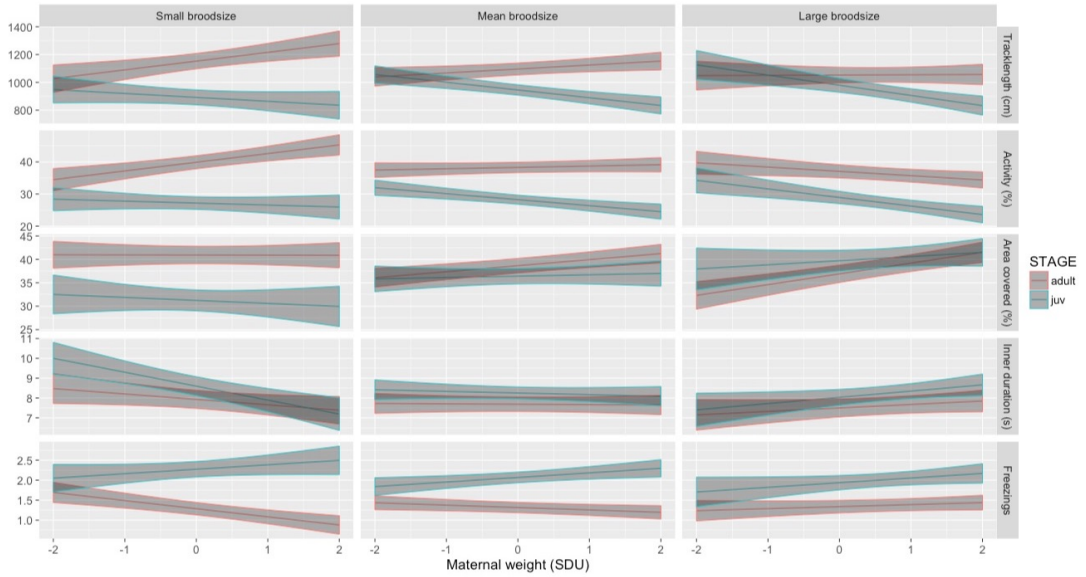


Figure 1: Predicted relationships between the 5 OFT traits in juveniles and adults and *Maternal weight* (in SDU) at small brood size (n=5), mean brood size (n=17.21) and large brood size (n=25). Shaded area indicates ± one standard error around the predicted relationship. Traits are shown in observed units except for Freezings, which are square root transformed. Maternal weight is in standard deviation units

Chapter 4

Sexual dimorphism and Genotype-by-Sex interactions of personality in the Trinidadian guppy, *Poecilia reticulata*

4.1 Abstract

Traits expressed in both sexes are likely to share a common genetic architecture. Yet the prevalence of sexual dimorphism in nature suggests that this sexual conflict can be resolved. Under sexually antagonistic selection, mechanisms are expected to evolve that reduce this conflict, resulting in Genotype-by-sex (GxS) interactions. While sexual dimorphism in behaviour and animal personality have been identified in a number of species, few studies have assessed the extent of shared genetic architecture across the sexes. Here, we assess the extent of sexual dimorphism in four risk-taking personality traits in the Trinidadian guppy, *Poecilia reticulata*, and apply a multivariate quantitative genetics approach to test for genotype-by-sex interactions. Specifically, we compared sex-specific genetic (co)variance matrices ($\mathbf{G_m}$ and $\mathbf{G_f}$) and tested for asymmetry of the cross-trait covariance matrix (**B**). As there is a clear sexual dimorphism in size, we also quantify the among-individual and genetic covariances between personality and size and growth and whether this differs between the sexes. We found significant sexual dimorphism in three of the four behaviours. r_{mf} values were significantly different from +1 in two cases, suggesting scope for future dimorphism evolution in these traits. While the variance structure of $\mathbf{G_m}$ and $\mathbf{G_f}$ were not significantly different from each other, we did find a large angle between male and female $\mathbf{G_{max}}$, which would also indicate scope for future multivariate dimorphism. Finally, one component of the **B** matrix was asymmetric across the diagonals, the

majority were symmetrical, however. From a single trait perspective, personality traits lack the sex-specific genetic architecture for future dimorphism to evolve. An expanded multivariate method has the potential to reveal additional avenues of constraint or divergence.

4.2 Introduction

Numerous adaptive models have been described to explain consistent among-individual variation in behaviour, or animal personality (Dingemanse and Wolf 2010; Wolf and Weissing 2010). But in order for behavioural variation to be adaptive, it must have a significant heritable basis. There is an increasing number of studies utilising a quantitative genetics approach to estimate the genetics of personality traits (van Oers et al. 2005), with low to moderate heritabilities found for behaviours such as boldness, aggression and sociality (Drent et al. 2003; Brommer and Kluen 2012; Ariyomo et al. 2013; Petelle et al. 2015). Confirmation of the genetic basis of personality is just the first step in assessing the evolutionary potential of personality, however, with more complex aspects of the genetic architecture to consider before we get a reliable interpretation of whether genetic variation is adaptive and maintained by selection.

Generally, traits under selection should evolve in a manner dependent on the genetic variance present, the genetic covariance structure with other traits and the strength of selection (Lande, 1979, Walsh & Blows, 2009). While males and females are often under sexually antagonistic (SA) selection (Reeve and Fairbairn 2001; Olsson et al. 2002; Cox and Calsbeek 2009; McPherson and Chenoweth 2012), traits expressed in both sexes are likely to share a common genetic architecture (Poissant et al. 2010). Although this shared architecture can

result in conflict and thus evolutionary constraint, the prevalence of sexual dimorphism across taxa and traits suggests that sexual conflict can, at least in part, be resolved (Cox and Calsbeek 2009). Indeed persistent SA selection is itself expected to favour mechanisms that reduce intra-locus sexual conflict, allowing the sexes to diverge towards their respective fitness optima (Lande, 1980, Rhen, 2000, Bonduriansky & Chenoweth, 2009). These mechanisms can include sex-linkage, sex-limited trait expression, sex-specific genetic modifiers and genomic imprinting (Rhen, 2000, Day & Bonduriansky, 2004, Fairbairn & Roff, 2006, Bonduriansky & Chenoweth, 2009). However, at the whole genome level, assessing the extent to which SA selection provides scope for further dimorphism requires determining the extent to which genetic variance is shared between the sexes.

Quantitative genetics provides several tools with which to test for and estimate genotype-by-sex (GxS) interactions, the presence of which implies that sex-limited genetic variance may facilitate conflict resolution and allow the divergence of the sexes (Wyman et al. 2013). The cross-sex genetic correlation (r_{mf}) between homologous male and female traits is most commonly used to quantify the extent of sex-specific genetic variance, where

$$r_{mf} = \frac{COV_{Amf}}{\sqrt{V_{Am}\, V_{Af}}} \quad (1)$$

V_{Am} and V_{Af} are the sex-specific (additive) genetic variances and COV_{Amf} is the cross-sex genetic covariance. Typically, an r_{mf} of +1 is viewed as maximally constraining for sex-specific adaptation under SA selection as any increase in fitness of one sex will result in a reduction in fitness of the other sex

(Bonduriansky & Chenoweth, 2009, Wyman *et al.*, 2013). Note r_{mf} =+1 does not imply an absolute constraint on trait evolution, as selection responses also depend on the magnitude of sex-specific additive genetic variances (V_{Am}, V_{Af}) which need not be equal when r_{mf} =+1. Only in the complete absence of GxS does it follow that both r_{mf} = 1 and V_{Am}=V_{Af} (Boulton et al. 2016).

Assessing GxS interactions on a trait by trait basis in this manner, while computationally and technically straightforward, gives a restricted view of trait evolution. This is because natural selection acts on suites of traits simultaneously, and many of these will be genetically correlated (Lande & Arnold, 1983, Walsh & Blows, 2009). Multivariate approaches that account for this among-trait genetic covariance structure in the form of a **G** matrix are therefore required (Lande, 1979, Blows, 2007, Walsh & Blows, 2009). In the context of understanding sexual dimorphism, one method has been to estimate sex-specific **G** matrices (subsequently $\mathbf{G_f}$ and $\mathbf{G_m}$) and compare them, using techniques such as eigen vector analysis. For instance, if $\mathbf{G_f}$ and $\mathbf{G_m}$ differ in orientation and/or magnitude of their leading eigen vectors ($\mathbf{G_{max}}$), then continued phenotypic divergence can be possible, even if homologous traits have high pairwise r_{mf} (Jensen *et al.*, 2003, Campbell *et al.*, 2010, Wyman *et al.*, 2013). Conversely, if the orientation of sex-specific $\mathbf{G_{max}}$ are similar, then this can constrain divergence between the sexes (Leinonen *et al.*, 2011, Wyman *et al.*, 2013).

Building on this multivariate approach, it is possible to further define a block matrix, $\mathbf{G_{mf}}$, that contains $\mathbf{G_m}$ and $\mathbf{G_f}$ as well as the cross-sex, cross-trait covariance submatrix (**B**). This latter matrix can reveal avenues for constraint or divergence between the sexes not detectable in the sex-specific **G** matrices alone

(Gosden *et al.*, 2012, Wyman *et al.*, 2013). The multivariate breeder's equation can then be modified to take into account SA selection (Lande 1980), such that

$$\begin{pmatrix} \Delta\overline{\boldsymbol{Z}}_{\boldsymbol{m}} \\ \Delta\overline{\boldsymbol{Z}}_{\boldsymbol{f}} \end{pmatrix} = \frac{1}{2} \begin{bmatrix} \boldsymbol{G}_{\boldsymbol{m}} & \boldsymbol{B} \\ \boldsymbol{B}^{\boldsymbol{T}} & \boldsymbol{G}_{\boldsymbol{f}} \end{bmatrix} \begin{pmatrix} \boldsymbol{\beta}_{\boldsymbol{m}} \\ \boldsymbol{\beta}_{\boldsymbol{f}} \end{pmatrix} \quad (2)$$

where $\Delta\overline{\boldsymbol{Z}}_{\mathbf{m}}$ and $\Delta\overline{\boldsymbol{Z}}_{\mathbf{f}}$ are the sex-specific vectors of predicted response for a set of traits and the $\boldsymbol{\beta}_{\mathbf{m}}$ and $\boldsymbol{\beta}_{\mathbf{f}}$ represent vectors of sex-specific (linear) selection gradients. The ½ coefficient accounts for both parents making equal genetic contributions to offspring of both sexes and $\mathbf{G}_{\mathbf{mf}}$ is the block matrix (shown in square brackets in equation 2) containing submatrices $\mathbf{G}_{\mathbf{m}}$, $\mathbf{G}_{\mathbf{f}}$, **B** as defined above (Lande 1980). Note that **B** may be asymmetric (i.e. the components above and below the diagonal in **B** are not equal, or $\mathbf{B} \neq \mathbf{B}^{\mathbf{T}}$), since for two traits (x and y), there is no expectation that $COV_{A(x.m,\ y,f)}$ should equal to $COV_{A(x.f,\ y,m)}$. Asymmetry in **B** therefore leads to predictions of unequal multivariate responses between the sexes (Steven *et al.*, 2007, Lewis *et al.*, 2011, Gosden *et al.*, 2012, Berger *et al.*, 2014).

Despite the availability of this multivariate framework, most empirical quantitative genetic studies of sexual dimorphism to date have focussed on single traits (but see work on insect models by Gosden *et al.*, 2012, Reddiex *et al.*, 2013, Berger *et al.*, 2014). Furthermore, GxS studies have most commonly been conducted on fitness (Chippindale et al. 2001; Brommer et al. 2007; Foerster et al. 2007), morphological (Steven *et al.*, 2007, Leinonen *et al.*, 2011, Potti & Canal, 2011, Gosden *et al.*, 2012) and life-history (Lewis et al. 2011) traits. Thus, while studies including average sex differences in personality traits are widespread (Aragón, 2011, Gyuris *et al.*, 2011, Koski, 2011, Mainwaring *et al.*, 2011), few also assess

the presence of GxS interactions and the potential for further dimorphism to evolve (Long & Rice, 2007, Berger *et al.*, 2014). This may be due, in part, to the inherent difficulty in measuring behaviour on the large number of individuals required for quantitative genetic analysis. Here, we aim to fill this gap by assessing the extent of GxS interactions for a suite of behaviours putatively indicative of risk-taking behaviour in the guppy, *Poecilia reticulata*.

One of the adaptive frameworks developed to explain among-individual variation in behaviour is the Pace of life syndrome. It states that behaviour should coevolve with physiology and life-history traits along a slow-fast axis (Réale et al. 2010). While our previous study measuring risk-taking behaviour, growth and metabolic rate in guppies did not support the pace of life model (White et al. 2016), this study was only conducted on one sex and did not assess the genetic associations between the trait. As strong size dimorphism is already well-known in the guppy, and size-personality associations are widely reported (Brown and Braithwaite 2004; but see (Harris et al. 2010), we compare the sex-specific among-individual and genetic correlations between risk-taking behaviour and growth.

In our lab population of guppies, derived from a high-predation site in the Aripo River (Trinidad), risk-taking behaviours are known to be significantly repeatable (White et al. 2016) and heritable in adults (White and Wilson, *Submitted*) and there is clear sexual dimorphism in post-maturity size and growth rate. Although we do not estimate selection in the current study, SA selection for risk-taking behaviour is expected in this species, with the degree of conflict likely to be mediated by predation risk. Males can increase reproductive success by being highly mobile, moving between shoals to find females (Griffiths & Magurran,

1998, Kelley *et al.*, 1999, Croft *et al.*, 2003a, b). We therefore expect male guppies to benefit from risk-taking behaviours through increased access to females. Godin and Dugatkin (1996) also found evidence that females preferred to mate with bolder males (as measured by approach distance to a predator). In contrast, risk-taking is expected to be selected against in females. When alone and away from a shoal, predation risk is high for females, with their larger size making them an energetically rewarding meal (Magurran 2005). High shoal fidelity and tighter shoaling behaviour in females reduces predation mortality risk and increases feeding efficiency (Griffiths & Magurran, 1998, Magurran & Garcia, 2000, Magurran, 2005, Richards *et al.*, 2010).

The aims of this study are twofold. Firstly, we assess the extent of sexual dimorphism for repeatable, risk-taking behaviours. We test the prediction that males will exhibit (on average) more risk-prone or 'bold' behaviours, before testing for dimorphism in the multivariate phenotypic (among-individual) covariance structure itself (i.e. do males and females differ in the extent or structure of (co)variation in risk-taking behaviour?). Secondly, we test for GxS interactions using both single-trait analyses and the fully multivariate approach outlined above. While our principal focus is on risk-taking behaviours, we also expand our analyses to include size and growth traits, noting that these are known *a priori* to exhibit strong dimorphism in guppies, and that risk-taking behavioural variation has been generally linked to body size across many taxa (Réale *et al.*, 2010, Wilson *et al.*, 2013).

4.3 Materials and methods

4.3.1 Husbandry and data collection

Data used here are derived from a larger quantitative genetic study and have been used in the previous chapter (all behavioural data, some size data) in a study of maternal effects across ontogenetic stages (White and Wilson n.d.). Since breeding design, general husbandry, and behavioural data collection have been described fully elsewhere (see White and Wilson, *Submitted*), they are described only briefly here (See Appendix 2).

Fish came from 81 known full-sib families nested within paternal half-sibships that were produced between April 2013 and July 2015. To produce families, parental individuals were haphazardly sampled from a captive wild-type population (originally descended from a 2008 collection at a high-predation site in the upper Aripo river, Trinidad) at the University of Exeter, Penryn campus fish facility. After initial rearing in family groups, adult fish (average age 132 days) were tagged using visible implant elastomer (anaesthetised in buffered MS222) and put into mixed family groups of 16 (8 males, 8 females). Mixing individuals from different families during development reduces the risk of common environment effects biasing additive genetic (co)variance estimates. This was not possible until here as small size preclude safe tagging.

Each adult fish underwent 4 open field trails (OFTs) over the course of two weeks. Each OFT comprised transferring a fish into an empty tank filled to 5cm depth with water. Movement was tracked for 4 minutes 30 seconds (following a 30 second acclimation period) using Viewer software (www.biobserve.com) and a

camera positioned above the tank. Four traits were extracted for analysis, *Activity* (*Act,* percent of the time the focal fish moved at more than 4cm s^{-1}), *area covered* (*AC*, the total percentage of the tank explored/visited by the fish), *time in middle* (*TIM*, total time spent in the inner zone away from tank walls) and *freezings* (*Fr*, the total number of times a fish' movement falls below 4cm s^{-1} for more than 2 seconds). This testing paradigm is widely used to assay "boldness" or risk-taking behaviour in fishes with the *a priori* expectation that risk-prone fish will be consistently more active and exploratory, freeze less often, and be less thigmotaxic (spend less time near the edges), although this pattern is only partly seen in this species (White et al. 2016). Order within group and water temperature (mean of 23.7°C) at the end of each behavioural trial were recorded for allowing statistical control for any variation. Water in the OFT tank was changed between groups. Standard length (henceforth *SL*, measured from snout to caudal peduncle in mm) measures were taken at tagging, at each OFT, and one month after the last behavioural trial. For a subset of fish, we opportunistically collected additional size data on known age individuals at monthly intervals for up to 13 months after the last OFT. This was not possible in all cases as tanks housing groups were required for other projects in the facility. A total of 2594 behavioural trials and 4493 body size measurements were collected on 831 adults (502 females, 329 males) in a 3 generation pedigree structure.

4.3.2 Statistical methods

Behavioural traits *Act*, *AC*, *TIM* and *Fr* were mean centred and rescaled into standard deviation units (using overall, rather than sex-specific, means and standard deviations). For *TIM* and *Fr* this was done after a square-root transformation to reduce positive skew and increase normality of residuals.

Scaling to overall standard deviation units allows better comparison of parameters among traits and facilitates convergence of multivariate mixed models while still preserving within-trait differences across sexes (in mean and/or variance). We denote traits by subscript m or f, when referring to male or female values specifically (e.g. Act_m, Act_f etc).

Data were analysed using linear mixed effect models fitted using restricted maximum likelihood in ASreml version 4 (www.vsni.co.uk). Conditional F statistics were used to test for significance of fixed effects where pertinent to biological hypotheses (e.g. to test for trait dimorphism). Note, however, that in most cases fixed effects were included principally to control for potential sources of variance not directly relevant to our hypotheses. In all behavioural models, fixed effects included *temperature* (of the tank water taken following each OFT), *age* (in days), *repeat* (a 4 level factor to control for habituation to the OFT arena over the 4 repeat trials), *order caught* (the order in which fish were caught from their home tank prior to the OFT, fitted as a continuous covariate) and *generation* (a 3 level categorical effect to control for any differences in husbandry and rearing among the generations of the pedigree, see White and Wilson, *Submitted*).

Significance of random effect (co)variance components was assessed using likelihood ratio test (LRT) comparisons of nested models, with twice the difference in log-likelihoods assumed to be χ^2 distributed with degrees of freedom equal to the number of parameters being tested. Random effects of *group* (a 40 level categorical effect to account for environmental and social sources of variation among home tanks) and *fish ID* were fitted to all traits in all models unless otherwise stated. To estimate genetic (co)variance parameters we used

animal models (Kruuk, 2004, Wilson *et al.*, 2009) allowing the partition of the among-fish (co)variance into additive genetic and permanent environment components. We assume an absence of maternal (identity) effects, noting that our previous study (White and Wilson, *Submitted*) showed maternal variance was non-significant for *Act* and bound to zero for all other OFT traits in these adult fish. Although previous analyses do suggest statistically significant effects of maternal weight and natal brood size on adult behavioural traits, their effects sizes are low (particularly relative to impacts on juvenile behaviour) and their omission here has little impact on the sex-specific covariance structures.

To model growth rate, we fitted random regressions of standard length over age in mixed model and animal model formulations, resulting in estimates of among-individual and additive genetic variation in both length (at average age) and growth. This reaction norm approach fits a random-by-covariate effect, allowing each level of a random effect to vary across a covariate and is an established technique in both behavioural and life-history studies (Nussey *et al.*, 2007, Dingemanse *et al.*, 2010, Roff & Wilson, 2014). In all length/growth models, fixed effects of *generation* and continuous effects of *age*, age^2 and age^3 were fitted, the latter to allow a curvilinear average relationship between length and age.

4.3.3 Sexual Dimorphism

Univariate models

To ascertain whether our traits were dimorphic on average, we fitted univariate mixed models for each behaviour and for the length/growth random regression (sexes pooled), with an additional fixed effect of *sex*. A significant sex effect coefficient ($P<0.05$) was considered evidence of average trait dimorphism. We

refitted the behavioural models with *SL* as an additional covariate to determine whether average differences between the sexes in behaviour could, at least in principle be explained entirely by size effects.

We fitted a series of models to test for sexual dimorphism in the variance components of observed traits (as opposed to their means). For each trait (X), we fitted bivariate mixed models with X_m and X_f as responses in which we allowed variance components of interest to differ between males and females, and compared the model log-likelihood to the corresponding fit with homogeneous variance imposed. This was done firstly with no random effects (i.e. just residual variance), allowing test for heterogeneity of total phenotypic variance between sexes for behavioural traits and length. Note it is not possible to estimate the total phenotypic variance of growth from the random regression framework used here, therefore this particular comparison was not done. Models including *fish ID* and *group* as random effects were then fitted to test for differences in among-fish variance (*Group* was fitted to control for among-group variation). LRTs were used to compare the unconstrained vs constrained (homogeneous variance across sexes) models on 1 degree of freedom (DF) for the behavioural traits and 3 DF for the length random regression.

Multivariate models

We then employed a multivariate approach to ask whether the **I** matrix (among-individual (co)variance matrix) of OFT behaviours differs significantly between the sexes. We fitted a multivariate model with all 8 sex-specific behaviours allowing estimation of $\mathbf{I_m}$ and $\mathbf{I_f}$ sub-matrices (noting that cross-sex terms are not statistically identifiable since every individual is either male or female) and

compared this to a refitted model in which we imposed the condition that $\mathbf{I_m} = \mathbf{I_f}$. For a more qualitative comparison, eigen vector decomposition was applied to the estimates of $\mathbf{I}_m$ and $\mathbf{I}_f$ matrices to see if the major axes of among-individual variation were broadly similar in males and females. More specifically, any differences in trait loadings on the first eigen vector ($\mathbf{I_{max}}$) were noted as well as the angle between $\mathbf{I_{max}}$ (the first eigen vector of **I**) in males and females.

Among-individual association between personality and size

We sought to determine whether phenotypic associations between behaviour and size and/or growth differed between the sexes. Further expansion of the multivariate behavioural model to include male and female *SL* as additional responses proved difficult, so we estimated the among-individual covariances (and corresponding correlations) with each sex-specific behaviour using a series of bivariate models. Statistical inference was by LRT comparison to constrained models in which among-individual covariance between behaviour and both size (random intercept for length) and growth (random slope) were fixed to zero.

4.3.4 Quantitative genetic analyses

Univariate models

Previous analysis of the OFT data with univariate animal models has shown all behaviours are significantly heritable in adults (pooled sexes, White and Wilson, *Submitted*) but did not test the covariance structure or estimate sex-specific parameters. For each trait we fitted bivariate animal models to estimate the genetic variance of the sex-specific sub-traits (V_{Am} and V_{Af}) and genetic correlation between them (r_{mf}). This was then compared to a model in which GxS interactions was assumed absent ($V_{Am} = V_{Af}$, $r_{mf} = +1$). We also compared

likelihood of model fits to two intermediate models: one where sex-specific V_A were constrained to be equal but r_{mf} was free to be <+1, and a second with r_{mf} constrained to be +1 but sex-specific V_A free to vary. Since these intermediate models are not nested, we also used AIC values from each model for additional comparison.

Multivariate models

Cross-sex multivariate animal models were fitted with the 8 sex-specific OFT sub-traits. First we compared the sex-specific **G** matrices without estimating the cross-sex, cross-trait terms, such that we estimated $\mathbf{G_{mf}}$ as:

$$\boldsymbol{G_{mf}} = \begin{bmatrix} \boldsymbol{G_m} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{G_f} \end{bmatrix} \quad (3)$$

This model was compared to one in which we impose the condition that $\mathbf{G_m = G_f}$ (using a LRT at 10 df). As in our comparison of $\mathbf{I_m}$ and $\mathbf{I_f}$, we also subjected the sex specific-submatrices to eigen vector decomposition to facilitate a qualitative comparison of trait loadings and also the angle between $\mathbf{G_{max}}$ of males and females. We then fitted the full multivariate model including all cross-sex cross-trait terms such that

$$\boldsymbol{G_{mf}} = \begin{bmatrix} \boldsymbol{G_m} & \boldsymbol{B} \\ \boldsymbol{B^T} & \boldsymbol{G_f} \end{bmatrix} \quad (4)$$

As noted earlier, asymmetry of the upper and lower diagonals of the sub-matrix **B** can offer additional opportunities for sexual divergence under sex-specific selection as well as constraint. Ideally, we would have compared the log-likelihood of our full multivariate model to a constrained fit in which symmetry of

B was imposed. We were, however, unable to obtain a stable model convergence with the latter constraint imposed. Therefore, to test for symmetry we calculated an estimate of $\mathbf{B} - \mathbf{B}^T$ as a square matrix, denoted as $\Delta\mathbf{B}$, noting that if **B** is symmetrical, then $\mathbf{B} - \mathbf{B}^T = \Delta\mathbf{B} = 0$. In order to generate approximate 95% confidence intervals on each element of $\Delta\mathbf{B}$ we performed a 5000 draw parametric bootstrap on the $\mathbf{G}_{mf}$ matrix (following the general approach outlined in Boulton et al. 2014), implemented within the R statistical environment (R core team, 2016), estimating $\Delta\mathbf{B}$ for each draw. It is important to note that this matrix bootstrapping procedure assumes multivariate normality.

Genetic association between personality and size

As we were unable to expand the multivariate animal model further to include size/growth as well as the 8 behaviours, we fitted a series of bivariate animal models between each sex-specific behaviour and length (again, modelled as a first order random regression of age for both additive and permanent environment effects). This was to determine whether behaviour-length/growth associations differed between males and females at the genetic level. As with the corresponding phenotypic analysis, the significance of genetic covariance with size/length was determined for each sex-specific behaviour using LRT and genetic covariances were standardised to correlations for easier interpretation.

4.4 Results

4.4.1 Sexual dimorphism

Univariate models

Visual inspection of raw data shows broadly overlapping distributions of male and female behavioural trait observations (Figure 1). Nonetheless, univariate

dimorphism models indicate that, conditional on other effects, all OFT traits except *Fr* differed significantly, on average, between the sexes. Females have significantly higher *activity* than males, but cover less tank *area* and spend less *time in the middle* zone (Table 1). As expected, sexual dimorphism is also present in *length* with females being larger on average (Figure 1, Table 1) and showing a steeper growth trajectory than males (Figure 2). We note that with the addition of the covariate of *length* to the behavioural models, it is apparent that the dimorphism in *Act* could, at least in principle, be explained by size-dependence and coupled with the larger average size of females (Supplemental table 3.1). Bivariate mixed models indicate significantly more total phenotypic variation (conditional on fixed effects) for *TIM* in males (χ^2_1=9.68, P=0.002) and for *length* in females (χ^2_1=1409.36, P=<0.001; Figure 1 & 2). For the other behaviours we found no evidence against the null hypotheses of homogeneous phenotypic variance (*Act* χ^2_1= 1.04, P= 0.308, *AC* χ^2_1=0.92, P= 0.337, *Fr* χ^2_1= 0.64, P= 0.424; Figure 1). Partitioning of sex-specific phenotypic variance into its among- and within-individual components showed there is evidence of more among-individual variance in females than males for *length*/*growth* (χ^2_3=199.2, P=<0.001), but the sex-specific estimates of V_I are very similar for each OFT trait (*Act* χ^2_1= 0.254, P=0.614, *AC* χ^2_1=1.22, P=0.269, *TIM* χ^2_1=0.088, P=0.767, *Fr* χ^2_1= 0.16, P=0.689).

Multivariate models

Sex-specific behavioural **I** matrices do not differ significantly from each other (χ^2_{10}= 10.62 P=0.388, Supplemental table 3.2). The first two eigen vectors account for 64% and 26% of the behavioural variance in males and 60% and 31% in females, respectively. There is little difference between the sexes in how observed behaviours load onto these first two eigen vectors (Table 2a). For

instance, in both sexes $\mathbf{l_{max}}$ describes an axis of among-individual behavioural variation along which *Act* loads antagonistically to *TIM* and *Fr*. The angle between sex-specific estimates of $\mathbf{l_{max}}$ is 5.70˚, indicating very close alignment (on the scale from perfectly aligned at 0 ˚ to perfectly orthogonal at 90˚).

Among-individual association between personality and size

There is support for among-individual covariance between OFT behaviours and standard length (modelled as comprising size at average age and growth rate) with patterns being at least qualitatively different between the sexes. *AC* is the only male behaviour to significantly covary with length (Table 3, see Supplemental table 3.3 for statistical inference), being negatively correlated with size at average age (weakly) and growth (moderately). In females, significant length-behaviour covariances are found for *Act*, *Tim* and *Fr. Length at* average age and *growth* are both positively correlated with *Act* and negatively so with *Fr* (Table 3). *TIM* was weakly correlated negatively with length at average size but more strongly positively correlated with growth.

4.4.2 Quantitative genetic analyses

Univariate models

Bivariate animal models of individual pairs of sex-specific homologous traits provided strong evidence for GxS interactions for two of the five traits. The full GxS model was a significantly better fit than the constrained (no GxS) model for *Length/growth* (χ^2_7= 61.92 P= <0.001) and *TIM* (χ^2_2=14.97, P= <0.001) but not the other behaviours (*Act* χ^2_2= 3.91 P= 0.141, *AC* χ^2_2= 3.18 P= 0.204, *Fr* χ^2_2= 0.70 P= 0.705). LRT comparison between the full and intermediate models also suggests that only Act and TIM had an r_{mf} significantly differ from +1. In no

behavioural trait did the full model differ significantly from one that assumes equality of sex-specific V_A (Supplemental table 3.4). Further comparison using AIC provides a slightly more nuanced picture (Table 4). The no GxS model was only preferred (lowest AIC) for *Fr*, while for *Act*, *AC* and *TIM* it was the intermediate model with homogeneous V_A but departure from r_{mf} =+1 allowed that was preferred. Although we note in these behavioural traits ΔAIC to at least one other model was <2, the fully unconstrained model (full GxS) is clearly the best fit for *length/growth*, with large ΔAIC between this and all other constrained models (Table 4). Overall, there was strong support for GxS interactions for *length/growth* and *TIM,* only weak support for GxS interaction in *Act* and *AC* and no support for GxS interactions in *Fr*.

Multivariate models

When modelled as sex-specific behaviours we found no evidence of overall significant differences between $\mathbf{G}_f$ and $\mathbf{G}_m$ (χ^2_{10}= 6.78 P=0.746). While reiterating the lack of significant matrix differentiation, visual inspection of these two sub-matrices of our $\mathbf{G}_{mf}$ estimate (Table 5, red and green matrices) is suggestive of more additive genetic variation in male *TIM* and a larger negative *Act-TIM* correlation. Conversely, in females there is a larger positive *Act-AC* correlation. Eigen vector decomposition of $\mathbf{G}_f$ and $\mathbf{G}_m$ shows that the first ($\mathbf{G}_{max}$) and second eigen vectors explain 54% and 40% in males and 68% and 27% of the additive genetic variation in females, respectively. In males, *AC*, *TIM* and *Fr* all load positively while *Act* loads negatively on $\mathbf{G}_{max}$. In females, it is *Fr* that loads antagonistically with respect to *Act*, *AC* and *TIM*. In addition, the angle between male and female $\mathbf{G}_{max}$ is close to being orthogonal, at 80.08˚ (Table 2b). For comparison we also calculated the angle between leading eigen vectors of the

corresponding correlation matrices as 60.74˚, indicating that the lack of alignment here arises largely from differences in among-trait genetic relationships between the sexes (as opposed to differing trait-specific genetic variances, since these are all set to one in the correlation matrix).

The full estimate of $\mathbf{G_{mf}}$ also yields **B**, the cross-sex, cross-trait genetic covariance matrix. Our estimate of **B** shows that the cross-sex genetic correlations are all positive but low for *TIM* (r_{mf} =0.110 (0.282)), higher for *Act* (r_{mf} =0.773 (0.147)) and *AC* (r_{mf} =0.677 (0.199)) and close to +1 for *Fr* (r_{mf} =0.974 (0.124); Table 5). These effect sizes are therefore in agreement with bivariate models that evidenced GxS in *TIM* and provided some (slightly equivocal) indication of $r_{mf} < +1$ in *Act* and *AC.* Calculation of $\Delta\mathbf{B}$ provides some evidence for asymmetry in **B,** although this is limited. Specifically, approximate 95% confidence intervals span zero for all the cross-sex elements of $\Delta\mathbf{B}$ except *Act-TIM* (95%CI: 0.005 - 0.245). The Act_m-TIM_f correlation being 0.177 (0.285), whereas the Act_f-TIM_m being -0.367 (0.202) (see Table 5 for the full $\mathbf{G_{mf}}$ matrix and Supplemental table 3.5 for the $\Delta\mathbf{B}$ matrix).

Genetic associations between personality and size

Finally, bivariate animal models revealed no support for significant genetic correlations between sex-specific behaviours and *length*/*growth* in either males or females (Table 3, supplemental table 3.3).

4.5 Discussion

Here we investigated whether personality, characterised as among-individual differences in risk-taking behaviours, is sexually dimorphic in a captive population

of guppies. We also scrutinised the relationship between behaviour and length and growth – traits known to be sexually dimorphic in this species, before employing quantitative genetic analyses to assess the extent of GxS interactions. We find clear evidence of sexual dimorphism in most traits and discuss this before addressing the evidence for GxS interactions provided by both the single-trait and multivariate approaches used. In what follows, we put our results into the context of the wider quantitative genetic literature and also seek to highlight the benefits of taking a multivariate view of sexual dimorphism in behavioural traits.

4.5.1 Sexual dimorphism in the guppy

Sexual dimorphism was present in OFT behaviours (except for Fr) as well as in length and growth. The latter result is already well known in guppies, with female fish tending to be larger for a given age, and grow faster post maturity, while males preferentially invest in mating opportunities over growth (Bronikowski *et al.*, 2002, Miller & Brooks, 2005). Females also had significantly higher total and among-individual variation in length (and growth) than males, which is not unexpected given that mature fish were used - females are indeterminate growers whereas males effectively stop growing soon after maturation. Females are likely under selection for larger size, with larger females being more fecund, they produce larger offspring (Reznick, 1983, Bronikowski *et al.*, 2002), and are preferred by males seeking to increase number of offspring sired (Dosen & Montgomerie, 2004, Herdman *et al.*, 2004). Males, on the other hand, are selected for (relatively) fast maturation to avoid loss of reproductive opportunities and are thought to gain little from larger size. Indeed there is some evidence that suggests that smaller males are also more successful at sneak matings than their

larger counterparts (Bisazza and Pilastro 1997). Thus the observed dimorphism is thought to be adaptive in the sense of reflecting divergent sex-specific optima.

Behavioural dimorphism is clearly present but only partially in line with our prediction that males would, on average, exhibit more risk-prone or 'bold' type behaviours than females within the novel OFT environment. We found that males tended to explore the tank more and spend more time in the middle zone. This tendency fits with previous studies in which, for instance, found that male guppies approached novel-objects and investigated more closely and quickly than females (Lucon-Xiccato and Dadda 2016). Harris et al. (2010) and Irving and Brown (2013) both showed that male guppies emerged from the safety of a shelter more quickly than females, with a similar result found in the closely related poeciliid, *Brachyraphis episcopi* (Brown et al. 2007a). However, females were more active than males and thus our prediction of how traits would differ between sexes was not fully upheld.

Our own previous work on female guppies (males were not tested) suggests that this could partially be explained by stress response. Although this interpretation is tentative (and perhaps subjective), high activity sometimes occurs because individuals swim rapidly up and down one or two sides of the OFT tank following introduction. This matches a general escape response found in most fish, where a fast-start swim profile consisting of rapid movement aids in predator escape (Walker et al. 2005; Marras et al. 2011). This generates a multivariate profile in which high activity is coupled with relatively low exploration (area covered) and high thigmotaxis (i.e., less time spent in the middle zone White et al. 2016). We speculate that such a behavioural approach to risky/novel situations may be more

common in females reflecting a stronger preference for finding shelter or a shoal (Griffiths & Magurran, 1998, Magurran & Garcia, 2000, Magurran, 2005, Richards *et al.*, 2010).

4.5.2 Cross-sex similarity of multivariate behavioural variation

Average differences in a trait are just one way that the sexes can differ. We also estimated and compared sex-specific **I** matrices to ask if the among-individual (co)variance structure of OFT traits differed. A meta-analysis conducted by Bell et al. (2009) found that, across taxa, there were significant sex differences in the repeatabilities of a wide variety of behaviours, with males being more repeatable than females. However, this pattern was actually reversed when mate choice was excluded from the analysis. Several recent studies have, however, reached varying conclusions as to which sex, if either, exhibits more within-individual consistency (Jenkins, 2011, Hedrick & Kortet, 2012, Debeffe *et al.*, 2015).

Here, we found no evidence that among-individual variation was greater in males, and trait repeatabilities were similar across sexes for homologous traits. Furthermore, multivariate analysis showed strong similarity of the full **I** matrix structure for OFT traits. Both males and females can therefore be differentiated along a similar continuum of behaviour, as shown by the low angle between male and female $\mathbf{I}_{\mathbf{max}}$, on which *activity* loads antagonistically relative to the other traits. Consequently, and in contrast to results from a similar testing paradigm applied to sheepshead swordtails (Boulton et al. 2014), the structure of behavioural variation here is not really consistent with predictions under a simple shy-bold axis. Rather, $\mathbf{I}_{\mathbf{max}}$ of OFT traits in guppies describes a continuum of behavioural variation ranging from ‘active escape response’ at one extreme to an exploratory

phenotype at the other. Average differences between the sexes (as discussed above) would therefore suggest that males inhabit the more exploratory or bold end of this axis, whereas females are closer to the escape response end of this axis.

While male and female **I** matrices were strikingly similar here, we suggest wider estimation of these structures will be generally useful in understanding among-individual (co)variation and multivariate sexual dimorphism. Certainly sexes can differ greatly in selection pressure, and in the contributions of social and abiotic factors to variation among-individuals at single behavioural traits (Croft *et al.*, 2006, Piyapong *et al.*, 2010). To our knowledge, extension to multivariate phenotypes has rarely been attempted. In a study of wild chacma baboons (*Papio ursinus*), Carter et al. (2012b) reported no difference between sex-specific principal components of (multivariate) responses to personality (boldness, novel object testing). In this case, the PCA was applied to observed data (rather than an **I** matrix) and so does not explicitly separate within- from among-individual covariance structure (Houslay and Wilson 2017). In contrast, Fresneau et al. (2014) used bivariate mixed models to show that the among-individual correlation between handling aggression and nest defence was significant (and negative) in female blue tits *Cyanistes caeruleus*, but not in males.

4.5.3 Evidence of size/growth-behaviour relationship

A number of primarily verbal models have postulated that personality will be associated with life-history and physiological traits, suggesting a need to take a more integrative view of the origin of among-individual variation in behaviour (Biro & Stamps, 2008, 2010, Réale *et al.*, 2010, Careau *et al.*, 2015). Links between

risk-taking behaviours and body size (and/or growth) have been reported previously in fish (Brown and Braithwaite 2004; Brown et al. 2007b). Here our univariate models indicated that while dimorphisms in (mean) AC and TIM were largely size independent, higher activity in females could, in principle, be explained by sexual size dimorphism. Thus, while we have no evidence of a causal effect of body size on activity, it is possible that bigger individuals (which tend to be female) exhibit more active escape responses regardless of sex when placed in the OFT arena.

Treating standard length as response variable (rather than a 'nuisance' predictor of behaviour), we found some support for sex differences in among-individual correlations between size and behaviour. In males, individuals that cover more area in the OFT are smaller and grow less. In a previous study we also detected a negative correlation between AC and growth in females from this population (White et al. 2016), but here it was not significant (though the estimate was, again, less than zero). The reason for this difference is not clear. The previous study was less powerful (just 32 females versus 502 here) but also used larger and thus, given indeterminate growth, putatively older females. In the present case, we did find that larger females tended to be more active, spend less time in the middle and freeze less. In other words, larger females tended to display a more 'escape response' type behavioural profile in the OFT. It is difficult to speculate further on the causes of this, or other size-behaviour relationships found, beyond stating that we do not find a simple correspondence between high growth rate and risk-taking or bold behaviour as is typically proposed, for example under the Pace of Life framework (Biro and Stamps 2008; Réale et al. 2010).

4.5.4 Evidence for genotype-by-sex interactions

Our analysis provided strong evidence of GxS interactions for length (modelled as *length* and *growth*) and *TIM* and some support for the presence of r_{mf} <+1 in two of the remaining OFT behaviours. The former result suggests that *length/growth* has scope for further sexual divergence if SA selection is acting, and mirrors recent findings for size at maturity in another poeciliid (*Xiphophorus birchmanni*; Boulton et al. 2016). Our study does not allow us to determine the mechanism underlying the length GxS interaction, though Postma et al. (2011) found evidence of autosomal/X-linkage of body size in male guppies. While it has been suggested that the X chromosome is likely to accumulate sex-specific genetic variation (Gibson et al. 2002), other work on closely related fish have suggested that the Y chromosome could also play a role (Lampert et al. 2010; Boulton et al. 2016).

GxS interactions in OFT behaviours were detected, notably in *TIM*. However, in the other behaviours support for GxS interactions was weaker and less well supported statistically. Consequently, if contemporary selection favours further divergence of male and female behaviour then the cross-sex genetic architecture is likely to be more constraining in these traits. Here, we see sexual dimorphism coupled with moderate to high r_{mf} values, a pattern that has been observed in other species (Long & Rice, 2007, Leinonen *et al.*, 2011, Potti & Canal, 2011). It is therefore important to note that the signature of historical GxS need not be permanent. For instance, while SA selection should favour mechanisms that allow divergence of the sexes (i.e. sources of GxS), following release from genetic constraint this same selection may erode sex-specific $V_{A,}$ causing a return of high values of r_{mf} (Meagher, 1992, Fairbairn & Roff, 2006, Delph *et al.*, 2011).

Nonetheless, across OFT traits our results are consistent with the generally negative relationship between degree of dimorphism and r_{mf} (Bonduriansky & Rowe, 2005, Poissant *et al.*, 2009). For instance, *Freezings* showed the least dimorphism and the highest cross-sex genetic correlation (sex difference of 0.026 SDU and r_{mf} of 0.974) while *TIM* was the most dimorphic behaviour with the weakest correlation estimate (sex difference of -0.507 SDU and r_{mf} of 0.110).

From a single trait perspective, a moderate to high r_{mf} would lead us to conclude that the scope for further behavioural dimorphism to evolve under SA selection is limited. However, a multivariate approach can reveal either additional avenues for the sexes to diverge or additional constraints on independent evolution (Kruuk et al. 2008; Gosden et al. 2012; Wyman et al. 2013). While several studies have found differences in the structure of sex-specific **G** matrices (Jensen et al. 2003; Rolff et al. 2005; Steven et al. 2007; Lewis et al. 2011), our model comparisons provide no statistical support for significant differentiation of $\mathbf{G_m}$ from $\mathbf{G_f}$. Nonetheless, inspection of $\mathbf{G_m}$ and $\mathbf{G_f}$ reveals the largest qualitative differences between elements are associated with *TIM* (both the additive variance, and additive covariances between *Act* and *AC*), the behavioural trait for which GxS was best supported in single trait models. Furthermore, we also estimate a large angle between male and female $\mathbf{G_{max}}$ vectors consistent with the two matrices differing in 'shape'. In fact, while $\mathbf{G_{max}}$ in males is similar to $\mathbf{I_{max}}$ in both sexes (described above), in females $\mathbf{G_{max}}$ trait loadings actually correspond to our *a priori* expectations for a shy-bold continuum (i.e. only freezing loading antagonistically to other behaviours).

The final assessment for multivariate GxS comes from our estimate of **B,** the submatrix of $\mathbf{G_{mf}}$ that describes the cross-sex, cross-trait genetic covariance structure. Though largely symmetrical, we found a difference in genetic association between Act_f - TIM_m (negative) and Act_m - TIM_f (weakly-positive). Predictions of (multivariate) sex-specific selection responses can be drastically altered by asymmetry in **B**, though how this manifests is necessarily dependent on the relative angles of SA selection (Wyman et al. 2013). Here, selection is not known so we cannot comment directly on the consequences. Nor are there sufficient empirical studies estimating **B** where selection is known (or estimable) to generalise from the literature. However, Lewis et al. (2011) initially found genetic constraints in the form of **G** deflecting the angle of response away from the direction of SA selection, but the inclusion of the **B** matrix reversed these predicted responses for females and greatly reduced predicted responses in males, resulting in extra constraint on sexual divergence. A similarly large effect was found for the cuticular hydrocarbons of *Drosophila serrata*, where consideration of **B** revealed significant constraints on continued sexual divergence compared to predictions from the sex-specific **G** matrices alone (Gosden et al. 2012). In the present study, the overall symmetry of the **B** matrix means it is unlikely to facilitate or constrain sex-specific evolution. It is important, however to estimate this matrix to get a fuller picture of how homologous traits expressed in the sexes will respond to SA selection.

4.6 Conclusion

Despite strong interest in sexual dimorphism this is, to our knowledge, the first study to estimate $\mathbf{G_{mf}}$ for a set of personality traits. We suggest that wider uptake of multivariate analyses will give us a fuller picture of how behavioural dimorphism

evolves (and why it sometimes may not). Here we show that guppies exhibit sexual dimorphism in size and growth, but also in average expression of heritable traits linked to risk-taking personality variation. Although the structure of among-individual behavioural (co)variation (as measured by **I**) is similar in males and females, single trait and multivariate analyses also provide evidence of some GxS interactions. These are detected as cross-sex genetic correlations of <1 in single trait analyses. Although the overall structure between $\mathbf{G_m}$ and $\mathbf{G_f}$ was similar, there was little alignment between the sex-specific $\mathbf{G_{max}}$ estimates. This suggests that sex differences in $\mathbf{G_{max}}$ are driven by the (co)variance of a small number of traits (in this case TIM and AC), differences that are not detectable when comparing the whole matrix. The **B** matrix was largely symmetrical with only one component that was asymmetrical. Lacking knowledge of (sex-specific) multivariate selection we cannot comment directly on how these genetic covariances will shape future evolutionary trajectories, although we broadly expect the GxS interactions and differences in $\mathbf{G_{max}}$ to facilitate some future dimorphism under SA selection.

Table 1: Estimated effect of sex (male relative to female) on trait means. Estimates are from pooled-sex univariate animal models, standard errors are shown in parentheses.

Trait	effect size	DF	F	P
Act	0.249 (0.053)	1, 779.6	21.960	<0.001
AC	-0.189 (0.050)	1, 782.3	14.38	<0.001
TIM	-0.507 (0.052)	1, 802.2	94.55	<0.001
Fr	0.026 (0.052)	1, 776.6	0.24	0.621
Length	1.527 (0.035)	1, 745.1	1934.86	<0.001

Table 2: Trait loadings on the first and second eigen vectors of male and female **I** matrices (a) and **G** matrices (b).

	Trait	Male Eigen 1	Eigen 2	Female Eigen 1	Eigen 2
a)	*Act*	-0.632	0.160	-0.640	0.253
	AC	0.102	0.813	0.193	0.779
	TIM	0.575	0.388	0.537	0.408
	Fr	0.510	-0.403	0.515	-0.404
b)	Act_m	-0.562	0.401	0.552	-0.384
	AC_m	0.320	0.644	0.584	0.377
	TIM_m	0.720	0.237	0.133	0.819
	Fr_m	0.250	-0.607	-0.580	0.201

Table 3: Estimated sex-specific among-individual and genetic correlations between each OFT trait and *length* (intercept) and *growth*. Standard errors are in parentheses and bold font denotes parameters where covariance between behaviour and standard length is statistically significant (see Supplemental table 2 for statistical testing).

	Trait	Male		Female	
		Length	Growth	Length	Growth
Among-individual	Act	0.150 (0.085)	0.190 (0.130)	**0.370 (0.057)**	**0.220 (0.113)**
	AC	**-0.104 (0.098)**	**-0.427 (0.142)**	0.032 (0.069)	-0.348 (0.123)
	TIM	-0.082 (0.088)	-0.244 (0.130)	**-0.199 (0.066)**	**0.092 (0.124)**
	Fr	0.031 (0.096)	-0.011(0.149)	**-0.205 (0.070)**	**-0.239 (0.130)**
Additive genetic	Act	0.110 (0.370)	0.060 (0.304)	0.247 (0.216)	0.247 (0.242)
	AC	-0.205 (0.389)	-0.453 (0.307)	-0.219 (0.394)	-0.482 (0.293)
	TIM	-0.001 (0.387)	0.098 (0.295)	-0.123 (0.382)	0.167 (0.25)
	Fr	-0.231 (0.375)	-0.049 (0.326)	-0.230 (0.381)	-0.055 (0.324)

Table 4: Comparisons of models in which for each pair of homologous traits full GxS is allowed (unconstrained model), homogeneity of sex-specific VA is imposed ($V_{Am}=V_{Af}$), r_{mf} of +1 is imposed, or no GxS is allowed ($V_{Am}=V_{Af}$ and rmf=+1). Shading denotes the preferred model based on AIC.

Trait	Model	AIC	ΔAIC
Act	unconstrained	1843.26	1.85
	$V_{Am}=V_{Af}$	1841.41	0
	r_{mf} = +1	1847.16	5.75
	No GxS	1843.18	1.77
AC	unconstrained	2033.90	1.91
	$V_{Am}=V_{Af}$	2031.99	0
	R_{mf} = +1	2036.57	4.58
	No GxS	2033.07	1.08
TIM	unconstrained	1915.18	0.86
	$V_{Am}=V_{Af}$	1914.32	0
	r_{mf} = +1	1926.53	12.21
	No GxS	1926.14	11.82
Fr	unconstrained	2311.05	3.30
	$V_{Am}=V_{Af}$	2309.21	1.46
	r_{mf} = +1	2311.53	3.78
	No GxS	2307.75	0
Length	unconstrained	-7659.74	0
	$V_{Am}=V_{Af}$	-7652.49	7.25
	r_{mf} = +1	-7649.80	9.94
	No GxS	-7611.83	47.91

Table 5: Estimated $\mathbf{G_{mf}}$ matrix from the full multivariate model of sex-specific OFT traits with coloured blocks corresponding to $\mathbf{G_m}$ (orange), $\mathbf{G_f}$ (green) and **B** (blue). $\mathbf{G_m}$ and $\mathbf{G_f}$ are necessarily symmetric and shown with variances on the diagonal (dark shading), covariance below, and correlations above. **B** is not necessarily symmetric so covariances are scaled to cross-sex genetic correlations in the upper right block, with grey shading denoting the estimates of r_{mf} for homologous traits. Standard errors on all estimates are shown in parentheses.

	Act_m	AC_m	TIM_m	Fr_m	Act_f	AC_f	TIM_f	Fr_f
Act_m	0.275 (0.085)	0.009 (0.203)	-0.681 (0.111)	-0.772 (0.095)	0.773 (0.147)	0.598 (0.199)	0.177 (0.285)	-0.744 (0.152)
AC_m	0.002 (0.054)	0.222 (0.055)	0.639 (0.130)	-0.373 (0.197)	0.161 (0.223)	0.677 (0.199)	0.207 (0.295)	-0.492 (0.202)
TIM_m	-0.205 (0.076)	0.173 (0.043)	0.329 (0.081)	0.338 (0.177)	-0.367 (0.202)	0.130 (0.231)	0.110 (0.282)	0.209 (0.217)
Fr_m	-0.184 (0.071)	-0.080 (0.504)	0.088 (0.063)	0.207 (0.076)	-0.889 (0.145)	-0.679 (0.226)	0.138 (0.297)	0.974 (0.124)
Act_f	0.176 (0.053)	0.033 (0.046)	-0.091 (0.057)	-0.176 (0.051)	0.188 (0.057)	0.598 (0.206)	-0.237 (0.234)	-0.875 (0.064)
AC_f	0.132 (0.051)	0.135 (0.048)	0.031 (0.056)	-0.130 (0.048)	0.109 (0.040)	0.178 (0.057)	0.424 (0.208)	-0.725 (0.181)
TIM_f	0.032 (0.052)	0.034 (0.049)	0.022 (0.058)	0.022 (0.050)	-0.036 (0.043)	0.063 (0.045)	0.123 (0.054)	0.103 (0.262)
Fr_f	-0.173 (0.055)	-0.103 (0.049)	0.053 (0.058)	0.196 (0.054)	-0.168 (0.054)	-0.135 (0.043)	0.016 (0.043)	0.195 (0.062)

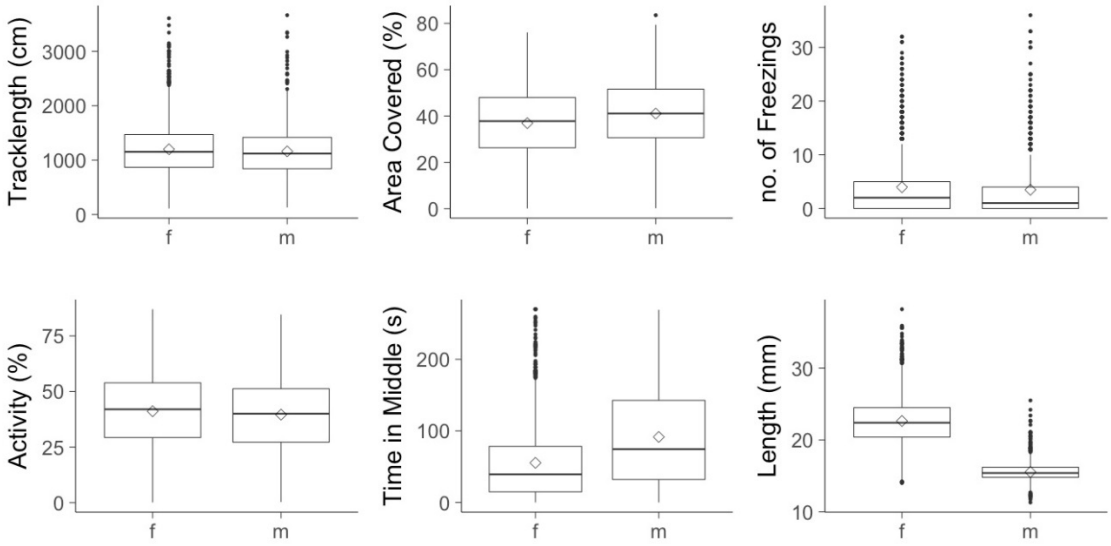


Figure 1: Boxplots of OFT raw data, comparing males (m) and females (f). Central horizontal line indicates the median, diamond indicates the mean.

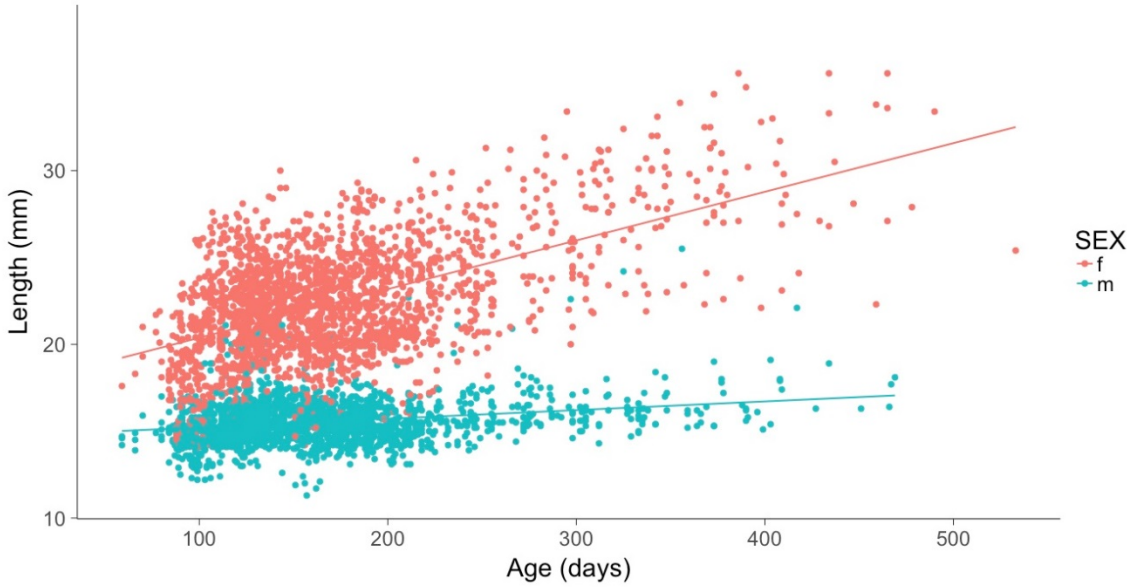


Figure 2: Scatterplot of individual length over age in males and females. Lines of best (linear) fit are shown for illustrative purposes only, noting that data points shown include multiple measures per individual and are non-independent.

Chapter 5

Phylogeny and among-individual variation in behaviour: a comparative approach to animal personality

5.1 Abstract

Personality axes can be viewed as latent, unobserved constructs to be identified by various behavioural assays developed to measure them. Observable behavioural traits measured in these assays are then used to infer underlying personality variables and can thus be viewed as proxies for personality. While personality traits have been studied in a great many species, there has been very little comparative work between species, testing if the standardised paradigms measure equivalent personality traits across taxa. In addition, much of the confusion over what personality traits are measured by these assays stems from the use of single observed traits, which are unlikely to fully represent personality variables that manifest in the expression of many observed behaviours. Here, we compare aspects of the among-individual (co)variance structure of 7 species of small, freshwater fish using multiple traits measured in an open field trial (OFT) - an assay designed to measure a shy-bold axis. Differences in total variation, alignment of first eigen vector ($\mathbf{I_{max}}$) and higher order dimensions are compared, along with the phylogenetic signal of these differences. We found that species differed in $\mathbf{I_{max}}$, with both shy-bold and stress response axes being captured. While this indicates that the OFT captures different personality variables in different species, when comparing across 2-trait dimensions, fish species were relatively similar. Furthermore, phylogenetic signal was low for most aspects of the comparison, with the exception of the higher order dimensions, with more closely related species having more aligned 2 dimensional trait space.

5.2 Introduction

Animal personality is widely defined by the presence of consistent, among-individual behavioural variation that is stable over time. Personality is, therefore, a broad term that covers a number of behavioural axes of variation assumed to have generality across populations or species, the most well studied being boldness, exploratory behaviour, aggression and sociality (Smith and Blumstein 2008; Bell et al. 2009; Toms et al. 2010). Personality axes can consequently be viewed as latent, unobserved constructs to be identified by various behavioural assays. Observable behavioural traits measured in these assays are used to infer underlying personality variables and can thus be viewed as proxies for personality (Walsh and Cummins 1976; Carter et al. 2013; Araya-Ajoy and Dingemanse 2014). Although personality axes have ostensibly been measured in a wide range of species, the observed traits used as proxies often differ between studies, making generalising across species or even populations within species difficult. The use of different traits is partly due to differences in definitions between studies and disagreement over what constitutes particular personality axes. For instance, boldness can be defined as either the propensity to exhibit 'risky' behaviours around novel objects, or as the behavioural response to a risky situation, not including response to novelty (Réale et al. 2007; Toms et al. 2010; Carter et al. 2013). Disagreement over definitions coupled to a wide range of proxy traits and assay types, has led to a lack of directly comparable personality studies across similar or related taxa. Consequently, while comparative analyses are widely and effectively used to test evolutionary hypotheses about other aspects of the phenotype, they are lacking for personality traits. This is problematic if we wish to make broad inferences about the maintenance and evolution of personality variation across taxa. Here, we aim to address this gap

by comparing the behaviour of 7 species of small freshwater fish in a commonly used personality assay.

Numerous behavioural assays have been developed for the measurement of personality traits. Due to the demanding data requirements for partitioning among-individual variation (the statistical signature of personality) from within-individual variation, many are primarily designed for quick and simple data collection. Such high-throughput behavioural phenotyping should allow replication of methods across studies, yet this opportunity has yet to be exploited. Furthermore, where similar methods have been applied to different species, there is often little agreement over what latent personality trait is being assayed. For example, the shy-bold continuum, arguably the most studied aspect of animal personality (Toms et al. 2010), is measured using several assays, including the emergence test, novel object tests and the open field trial (OFT). Although there is still discussion about whether these assays do actually measure boldness, or other personality variables such as anxiety (Carter et al. 2012c), the OFT is still considered by many as a reliable measure of boldness. Despite its widespread application, however, debate continues over whether the OFT effectively assays variation in boldness as opposed to other latent variables (Carter et al. 2012c). Recent work certainly suggests inconsistency across species either in the nature of among-individual behavioural variation being revealed by the OFT or in the interpretation of what personality axes this variation represents. Unfortunately, data sets are rarely comparable enough to distinguish these two possibilities. For instance, the OFT was interpreted as capturing both boldness and exploration in the common mynas, Acridotheres tristis and the great tit, *Parus major* (Dingemanse et al. 2002; Perals et al. 2017), but activity in the red squirrel

Tamiasciurus hudsonicus and brown trout *Salmo trutta* (Taylor et al. 2012; Adriaenssens and Johnsson 2013), anxiety in the zebrafish *D. rerio* (Champagne et al. 2010) and anxiety or exploration in sighted and non-sighted cavefish, respectively (Sharma et al. 2009). Here, different conclusions can be ascribed in part to different definitions of boldness between studies. There will also be actual differences among species in how they react to an open field environment, both in terms of average behaviours and in variation among individuals. We suggest that while continued debate over the former is unlikely to contribute major biological insights, personality research is likely to benefit greatly from more comparative studies. For example, while adaptive explanations for personality variation dominate the literature, there has been little formal attempt to compare levels of variation among populations differing in (expected) selection regimes. We also know little of the extent to which phylogenetic signal is found in behavioural traits generally - whether more closely related populations or species have more similar pattern of among-individual (co)variation, or whether patterns among species are unrelated to phylogenetic distance entirely.

In order to adopt more formal comparative approaches to personality research, empiricists need to fully embrace multivariate mixed modelling. The reasons for this are twofold. First, since latent personality axes are expected to be manifest in the expression of many observed behaviours (Walsh and Cummins 1976; Carter et al. 2013), it is intuitive that observing more behaviours should lead to their more robust inference. Therefore considering multiple observed traits and their covariances together should give a fuller picture of the underlying latent variable they represent (Wright et al. 2006; Toms et al. 2010). Second, by using multivariate approaches and a common set of observed traits in a comparative

context, we can employ quantitative comparison of the covariance structures among observed traits (Dochtermann and Roff 2010; Wilson et al. 2011a; Brommer 2013; Houslay and Wilson 2017). This will facilitate much more nuanced questions about the "shape" (as opposed to simply the amount) of among-individual behavioural variation and how it differs among populations/species.

Despite the potential advantages of multivariate methods, most studies have used a single observed trait to represent a particular personality variable, with the choice typically justified by a verbal model. Although this is conceptually and practically convenient, studies that have tried to validate and reconcile single observed traits with the personality variables they putatively assay have revealed many inconsistencies (Burns 2008; Toms et al. 2010; Perals et al. 2017). Increasingly, researchers are seeking to validate the link between observed traits and latent personality variables, for instance by testing for correlation of the former with other (already accepted) proxies. While useful, this approach obviously depends on having a universally accepted proxy or measure of personality against which to validate any new assay or observed trait (Walsh and Cummins 1976).

A growing alternative to the use of single traits is to collect data on multiple behavioural traits and then apply dimensionality reducing techniques, most commonly principle component analysis (PCA). In this context, PCA identifies the major independent axes of behavioural variation across multiple traits. This reduces the need for validation of observed traits as the main axes of variation (i.e. principle components), can themselves be viewed as representing

personality variables (Carter et al. 2013). An important criticism is that, if applied directly to raw data containing repeated measures on individuals, the principle components of phenotypic variation will not necessarily be the major axes of among-individual variation (see Houslay and Wilson 2017) for further discussion). Aside from this issue, PCA has proven useful for identifying personality variation captured by sets of observed traits from single (Menzies et al. 2013; Castanheira et al. 2016) and multiple assays (Rödel and Meyer 2011; Watanabe et al. 2012; Ibarra-Zatarain et al. 2016).

In this study we aim to address the need for comparative analyses of personality by comparing the among-individual behavioural variation structure of 7 species of freshwater fish from the families *Poeciliidae*, *Goodeidae* and *Cyprinidae*. We take a fully multivariate mixed model approach that correctly utilises repeated measures data to identify and compare among-individual axes of behavioural variation captured by the OFT. The species used in this study all inhabit freshwater streams where they may get swept to new and risky areas away from the shoal (Magurran 2005), thus an OFT provides an ecologically relevant test of behavioural response to risk in these species. We measure a common set of observed traits - tracklength (*Tl*), activity (*Act*), area covered (*AC*) and time in a middle zone (*TIM*) across all species, and evaluate the extent to which the multivariate among-individual variation, estimated as an **I** matrix, matches our *a priori* expectations of a "shy-bold" personality axis. Our expectation is that all traits will vary, and positively covary, among individuals, as shown in the sheepshead swordtail *Xiphophorus birchmanni* (Boulton et al. 2014). We note this expectation is naïve since our recent work on the closely related guppy, *Poecilia reticulata*, suggests the OFT reveals variation more readily interpretable as being linked to

stress response than boldness (an informal and subjective conclusion that in part has motivated the current comparative study).

We then compare **I** matrices among species, testing for differences and employing several approaches to quantify (dis)similarity objectively. These include comparing the traces of **I** to compare total variation, the leading eigen vector ($\mathbf{I}_{max}$) to ask whether a similar main axis of personality variation is apparent in each species and we use the Krzanowski index to assess higher dimensional subspace similarity. This metric is often used in quantitative genetics to identify and compare the effective dimensionality of **G** matrices and its role as a constraint to evolution (Krzanowski 1979; Hine and Blows 2006; Aguirre et al. 2014). Finally, we test for phylogenetic conservatism in **I**, a phenomenon that is typically controlled for in comparative studies of other trait types (Uyeda et al. 2015) but has yet to be examined in the context of behavioural (co)variation. All else being equal, we would expect more closely related species that share more of their evolutionary history, to have more similar behavioural structure than those more distantly related. To test this, we estimated a phylogenetic relatedness matrix between all species based on cytochrome b sequences and compared it to the (di)similarity measures of **I**.

5.3 Materials and methods

5.3.1 Study species and husbandry

We obtained repeated measures data from open field trials (OFT) of 7 species of small freshwater fish from 3 families. These were the Trinidadian guppy (*Poecilia reticulata*), black-barred limia (*Lima nigrofasciata),* sheepshead swordtail *(Xiphophorus birchmanni),* green swordtail (*Xiphophorus helleri*) and common

platy *(Xiphophorus maculatus)* from the family *Poeciliidae*; the red-tailed splitfin *(Xenotoca eiseni)* from the family *Goodeidae;* and, the zebrafish *(Danio rerio)* from *Capriniidae*. All fish used were captive bred wild-type strains except for *X. maculatus* (which was an ornamental "blue tuxedo" strain). Data were collected at the University of Edinburgh, Ashworth laboratories (*X. birchmanni* and *D. rerio*) and the University of Exeter, Penryn Campus (all other species) over various time periods between August 2010 and November 2016. Fish were kept at 21- 25˚C (species dependent) on a 12:12 light:dark cycle and fed twice daily with commercial flake food and live brine shrimp nauplii (*Artemia salina*), frozen bloodworm or frozen adult brine shrimp (dependent on fish size). To allow individual recognition for repeated behavioural trials all fish used were tagged with either PIT tags implanted sub-dermally (*X. helleri* using the P-Chip system at www.pharmaseq.com) or visible implant elastomer (all other species). All fish were tagged under anaesthetic using a buffered MS222 solution as described elsewhere (White et al. 2016).

The open field trial (OFT)

Behavioural data collection was broadly similar across species, but with variation in numbers of fish (range of 26-831), average observations per fish (range 4-6) and experimental period (range 2-28 weeks; See Supplemental table 4.1). Across all species we collected data from 5109 OFT trials on 1479 individuals. Data on *X. birchmanni* and *P. reticulata* have been published previously (Boulton et al. 2014; White et al. 2016). *X. birchmanni* was unique in being assayed more times and over a longer period of 28 weeks as part of another study comparing short vs long term measures of personality (Supplemental table 4.1, Boulton et al. 2014). Data from other species have not previously been published.

The OFT procedure used has been detailed previously (White and Wilson, *Submitted*; Boulton et al. 2014; White et al. 2016) and we therefore abbreviate the current description. For all species, individual fish were assayed with multiple OFT, with at least 48 hours between trial. Each OFT comprised an individual fish being transferred into a 'bare' trial tank, lit from below using a lightbox and filled with 5cm of water. For most species the tank had a base of 45 x 25 cm, but for the smallest two (*D. rerio, P. reticulata*) we elected to use a smaller tank (30 x 20 cm base). Following a 30 second settling time, fish movement was tracked using an overhead camera and Viewer software (www.biobserve.com) over a 4 minute 30 second time period for most species (trials lasted 5 minute for *X. birchmanni* and *D. rerio*). *Tracklength* (*Tl*, defined as the total length (cm) that the individual swam), *activity* (*Act,* percent of the time spent moving over 4cm s^{-1}) and *area covered* (*AC,* percentage of the tank area covered) were extracted from the tracking data. In addition, central and outer zones (of equal area; see Boulton et al. 2014) were imposed on the tank using Viewer software and the *time spent in the middle zone* (*TIM,* measured in seconds) was also recorded. The OFT water was not changed after each trial, but rather after each group of individuals (with different group sizes across species; Supplemental table 4.1). Effects of order (within group) could arise from cumulative effect of netting stress from the home tank (groups corresponded to sets of fish housed together) and/or build-up of chemical cues in the OFT tank so are controlled for statistically (see below).

5.3.2 Statistical methods

As the species used varied in average size (smallest by standard length being *P. reticulata* at 19.47mm and largest being *X. eiseni* at 48.25mm), we decided to scale each individual *Tl* measure by average species length to produce distance

swam in (average) body lengths. We elected to do this, rather than dividing each individual's *TI* by its own standard length as the latter risks conflating personality with within-species size variation (i.e. our scaling retains any size-dependent among-individual variation within each species). For all species, *TIM* was square root transformed to better fit the assumption of residual normality required for our linear mixed effect models (see below). All (transformed) traits were then mean centred and scaled to standard deviation units (SDU). In doing this we use the global (i.e. across all individuals of all species) mean and standard deviations. This puts all traits on a similar scale, aiding convergence of multivariate mixed models while still maintaining differences between species.

Data were analysed using linear mixed effect models fitted in ASReml-R (Butler et al. 2009) using restricted maximum likelihood (REML). The four traits assayed have been shown to be significantly repeatable in *P. reticulata* (White et al. 2016) and *X. birchmanni* (Boulton et al. 2014). To get comparable estimates (and tests of) repeatabilities in all 7 species, we first fitted univariate mixed models to each trait in each species (unscaled and uncentred). Each model included a random effect of individual identity (FishID). A fixed factor of repeat (cumulative number of trials experienced) and continuous linear effect of order within-group were also fitted. These were included to control for any across-trial habituation to the OFT and/or trends within groups respectively. Repeatability, conditional on these fixed effects, was estimated as the intraclass correlation coefficient (V_I/V_I+V_R) where V_I is the among-individual variation and V_R is the residual variance. The significance of V_I was determined by likelihood ratio test (LRT) comparison to a simpler model with no random effect. As a single random effect was tested, the

test statistic was assumed to follow a 50:50 mix of χ^2 with 0 and 1 degrees of freedom (Visscher 2006).

Among-species variation in mean behavioural phenotype

Before comparing the among-individual (co)variance structures among species, we next describe among-species variation in average multivariate phenotype. Canonical variate analysis (CVA) was used to do this, visualising the spread of the species across multivariate trait space. Having verified that all traits in all species are repeatable, we calculated a within-individual mean behaviour for each trait to reduce the repeat measures structure of the data to a single (multivariate) phenotype per individual. CVA was then applied as a data reduction approach to identify and visualise the main, orthogonal axes of variation (canonical variates) across pre-specified groups (in this case, species). This is done by sequentially maximising the differences between the groups in a similar fashion to principle component analysis (Campbell and Atchley 1981). This technique has been used to describe multivariate differences in both behavioural and morphological traits (Carter and Feeney 2012; Figueirido et al. 2016).

Multivariate models to estimate species-specific **I** *matrices*

For each species (s), we then estimated the among-individual behavioural (co)variance matrix $\mathbf{I_s}$ for the set of four traits (*TI, Act, AC* and *TIM*) for each species using multivariate mixed models. As with the univariate models above, fixed effects of repeat and order caught were fitted along with a random effect of FishID. We tested for significant among-individual covariance structure in each species by comparing the full model fit to a reduced model in which all covariance terms in $\mathbf{I_s}$ were fixed to zero using LRT at 6 DF. While acknowledging that this

creates a multiple testing issue, we then formally tested the null hypothesis that $\mathbf{I}_{s1}=\mathbf{I}_{s2}$ for each pair of species (21 species pairs in total). To do this we fitted a series of 8-trait cross-species multivariate models (i.e. each of the 4 OFT traits for species 1 fitted with the 4 traits for species 2) with fixed and random effects as described above (but with no cross-species covariance terms in **I** or the corresponding residual structure). Thus for each species pair we estimate **I** as a blocked matrix where

$$\mathbf{I} = \begin{bmatrix} \mathbf{I}_{s1} & 0 \\ 0 & \mathbf{I}_{s2} \end{bmatrix} \quad (1)$$

The fit was then compared (LRT at 10 DF) to a simplified model in which we impose the constraint that $\mathbf{I}_{s1}=\mathbf{I}_{s2}$.

Among-species comparison of **I** *matrices*

While the above provides a formal test for equality of **I** between species, there are many ways to describe (dis)similarity between matrices. We chose three complementary approaches to assess several aspects of similarity among the 7 estimates of $\mathbf{I}_s$: trace comparison, $\mathbf{I}_{max}$ comparison and the eigen subspace comparison. Firstly, the trace of $\mathbf{I}_s$ (calculated as the sum of the trait-specific V_I variances on the diagonal) was used to characterise the total amount of multivariate among-individual behavioural variation. For each species, approximate 95% CI of the trace were determined from a 5000 draw parametric bootstrap (using the approach described in chapter 4 and Boulton et al. 2014). For each pair of species, we also calculated the difference in traces, which we denote Δ, (with approximate 95% CI) to test whether species differ in the amount of among-individual variation.

Second, for each species pair we calculated the angle between the first eigen vectors (referred to as $\mathbf{I}_{max}$) of the corresponding $\mathbf{I}_s$ estimates. Eigen vector decomposition allows identification of the leading axis (or axes) of among-individual variation which can be useful in determining the extent to which observed traits map to an underlying model or expectation of personality. Thus, for example, if all four observed traits represent valid proxies of a single latent personality trait (e.g. boldness), and that personality trait is similar in two species, we predict the angle between leading eigenvectors of $\mathbf{I}_s$ will be low. The angle θ ranges from zero in the case of fully aligned vectors to 90 degrees when maximally non-aligned.

Comparing the angles between the $\mathbf{I}_{max}$ of two matrices in this way quantifies the alignment (or lack thereof) between 1-dimensional subspaces defined by the leading eigen vectors. If $\mathbf{I}_{max}$ does not contain the majority of among-individual variation, however, comparing species matrices by the alignment of a higher dimensional subspace may be more biologically appropriate. For instance, species may differ dramatically in the alignment of their leading vector in trait space, but be very similar across two (or more) dimensions. This could happen in the case that a common set of observed traits assayed variation in two (rather than one) personality axes that were conserved across species but differed in their relative contributions to total variance. Our third approach was therefore to use the Krzanowski test, which provides a general index of similarity between eigenvector subspace.

Take two matrices, each is made up of *n* traits and therefore has a total of *n* dimensions. If the majority of variation within the matrices is contained within a

subset of *x* eigen vectors we can compare the similarity of *x*-dimensional subspace between different matrices (Krzanowski 1979). The **S** matrix forms the basis of this similarity index and is calculated by rotating the chosen eigenvectors within the subspace in the two matrixes to minimise the angle between them:

$$\boldsymbol{S} = \boldsymbol{A}^T\boldsymbol{B}\boldsymbol{B}^T\boldsymbol{A}$$

Where **A** and **B** contain the subset of *x* eigenvectors of the two original matrices (in the present context a pair of species-specific **I** estimates) that define the subspace. The sum of the eigen values of the **S** matrix gives an overall index of similarity of the subspaces, with a lower limit of 0 meaning they are unrelated and orthogonal and an upper limit equal to *x*, meaning they are completely aligned (Krzanowski 1979; Blows and Walsh 2007; Aguirre et al. 2014). Here, we used the first two eigen vectors as our vector subset, with the Krzanowski test will compare 2-dimensional subspace between the matrices. The resulting index of similarity, which we denote K, will range from 0 (subspaces are orthogonal) to 2 (subspaces are aligned).

Testing for phylogenetic signal in $\mathbf{I}_s$

The matrix comparison tools described above thus yield three different measures of **I** matrix (dis)similarity for each pair of species (s1, s2) – the difference in traces ($\Delta_{s1,s2}$), the angle between leading eigen vectors ($\theta_{s1,s2}$), and the Krzanowski similarity index ($K_{s1,s2}$). While the small number of species (n=7) limits the potential for formal comparative analysis our final analyses sought to test for phylogenetic signal in **I** by asking whether these measures were predicted by phylogeny. While the phylogenetic relatedness between some of the species

used here has been studied (Marcus and McCune 1999; Hamilton 2001; Jones et al. 2013), there is no published phylogeny including all 7 species used here. Therefore, in order to try and incorporate branch length information for the 7 species, we estimated a phylogeny based on cytochrome b sequences obtained from Genbank. Unfortunately, we were limited to the cytochrome b gene due to limited sequences being available for *X. eiseni* and *D. rerio*. Sequences used were the partial tRNA-Glu gene, cytochrome b gene and partial tRNA-Thr gene for *L. nigrofasciata, X. birchmanni*, *X. helleri*, *P. reticulata*, the cytochrome b gene and TRNA-Thr gene from *X. maculatus* and partial cytochrome b gene sequence only from *X. eiseni* and *D. rerio*. The phylogenetic tree was constructed from these aligned and trimmed sequences using a maximum likelihood method implemented on the PhyML server (http://www.atgc-montpellier.fr). The relatedness matrix was then calculated as the number of nucleotide substitutions per site between each species. For the purposes of comparison with the phylogenetic measure of dissimilarity, the K index of subspace similarity was inverted to produce an index of dissimilarity. The $\Delta_{s1,s2}$, $\theta_{s1,s2}$ and inverted $K_{s1,s2}$ values of every species pair were arranged into 3 dissimilarity matrices, with the correlation between each of these matrices and the phylogenetic matrix calculated. Significance of these matrix correlations was calculated using permutation based Mantel tests using the Mantel function in the r package "vegan".

5.4 Results

Our univariate mixed models showed that all traits are significantly repeatable in all species, with low to moderately high repeatabilities ranging from 0.157 (Act in *D. rerio*) to 0.562 (TIM in *D. rerio*) with a median value of 0.366 (all estimates

presented in Supplemental table 4.2). The CVA shows clear separation of (average) behavioural phenotypes between some, but not all species (Figure 1). Based on the first two canonical variates, *X. helleri*, *L. nigrofasciata, X. maculatus* and *X. eiseni* appear quite similar to each other in behavioural phenotype, forming a 'core group' of species with large overlap of confidence ellipses (Figure 1). *X. birchmanni* and *P. reticulta* are moderately differentiated from this group, and more strongly from each other along CVA1. This axis captures most of the among-species variance (88.9%) and loads antagonistically on mean TI and mean Act (Coefficients of linear discriminants for within-individual mean behaviours: TL =0.124, Act=-0.176, AC=0.023, TIM=-0.033). Therefore, relative to X. birchmanni, it is the case that *P. reticulata* tends to exhibit longer *TI* but lower *Act*. *D. rerio* is strongly differentiated from the core group as well as from *P. reticulata,* but there is some overlap of confidence ellipses with *X. birchmanni*. Differentiation of *D. rerio* is primarily on CVA2, which captures 9.5% of the among-species variance and loads primarily on TIM (Coefficients of linear discriminants for within-individual mean behaviours: TI =0.034, Act=0.025, AC=0.026, TIM=0.148). Thus separation of *D. rerio* is largely driven by an increased tendency of this species to spend more time in the middle of the OFT arena.

Multivariate mixed models provided evidence of significant among-individual (co)variance structure in **I** for all Poeciliids and *X. eiseni* (LRT comparison of full and reduced models, all P<0.001; Supplemental table 4.3). *D. rerio* provided an exception to this pattern (LRT comparison, χ^2_{6DF}=10.46, P=0.107). Note however that while we cannot statistically exclude the possibility of among-trait covariance being entirely due to within-individual effects in this species, our unconstrained

estimate of $\mathbf{I}_{Dr}$ (i.e. with covariance terms modelled) was used in subsequent matrix comparisons.

5.4.1 Comparison of **I** matrices

Estimates of $\mathbf{I}_s$ obtained for all species (s) are shown in Supplemental table 4.4. Pairwise testing for equality of **I** using 8-trait multivariate models proved somewhat problematic as we were unable to obtain stable model convergence when *L. nigrofasciata* was one of the species in the pair. However, where tests were possible all species pairs differed significantly in $\mathbf{I}_S$ (at nominal $P<0.05$ with no correction for multiple testing; Supplemental table 4.5) with the exception of the *X. helleri* and *X. eiseni* comparison ($\chi^2_{10}=5.41$, $P=0.862$). We therefore conclude that there is evidence of among species variation in **I**.

5.4.2 Trace comparisons

Matrix traces provide some support for species differences in the amount of among-individual (multivariate) variance (Figure 2). *X. helleri*, *L. nigrofasciata*, *X. eiseni* and *X. birchmanni* have very similar traces (ranging from 0.415-0.513 standard deviation units (SDU)). Estimates of Δ between these species have 95% CI containing zero, Table 1). The common platy, *X. maculatus*, has a slightly larger trace of 0.828 SDU, which is significantly greater than both *L. nigrofasciata* and *X. birchmanni* (95% CI of Δ do not overlap zero; Table 1). *P. reticulata* is most dissimilar to the other species, having the highest trace (1.463 SDU) and showing significantly more among-individual variance than all species except *D. rerio*. Due to the large 95% CI surrounding the trace of *D. rerio*, it is difficult to comment on its similarity with the other species, but it is at least qualitatively more similar to *P. reticulata* than the other species. A closer examination of the **I** matrix

estimates (Supplemental table 4.4) shows that the greater traces in these two species are due to much more V_I for Tl and to a lesser extent for AC and TIM (note that trait units are global, i.e. across all species, SDUs and this pattern is not seen in repeatabilities which are standardised by within-species variance).

5.4.3 Eigen vector decompositions, θ and K comparisons

Eigen vector decomposition reveals $\mathbf{I_{max}}$ accounts for between 58% (*D. rerio*) and 85% (*X. maculatus*) percent of variance in $\mathbf{I_s}$ with a median of 60.4% across species. Based on trait loadings for $\mathbf{I_{max}}$ a qualitative interpretation could be made that the OFT is revealing 3 different types of personality variation across the 7 species (Table 2). The $\mathbf{I_{max}}$ of *X. birchmanni*, *L. nigrofasciata* and *X. maculatus* match the traditional 'shy-bold' axis, with all OFT traits loading positively (Figure 3a). In these species, individuals that have longer Tl also have higher values for Act, AC and TIM. By contrast, in *X. helleri* and *P. reticulata*, Tl and Act load antagonistically to AC and TIM on $\mathbf{I_{max}}$. This type of variation may be linked to stress response, as previously suggested by work on *P. reticulata* females from the same population (White et al. 2016). Here, some individuals exhibit "escape response" type behaviours, swimming rapidly along one or two sides of the tank (leading to high Tl and Act but low AC and TIM; see Figure 3b for an example).

The third axis type, seen in *D. rerio* and *X. eiseni*, has TIM loading antagonistically to all other traits. This then differs from our initial expectations under a shy-bold paradigm (Figure 3a) because active and exploratory individuals also tend be more thigmotaxic. More quantitatively, estimates of θ support this somewhat subjective interpretation with relatively low angles between species of a similar axis type (e.g., $\theta_{Xm,Lm}$=20.3˚, $\theta_{Xh,Pr}$=31.6˚; Table 3) but very poor alignment

between species specific $\mathbf{I_{max}}$ in other cases with θ as high as 89.2˚ (i.e., effectively orthogonal) in the comparison of *X. birchmanni* and *X. helleri*.

As noted above, $\mathbf{I_{max}}$ captures approximately 85% of the variance in $\mathbf{I}_{XM}$. While in the other species, **I** was less obviously dominated by a single axis of variation; $\mathbf{I_{max}}$ accounted for less variance and there was a corresponding increase in importance of the second eigenvectors (accounting for 27.8% to 35.3% of the variance; Table 2). The first two eigenvectors together capture the great majority (88-99%) of variation in all species (Table 2), justifying our decision to compare 2-dimensional subspaces between the matrices with the Krzanowski tests. Across species comparisons, K ranged from 1.43 to 1.96 (on a scale from 0-0-2) suggesting two dimensional subspaces are rather similar (Table 4, Figure 4).

5.4.4 Testing for phylogeny signal in $\mathbf{I}_s$

Our phylogenetic analysis showed that the generalised time reversible (GTR) nucleotide substitution model with additional gamma and 'I' parameters (GTR + G + I) had the lowest AIC and therefore the best fit. GTR is one of several nucleotide substitution models used to estimate genetic distance between markers. Gamma refers to the gamma distribution used to estimate the variation in substitution rate of nucleotides among different sites in the sequence. 'I' refers to the proportion of invariant sites that are fixed to have zero evolutionary change and facilitates model convergence.

While noting that the available sequence data across species was limited, the branch order of the resultant phylogeny (Figure 5) is fully consistent with expectations from other studies where comparison is possible (Marcus and

McCune 1999; Hamilton 2001; Jones et al. 2013; Figure 5). Expressed as a phylogenetic distance matrix among species (Table 5), we estimated correlations of r=0.207 (P=0.195) and r=0.054 (P=0.305) with Δ and θ respectively. However, a much stronger, though marginally non-significant, correlation of 0.554 (P = 0.074) was estimated between phylogenetic distance and subspace dis-similarity (defined as $2\text{-}K_{s1,s2}$ for each pair of species). This suggests that the phylogenetic distance between species explains little of the among-species patterns of variation in trace and $\mathbf{I_{max}}$, but when considering the first two eigenvectors together as a 2-dimensional subspace, there is a much larger phylogenetic signal.

5.5 Discussion

We tested whether the OFT, a commonly used personality assay, effectively captures a simple shy-bold axis of personality variation in 7 species of small fish. Using a repeated measures approach, we estimated and compared **I** matrices among species (using Δ, θ and K) and asked whether species similarity was predicted by phylogenetic relatedness between the species. Trace comparisons showed that *D. rerio* and *P. reticulata* differed from the other species most in the amount of among-individual variation present. Conversely, eigenvector decomposition reveals that species differ in what main axis was captured. A shy-bold $\mathbf{I_{max}}$ was found in some species, while a stress response $\mathbf{I_{max}}$ was found in others. Despite differences in the leading eigenvector, all 7 species had a moderate to high similarity in the orientation of 2-dimensional subspace and the first two eigenvectors in all species were consistent with both shy-bold and stress response axes. Phylogenetic relatedness was not correlated with similarity in matrix trace or alignment of $\mathbf{I_{max}}$, suggesting that processes other than the shared

evolutionary history are important in shaping these aspects of **I**. There is, however, a stronger (though marginally non-significant) correlation between phylogenetic distance and similarity in 2-dimensional subspace. We view this as evidence for some phylogenetic signal in the structure of **I**. We discuss these findings within the context of the personality literature, with particular attention paid to the methodologies employed within animal personality research and the benefits of utilising a multivariate approach.

5.5.1 Trace comparison

Direct comparison of the matrices showed that both *P. reticulata* and *D. rerio* had significantly larger matrix traces than all other species, driven mainly by high variance in TI and TIM. Very little work has been done on how species differ in among-individual variation of behaviour and it is unclear why *P. reticulata* and *D. rerio* should have much higher traces than the other species. We note that these two species were assayed in a smaller tank (in absolute terms) and so cannot rule out the possibility that methodological differences in the assay are important. We note however, that the smaller tanks were used to reduce (among-species) variation in arena size relative to average body size. Thus despite the smaller tank size *P. reticulata* was actually in OFT with the largest tanksize:bodysize ratio (Table 1). However, this ratio was actually very similar to those experienced by other species such as *X. birchmanni* and *X. maculatus* so it is not possible to conclude it is the driver of high variance in TI.

5.5.2 Leading axis of variation ($\mathbf{I_{max}}$) comparison

Across the 7 species assayed, in only three (*X. birchmanni*, *L. nigrofasciata* and *X. maculatus*) did $\mathbf{I_{max}}$ correspond qualitatively to our *a priori* expectations, under

a verbal model, of boldness. In two others (*X. helleri* and *P. reticulata*) $\mathbf{I}_{\mathbf{max}}$ could be interpreted as a stress response, while in the remaining species (*D. rerio* and *X. eiseni*) there is no clear interpretation of $\mathbf{I}_{\mathbf{max}}$. Although in *X. eiseni*, this pattern of trait loadings on $\mathbf{I}_{\mathbf{max}}$ is, in fact, driven by sexual dimorphism in $\mathbf{I}_{\mathbf{max}}$. Males fall on a shy-bold axis, whereas females fall on a stress response axis (results not shown). The addition of sex as a fixed effect to control for these differences produced very similar $\mathbf{I}_{\mathbf{max}}$ estimates for all species, however, so it is still unclear as to the identity of this final axis of variation. Ultimately, this means the use of the OFT to assay boldness variation in this way is not equally effective in all species, or the expectation of a simple shy-bold continuum is not appropriate to all species.

The lack of a clear, consistent shy-bold axis in **I** within- and among-species is consistent with other studies that are increasingly finding a lack of correlation among observed traits thought to represent the same underlying personality axis. For instance, Beckmann and Biro (2013) found that emergence time in a novel environment and emergence time after a simulated predator attack in the home tank, both assumed to represent boldness, are uncorrelated in damselfish, *Pomacentrus wardi* and *P. amboinsensis*. A similar pattern was found in the chacma baboon *Papio ursinus*, where responses to a threatening object and novel object, again both assumed to measure boldness, were uncorrelated (Carter et al. 2012c) and behaviours from two exploration test set ups were unrelated in the great tit, *Parus major* (Arvidsson et al. 2017). The failure to find expected correlations among different proxies even within populations raises serious questions about the extent to which univariate studies of boldness (or

other personality traits) can be assumed to be assaying the same biological phenomenon.

5.5.3 Eigen subspace comparison

As discussed above, focusing on $\mathbf{I}_{max}$ we found the OFT revealed a shy-bold type axis in some species but not others. However, consideration of the leading axis alone is potentially misleading, since only in *X. maculatus* was **I** strongly dominated by the first eigen vector. In most species, $\mathbf{I}_{max}$ represented just over half of the among-individual variation, with the second eigenvectors capturing the vast majority of the remainder. Furthermore, we found that across 2-dimensional subspace, species were remarkably similar (as measured by the Krzanowski similarity index), a conclusion that differs from that based on $\mathbf{I}_{max}$ alone. In other words, while alignment of $\mathbf{I}_{max}$ was generally poor, the first two eigenvectors together actually captured quite similar (multivariate) variation in all species. This study is the first, to our knowledge, to use the Krzanowski similarity index to compare behavioural matrices among species, although it is quite widely used in comparative quantitative genetics (as one of several approaches to comparing genetic covariance matrices; (Hine and Blows 2006; Teplitsky et al. 2013; Aguirre et al. 2014; Puentes et al. 2016)).

It is important to bear in mind that eigen vectors (or principal components of raw data) are statistical properties of the multivariate trait distributions that reflect correlation. They can be consistent with, but not proof of, latent variables driving observed trait variation. With this caveat in mind, one interpretation of our data is that the OFT captures two aspects of personality. Those species whose $\mathbf{I}_{max}$ captured a shy-bold axis had a stress response second axis and vice versa. *X.*

maculatus was the only species that deviated from this pattern, where the vast majority of the among-individual variation was captured by $\mathbf{I}_{max}$. In this species, the uniformly strong positive correlations among traits in **I** generate a clear $\mathbf{I}_{max}$ matching our shy-bold expectations. For all other species, it must be remembered that because each successive eigen vector is defined as being orthogonal to the one proceeding it, the direction of the second eigen vector is not entirely independent of the first. Despite this, it is quite often useful to consider what the second eigen vector may represent, particularly when considering latent personality variables. In a review of assays and analysis techniques for personality, Toms et al. (2010) concluded that boldness and anxiety or fearfulness are difficult to tease apart in the OFT and emergence assays and responses in these assays may depend on the ecological background from which the species evolved.

5.5.4 Personality and phylogeny

Finally, we found that phylogenetic relatedness between the species explained very little of the pattern of similarity in trace, and likely none of the pattern of similarity in $\mathbf{I}_{max}$. While few studies compare among-individual covariance structures between species within a phylogenetic framework, work at the inter-species level generally finds a weak relationship between phylogenetic relatedness and behaviour. For instance, there was no significant phylogenetic signal detected for a relationship between exploratory behaviour, age at first reproduction and metabolic rate in 17 species of muroid rodents (Careau et al. 2009) and foraging behaviour varied according to habitat use rather than relatedness in 31 species of West Indian *Anolis* lizards (Johnson et al. 2008). More generally, behaviour is considered more 'evolutionarily labile' and subject

to strong and immediate environmental effects compared to morphological and physiological traits, resulting in a generally low phylogenetic signal for behavioural traits (Blomberg et al. 2003; Garamszegi et al. 2013). In the present study, the species vary in their life-history and reproductive behaviour. *P. reticulata* are a shoaling species with very little agonistic or aggressive behaviour towards conspecifics, whereas the swordtail species *X. birchmanni* and *X. helleri* are much more aggressive, with males competing for access to females and food resources (Magellan and Kaiser 2010; Boulton et al. 2012; Wilson et al. 2013), behaviour mirrored in *L. nigrofasciata* (pers. Obs.). While the groupings of species according to the direction of $\mathbf{l_{max}}$ does not necessarily fit with differences in shoaling tendency or aggressive nature, the selection that produced these different strategies may have indirect effects on boldness (Piyapong et al. 2010; Briffa et al. 2015).

Unlike with the other comparison metrics, the correlation between species relatedness and subspace similarity was moderate, suggesting that there may be some phylogenetic signal for higher dimensional subspace. Lower order aspects of covariance matrices such as trace and the leading eigen vector may be altered by strong stabilising or directional selection, thus removing any phylogenetic signal. Speculatively, the larger phylogenetic signal in 2-dimensional subspace would suggest that higher order metrics of comparison may be relatively resilient to selection, at least in the few species assayed here. This is not supported in the literature, however, with subspace comparisons of mating calls among 7 species of field cricket having low phylogenetic signal (Blankers et al. 2016). It is important to note that we were unable to estimate error around the subspace comparisons and the phylogenetic estimate via maximum likelihood similarly lacks error, so

significance of the relationship between relatedness subspace similarity is not available. At least qualitatively, this relationship is higher than the lower order metrics used.

5.6 Conclusion

In conclusion, the OFT does not capture the same main axis of among-individual variation across the 7 fish species used. While the traditional shy-bold axis was found in 3 species, the remaining 4 species exhibited a stress response axis. Comparisons across the effective dimensions, however, shows that species are relatively similar with shy-bold and stress response axes being found in all species across the first two eigen vectors. The low phylogenetic signal for trace and $\mathbf{I_{max}}$ differences among species suggests that strong selection or environmental effects causes species to differ above and beyond what we would expect from relatedness alone. This study advocates the use of multivariate mixed modelling and eigen vector decomposition of the among-individual (co)variance matrix, rather than a single trait approach, as valuable tools for identifying latent personality variables. Future work should apply this multivariate approach to other species to estimate personality axes in a more direct way, from single personality assays. Progression to including traits from multiple different assays that are known to measure the same or different personality axes may offer a more robust and multivariate way of validating observed traits.

Table 1: Estimates of Δ, the difference in **I** matrix traces (with 95% CI) between each species pair.

	D. rerio	*L. nigrofasciata*	*P. reticulata*	*X. birchmanni*	*X. eiseni*	*X. helleri*
L. nigrofasciata	0.783 (0.147, 1.498)					
P. reticulata	0.258 (-0.893, 0.441)	1.042 (0.795, 1.28)				
X. birchmanni	0.790 (0.095, 1.405)	0.007 (-0.25, 0.207)	1.049 (0.882, 1.197)			
X. eiseni	0.692 (-0.016, 1.405)	0.091 (-0.235,0.443)	0.951 (0.651, 1.232)	0.098 (-0.165,0.368)		
X. helleri	0.700 (0.021, 1.387)	0.084 (-0.213,0.372)	0.958 (0.699, 1.174)	0.090 (-0.117,0.309)	0.008 (-0.31, 0.325)	
X. maculatus	0.377 (-0.299, 1.122)	0.406 (0.061, 0.739)	0.636 (0.327, 0.916)	0.413 (0.135, 0.715)	0.315 (-0.053,0.682)	0.323 (-0.01, 0.66)

Table 2: The first (a) and second eigenvector (b) of **I** each species, with associated eigen values, percent of total among-individual variance explained and the loadings of each trait on the vectors.

a)	*D. rerio*	*L. nigrofasciata*	*P. reticulata*	*X. birchmanni*	*X. eiseni*	*X. helleri*	*X. maculatus*
Eigen value	0.704	0.261	0.842	0.250	0.348	0.305	0.706
Percentage	58.388	62.010	57.570	60.391	67.892	60.352	85.349
Loadings							
TI	0.760	0.165	-0.593	0.563	0.321	-0.192	0.237
Act	0.173	0.354	-0.436	0.583	0.728	-0.423	0.383
AC	0.469	0.853	0.353	0.495	0.310	0.134	0.644
TIM	-0.416	0.346	0.578	0.314	-0.521	0.875	0.619
b)							
Eigen value	0.368	0.149	0.407	0.139	0.156	0.178	0.112
Percentage	30.576	35.352	27.785	34.477	30.472	35.313	13.561
Loadings							
TI	0.192	0.233	0.541	0.376	0.099	0.304	0.333
Act	0.192	0.540	0.357	0.337	0.171	0.615	0.513
AC	0.395	0.057	0.614	-0.315	0.682	0.682	0.283
TIM	0.878	-0.806	0.450	-0.804	0.704	0.258	-0.739

Table 3: Angle θ between estimates of $\mathbf{I_{max}}$ for each species pair (with approximate 95% CI in parentheses).

	D. rerio	*L. nigrofasciata*	*P. reticulata*	*X. birchmanni*	*X. eiseni*	*X. helleri*
L. nigrofasciata	63.7 (32.2, 90.0)					
P. reticulata	73.1 (6.5, 83.1)	75.6 (37.4, 89.9)				
X. birchmanni	50.9 (24.3, 89.3)	33.9 (22.7, 79.7)	76.6 (56.9, 89.9)			
X. eiseni	42.9 (31.9, 83.6)	66.8 (15.2, 89.8)	45.6 (26.1, 76.7)	53.5 (24.2, 86.4)		
X. helleri	58.7 (32.9, 89.9)	76.4 (17.650, 89.9)	31.6 (24.7, 66.3)	89.2 (33.8, 89.9)	38.4 (4.7, 84.2)	
X. maculatus	73.1 (26.0, 89.9)	20.3 (9.1, 74.2)	73.9 (62.4, 85.5)	29.6 (9.1, 51.5)	76.6 (34.7, 89.9)	65.1 (21.0, 89.9)

Table 4: Krzanowski's index of two-dimensional subspace similarity (K) among species specific **I** matrix estimates.

	D. rerio	*L. nigrofasciata*	*P. reticulata*	*X. birchmanni*	*X. eiseni*	*X. helleri*
L. nigrofasciata	1.433					
P. reticulata	1.548	1.536				
X. birchmanni	1.635	1.681	1.918			
X. eiseni	1.453	1.961	1.628	1.806		
X. helleri	1.518	1.934	1.621	1.830	1.991	
X. maculatus	1.676	1.904	1.560	1.788	1.933	1.963

Table 5: Phylogenetic distance between species, given as the difference in the number of nucleotide substitutions. A larger value between two species indicates greater phylogenetic distance.

	D. rerio	*L. nigrofasciata*	*P. reticulata*	*X. birchmanni*	*X. eiseni*	*X. helleri*
L. nigrofasciata	1.605					
P. reticulata	1.738	0.509				
X. birchmanni	1.577	0.549	0.682			
X. eiseni	1.463	0.980	1.112	0.952		
X. helleri	1.571	0.542	0.675	0.167	0.945	
X. maculatus	1.564	0.536	0.668	0.161	0.939	0.106

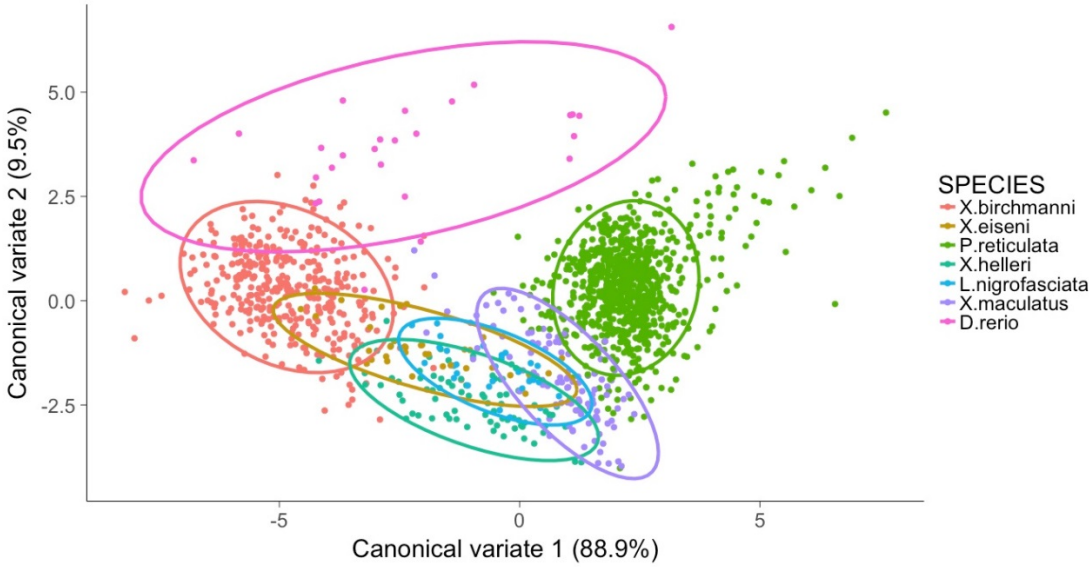


Figure 1: CVA of all 7 species, with individuals plotted on the first two canonical variates. Confidence ellipses, assuming multivariate normality, are shown.

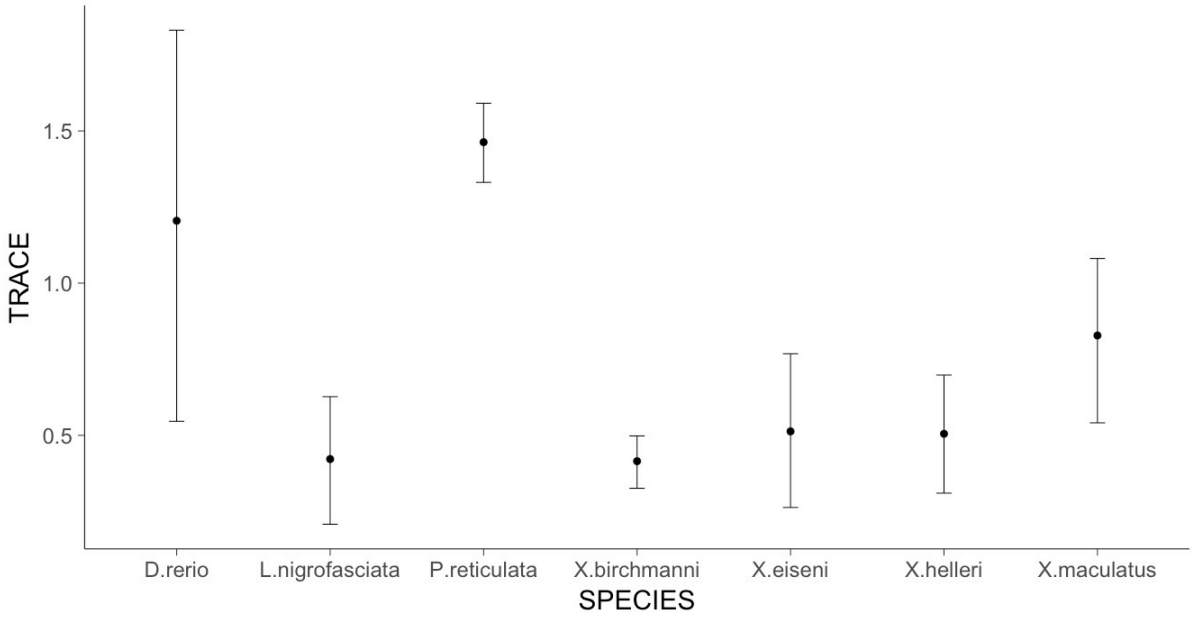
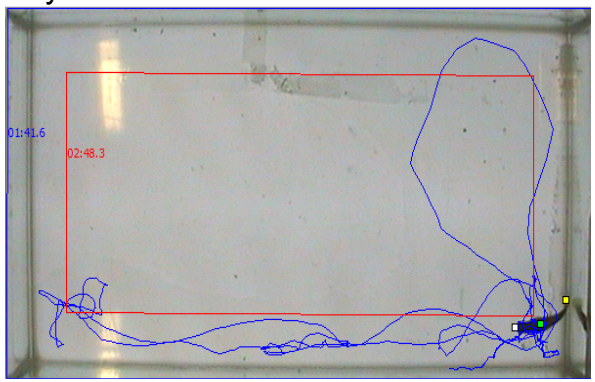


Figure 2: Total multivariate variance (trace) for each species. 95% CI shown.

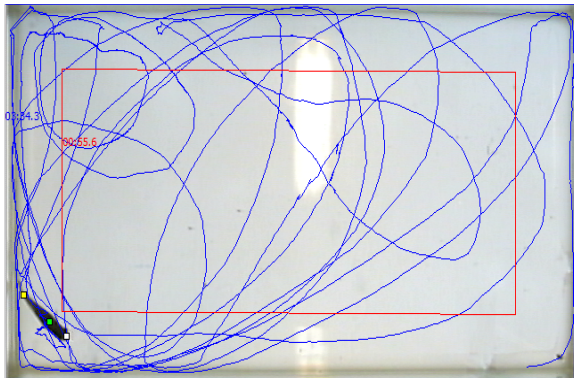
Figure 3: Lower and upper extremes of the two main axes of behavioural variation captured by the OFT: (a) shy-bold and (b) stress response. The blue line is the track of the fish over the 4.5 minute OFT. The red line separates the inner zone from the outer zone. In (a), all OFT traits load positively onto $\mathbf{l_{max}}$, with a shy individual in the left panel and bold individual on the right panel. In (b), TL and Act load antagonistically onto $\mathbf{l_{max}}$, driven by reactive individuals 'frantically' seeking shelter (left panel) vs less reactive individuals exploring more of the tank area (right panel). These screenshots are taken from the Viewer software used to track individual movement during the OFT. Example trials are taken from (a) L. nigrofasciata and (b) X. helleri.

(a) Shy-bold axis

Shy

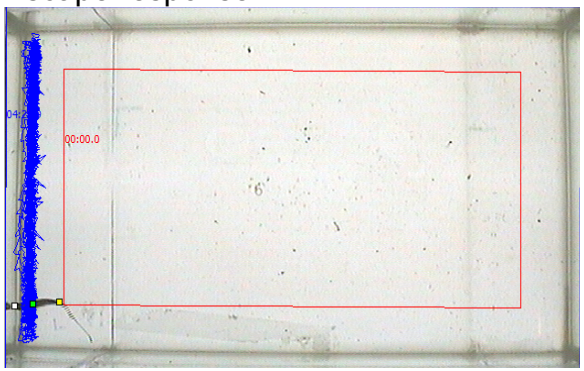


Bold

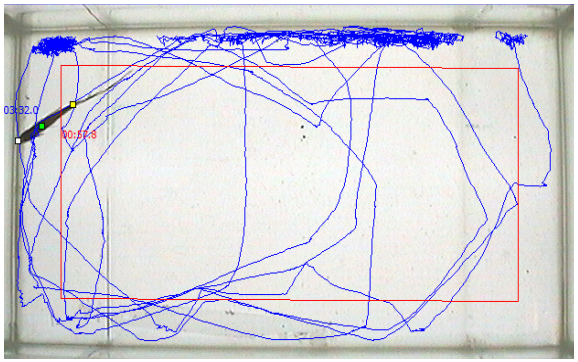


(b) Stress response axis

Escape response



Bold



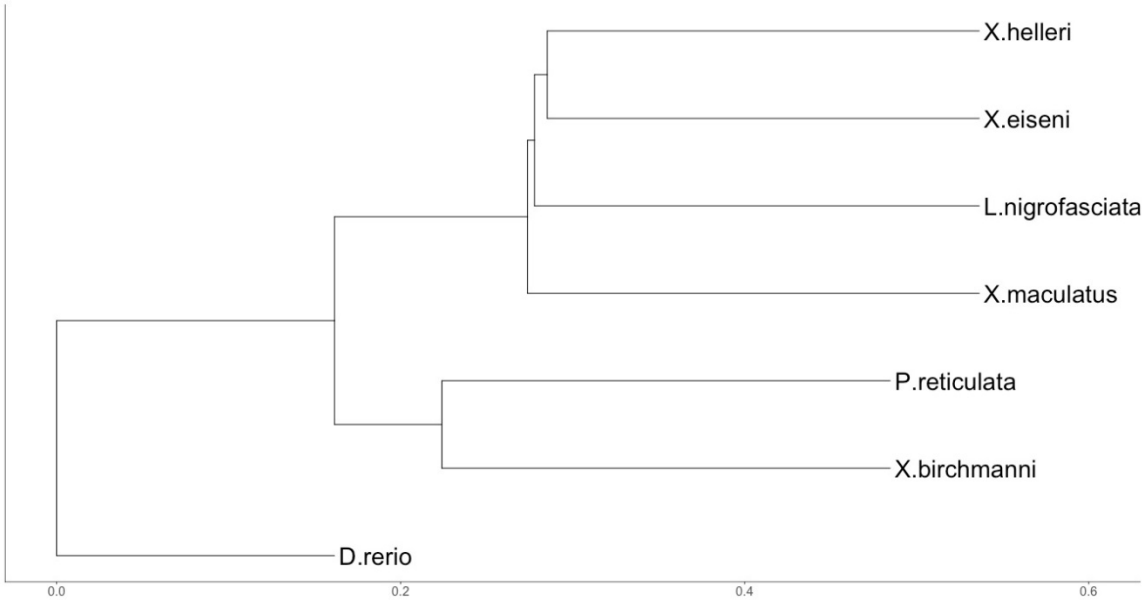


Figure 4: Cladogram of behavioural distance between species using an inverted from of the Krzanowski index.

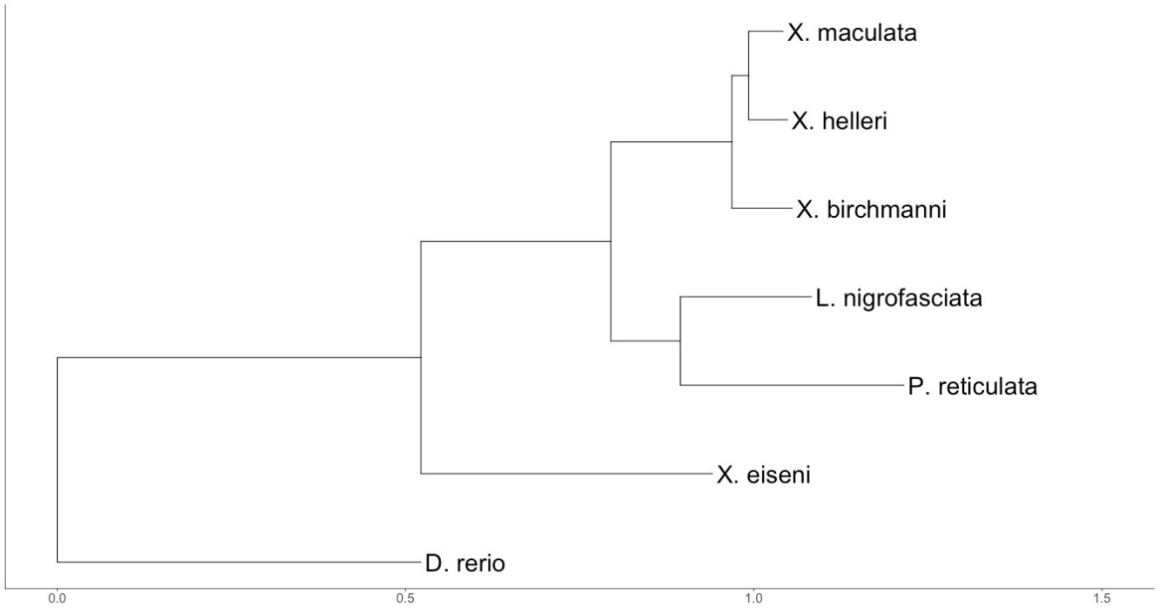


Figure 5: Phylogram of phylogenetic distance between the species based on the cytochrome b gene.

Chapter 6

General discussion

6.1 Overview

The broad aims of this thesis were to identify patterns of consistent among-individual (co)variation in behaviour and to examine in detail the genetic (co)variance structure underpinning this variation. Adaptive models, both verbal and mathematical, seek to resolve the questions of why individuals are consistent and variable in their behaviour – or, in simple terms, what maintains personality variation? A fundamental assumption of these models is that the behavioural traits in question have a significant genetic basis of variation. Indeed, an essential element of the evolutionary study of any complex trait is a comprehensive appraisal of its genetic basis of variation. Estimates of heritability are becoming more common for personality traits as quantitative genetic tools become more accessible, but in order to truly assess the adaptive nature (or otherwise) of animal personality, more comprehensive treatments of the genetic architecture of behavioural traits are required. Studies on the fitness consequences of personality traits are, of course, important in expanding our understanding of the potentially adaptive nature of personality. It is vital, however, that this selection information is interpreted with knowledge of the genetics underpinning these traits. Univariate estimates of heritability, alone, are insufficient for this task given the complexity of genetic associations among traits expressed within individuals as well as associations across individuals in different generations or of a different sex. Here, I summarise the results of each chapter before suggesting

improvements to current statistical methodologies and future work for assessing the adaptive nature of animal personality.

6.2 Metabolism, personality and pace of life in the Trinidadian guppy, *Poecilia reticulata*

The aim of this chapter was to test the importance of pace-of-life as a mechanism for maintaining among-individual variation in behaviour in the guppy. I quantified the among-individual covariance structure between growth, metabolic rate and behavioural traits thought to represent a shy-bold axis. The Pace of Life syndrome (POLS) hypothesis predicts that boldness, growth and metabolic rate should all covary positively, which is assumed to be the result of correlational selection. While an intuitive route through which behavioural variation might be maintained, there is mixed support for the POLS hypothesis. Previous studies have quite often used measures of resting metabolic rate and tested how it covaries with behaviour, however. Here, I used metabolic scope, which can be considered a better proxy for the metabolic capacity available for behaviours to be expressed.

I found that there was no among-individual variation for any metabolic trait after accounting for individual size. This means that metabolism is unlikely to be a plausible driver of among-individual variation in behaviour in this instance. In addition, there was no support for covariance between behaviour and growth rate. This chapter therefore does not support the Pace of Life hypothesis. The behavioural traits observed in the open field trials (OFT) were predicted to positively covary among-individuals based on (i) an *a priori* expectation that the OFT captures a “shy-bold” axis of variation, and (ii) literature-derived

expectations of what constitutes bold behaviour in a small fish. This was not the case however, and I interpreted observed patterns as being more consistent with an axis of variation in stress (and/or) escape response in the guppy.

Overall, the POLS framework does not readily explain the maintenance of among-individual variation in behaviour in this system. This suggests that perhaps there are other mechanisms at work either instead of, or in tandem with, the effects of correlations among behavioural, metabolic and life-history traits in maintaining personality in guppies. While some studies have found support for the POLS framework, my results together with other recent work suggests that POLS is not a universally robust framework for understanding why individuals vary behaviourally. Nevertheless, it could be argued that it remains a good starting point in attempting to understand the integration of behavioural variation with other aspects of the phenotype. Regardless of whether the framework's specific predictions are upheld in any instance, the approach places strong emphasis on considering how the phenotype as a whole evolves under selection.

This chapter also draws attention to the value and power of a multivariate framework in quantifying not only the covariance structure between personality and other traits but also in the estimation of personality itself.

6.3 Maternal and genetic effects on personality over ontogeny in the Trinidadian guppy, *Poecilia reticulata*.

In the quantitative genetics literature, the strength of maternal effects is predicted to wane as individuals age and mature, giving way to the increasing influence of additive genetic effects. This pattern is commonly observed in morphological and life-history traits, but little work has been done on investigating this pattern in behavioural traits. The main aim of this chapter was to test the above prediction

and assess the relative contributions of maternal and additive genetic effects to behavioural variation, specifically in 'boldness', personality over ontogeny. I used a "hybrid" approach to estimate maternal effects, in which effects of both specific (I.e. known maternal traits) and non-specific maternal identity effects (arising from unknown traits) were estimated at two ontogenetic stages. This provides the advantage of testing the hypothesised effects of specific maternal phenotypes (and associated genotypes) on offspring behaviour without assuming there are no other sources of maternal effects. Following on from this, an additional aim of the chapter was to evaluate the estimated upward bias of estimated heritability that could arise if maternal effects were not explicitly modelled. This is important since, to date, few researchers have attempted to test for or estimate maternal effects in quantitative genetic analyses of personality. Finally, I estimated whether the maternal traits estimated above, themselves, had a genetic basis, also termed maternal and grand-maternal genetic effects.

In agreement with my first prediction, I found that both maternal identity variance and specific maternal traits had a lower influence on adult offspring behaviour, relative to juveniles. I also found support for the widely accepted premise that failure to model maternal effects leads to upward bias of heritability estimates. Finally, I found that maternal weight is genetically variable (and so a likely source of maternal genetic effects on offspring behaviour) but also subject to maternal effects of its own. These results together indicate that in order to predict selection response, we not only need to control for maternal effects when estimating heritability, but we also need to estimate quantitative genetic parameters over multiple life stages. Furthermore, the presence of maternal and grand-maternal (genetic) effects indicates that offspring personality is capable of

responding to selection on weight in previous generations. Ultimately, this means that a more holistic approach, in terms of generations considered and point long the life-cycle measure, is required in order to improve our understanding of the evolutionary dynamics of personality traits.

6.4 Sexual dimorphism and Genotype-by-Sex interactions of personality in the Trinidadian guppy, *Poecilia reticulata*

While sexual dimorphism in average behaviour (as well as morphology and life-history) is common, few studies have examined, in detail, the sex differences in the among-individual (co)variance structure of behavioural traits that characterise personality. Furthermore, to date there has been very little attempt to test for and characterise within- and across-sex additive genetic (co)variance structures, that ultimately determine the potential for sexually antagonistic selection to drive the evolution of dimorphism. In this chapter, I address these gaps for growth and 5 behavioural traits thought to represent a shy-bold/stress axis. The first part of this chapter consisted of a comprehensive treatment of evolved sexual dimorphism in behaviour and growth using univariate and multivariate frameworks. The second part utilised a quantitative genetic analysis to assess the future scope for sexual divergence. If the sexes differ in the genetic variance available for traits or the genetic covariance structure between multiple traits (i.e. GxSex interactions), then a sex-specific response to sexually antagonistic selection is possible. If the sexes are uniform in their additive genetic (co)variance structure, then sexually antagonistic selection could be a possible mechanism through which genetic (and among-individual) variation can be maintained over the long-term.

Here, I showed that guppies exhibit sexual dimorphism in size and growth as expected, but also in average expression of heritable traits linked to risk-taking behaviour. Although the structure of among-individual behavioural (co)variation (estimated as an **I** matrix) was similar in males and females, single trait and multivariate analyses also provide evidence of some GxS interactions. This would suggest that there is some capacity for the sexes to diverge further (under appropriate selection). Multivariate analyses show that while there was little overall difference between the $\mathbf{G_m}$ and $\mathbf{G_f}$ matrix structures**,** the $\mathbf{G_{max}}$ axes were almost orthogonal. However, estimation of the **B** matrix revealed only very slight asymmetry between the sexes, so this is unlikely to provide extra avenues of divergence or constraint.

While these results indicate that the sexes can diverge in multivariate phenotype to some degree, the overall picture is one of shared genetic architecture between the sexes. Thus the potential for further evolution of sexual dimorphism is likely to be moderate. This chapter highlights the potential of sexual antagonism when considering what mechanisms might maintain variation in behaviour. In guppies, there is still some possibility of sex-specific responses to selection, but common genetic architecture may be a factor in maintaining behavioural variation, if selection in the field is antagonistic. Moreover, the use of fully multivariate approaches, while computationally demanding, gives a more complete estimate of how traits are expected to interact with selection, when compared to more widely used univariate analyses.

6.5 Phylogeny and among-individual variation in behaviour: a comparative approach to animal personality

In the personality literature, verbal arguments are generally used to justify the choice of specific observed behaviours to represent a particular latent personality variable. Very commonly, a single observed behaviour is used as a proxy for a personality variable. However, recent work has resulted in questions being raised about whether this approach allows valid generalisation of inferences about personality across populations or species, even when similar assays are used. In fact, there have been few comparative studies of personality among related species and almost no attempt at incorporating a phylogenetic component. This is an important omission because a phylogenetic approach allows us ask whether patterns of among-individual variation are changing with species divergence over evolutionary time (giving a high phylogenetic signal), or whether they change more rapidly. In the latter case we can begin to ask, what differences in selective environments experienced by closely related species could drive this divergence in (co)variance structure?

In this final chapter, I quantified among-individual (co)variation structure for four traits from a common personality assay, the open field trial (OFT), across 7 species of small tropical fish. I compared aspects of the among-individual (co)variance structure (**I**) and asked whether the assay was capturing the same personality axis across all species. I predicted that the OFT would capture a shy-bold axis, specifically manifest as positive among-individual covariance between the measured traits (which would all be repeatable). In addition, I compared the trait subspace characterised by the first two eigen vectors of **I**. Following on from this, I approximated the strength of phylogenetic signal in personality. I found that

the OFT does not capture the same major axis of among-individual variation across the 7 fish species. While $\mathbf{I}_{max}$, the leading vector of **I**, was consistent with predictions of a shy-bold personality axis in 3 species, in the remaining 4 species it was better interpreted as reflecting variation in stress response. Nonetheless, more holistic comparisons using two-dimensional subspace (rather than just the leading axis of variation), revealed that all species are actually relatively similar. Therefore, the OFT can be interpreted as capturing both shy-bold and stress response variation in all species, although they differ in which signal dominates **I**. Finally, phylogenetic signal was strongest (though marginally non-significant) for this higher dimensional comparison, than it was for other measures of cross-species **I** matrix (dis)similarity. Although larger studies (with more species) are likely required, my results are at least suggestive of patterns of behavioural variation in higher dimensions being evolutionarily conserved across species. We can speculate that the total behavioural repertoire required by these related species is very similar, but the most important axis of variation in each case is determined by local selection conditions.

6.6 Concluding remarks and directions for the future

There have been several adaptive frameworks developed to explain the presence of among-individual variation in behaviour, but in order to fully test these adaptive models we require both knowledge of selection and a detailed assessment of the underlying genetic architecture. Estimates of selection on behavioural traits are relatively common in the literature, but the presence of a significant heritable basis to traits is often assumed rather than directly measured. This is not surprising owing to the large sample sizes required for robust quantitative genetic analysis. Consequently, among-individual variation is often argued to be a

suitable proxy for underlying additive genetic variation available to selection, but this has rarely been tested. While I found among-individual and genetic covariance structures to be strongly related in guppies, this may not always be the case. If the goal is to make micro-evolutionary inferences, then it is always better to directly estimate the additive genetic variation where possible, and caution is required when taking phenotypic patterns of trait (co)variance as a proxy. Clearly this view is shared by others and the past few years have seen an increasing number of studies estimating the quantitative genetic parameters required for estimating heritability of behaviour. However, while most studies have been univariate, in this thesis I have shown that a single estimate of trait heritability is not sufficient to describe the quantitative genetic architecture.

Changes in additive genetic variance over ontogeny, maternal (genetic) effects and GxSex interactions will all impact how traits will respond to selection, often in unintuitive ways. In addition, the univariate focus on heritability ignores the reality among-trait genetic covariance structure can both constrain and facilitate responses to selection. Genetic (and therefore among-individual) variation could well be maintained by conflict between multiple, genetically correlated traits. The majority of this thesis represents a thorough treatment of the genetic architecture underlying behavioural traits in guppies. This is, however, only half of the puzzle, as I have not estimated selection (neither in the lab nor in ecologically relevant field conditions). It is therefore difficult to comment precisely on how such genetic nuances will affect evolutionary trajectories or the maintenance of variation in this species.

What is required moving forward are model systems that allow us to obtain both ecologically relevant selection estimates over time and in depth assessments of the genetic architecture underlying behavioural traits. This can

be expanded to comparative studies across populations and species, the latter incorporating phylogenetic relatedness, to identify general patterns of among-individual variation and ask how these are related to particular selection regimes.

In tandem with these approaches, the field of personality research will benefit greatly from trying to understand how much among-individual variation in behaviour is expected if these traits were not under selection at all. As mentioned earlier, many of the frameworks and models for explaining personality are adaptive and assume some form of balancing or antagonistic selection on traits, maintaining variance. Because simple directional and/or stabilising selection quite often erodes additive genetic (and presumably among-individual) variation, an alternative explanation for the presence of variation could be that these traits are largely neutral, arising from stochastic developmental or environmental conditions. Arguably, the true test of whether an adaptive explanation for personality is really needed would be to determine whether we see more phenotypic variance than expected if there were no selection acting. This is, however, a difficult question to answer.

From a methodological point of view, I have highlighted quantitative genetic modelling approaches as providing an ideal framework for investigating among-individual variation in behaviour, even in the absence of pedigree data. They allow patterns of among-individual (co)variation in behavioural traits to be estimated and, through the use of multivariate mixed models and eigen decomposition, direct estimation of axes of among-individual variation to be summarised. This multivariate approach is especially useful when we consider the persistent semantic and practical confusion in the literature about what personality axes are, and how they can best be characterised from observed

behavioural traits. The field is moving in the right direction with increasing use of validation techniques borrowed from psychology and multivariate methods (e.g. PCA). I advocate the use of multivariate modelling practices for identifying axes of personality variables rather than using a single trait approach with verbal justification. Future work should apply this multivariate approach to testing common personality assays in other species. Work should also include traits from different assays that are argued to measure the same or different personality axes, and in doing so, gradually build a more robust and multivariate way of validating observed traits as indicators of personality.

This argument for multivariate modelling extends equally to the concept of 'behavioural syndromes', or among-individual covariation between traits that themselves represent different functional behaviours/personalities. Currently, there is much confusion over how to interpret correlations between personality traits: does this indicate a behavioural syndrome potentially resulting from coadaptation of two functionally different traits, or does it more commonly mean the observed traits used are two proxies of the same underlying personality variable? Without multivariate analyses, the distinction between these scenarios risks becoming semantic and largely a matter of researcher preference. Animal personality is ultimately a multivariate phenomenon. If we are to make gains in understanding its causes, its consequences and the extent to which it is truly an adaptive phenomenon, we must more widely adopt empirical methods that fully reflect this in both our descriptive and inferential studies.

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Appendix 1

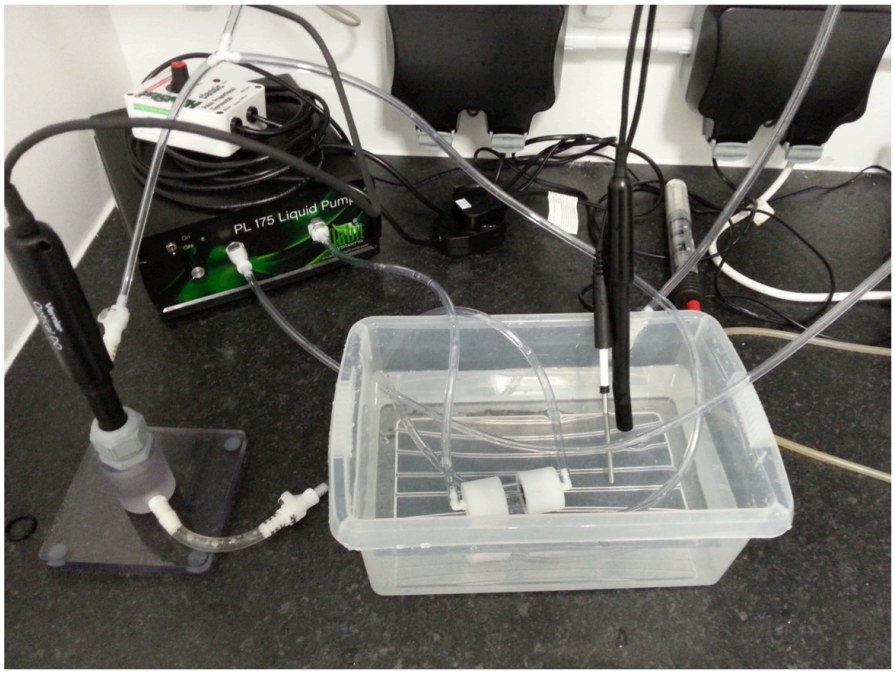
Metabolic rate estimation

A pump was used to deliver water from the water bath through to the respiration chamber past the optical dissolved oxygen (DO) probe and back into the water bath, in what is termed the 'flush' state. For oxygen consumption measures to take place the system was switched to a 'closed' state in which water only flowed between the pump, respiration chamber and DO probe, reverting back to the flush state upon completion of the measurement. This allows precise measurement of oxygen consumption while preventing the build-up of CO_2 and other waste products in the respiration chamber.

To account for bacterial respiration in the system, oxygen consumption of the empty respiration chamber was taken either before or after each fish measure and subtracted from corresponding fish metabolic rate measures. Finally, the fish volume relative to the system volume was corrected to produce whole animal metabolic rate in mg O_2
hr^{-1}:

$$\mathbf{VO2 = DO\ slope*(Vol_R - Vol_A)*3600}$$

Where **VO2** is the oxygen consumption rate ($mgO_2\ hr^{-1}$), **DO slope** is the rate of decrease of dissolved oxygen ($mg\ O_2\ L^{-1}\ s^{-1}$), $\mathbf{Vol_R}$ is the volume of the active respirometer in L (0.069L) and $\mathbf{Vol_A}$ is the volume of the fish also in L.



Aquatic respirometer setup, showing water bath and respiration chamber.

Appendix 2

Breeding design and pedigree management

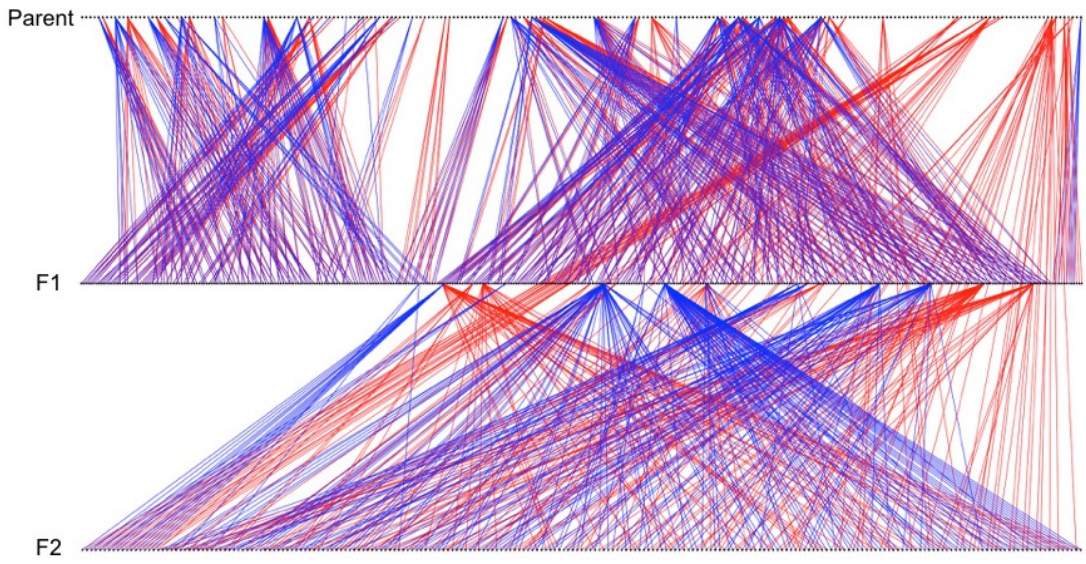
To create a pedigreed sub-population, female fish were haphazardly sampled from stock and isolated from male contact for 3 months. They were then tagged under anaesthetic (buffered MS222 solution) using visible implant elastomer (VIE) to allow individual identification and assigned to breeding groups of 4 females to one stock male, housed in 15L breeding tanks (18.5cm x 37cm x 22cm). Females were inspected daily, and heavily gravid individuals (as determined from swollen abdomens and an enlarged 'gravid spot') were isolated in 2.8L brood tanks to give birth. Although sperm storage from previous matings may persist, strong sperm precedence is also known in this species, thus we assume subsequent broods produced were sired by the known male. Once a brood was produced, maternal standard length (measured from tip of snout to caudal peduncle, mm), weight and brood size were recorded. The female was then returned to the breeding tank (with offspring raised initially in the brood tank; see below). Any females that did not produce a brood within two weeks of being isolated were returned to their breeding tank. Any offspring born in the breeding tank were returned to general stock, as we could not be sure of maternal identity.

In total 133 females and 38 male parental fish (P generation) were sampled from stock, of which 54 females and 33 males contributed to the first generation of offspring (F1 generation), which comprised 566 individuals from 72 broods in total. The F1 generation was produced in two breeding bouts, the first between April and November 2013 and the second between February and April 2014. A further offspring generation (F2) was then produced using adults from the F1

generation assigned to breeding groups (haphazardly sampled after isolating females for 3 months, but ensuring no inbreeding) between February and July 2015. For the F2 production we altered the protocol slightly - each female was kept in its own 2.8L tank, with a single male moved between 3 females in the breeding group on a weekly basis. This meant it was unnecessary to isolate females to collect broods, and removed the problem of unknown maternity for broods being produced in the larger tanks. A total of 25 females and 12 males contributed 281 F2 offspring from 34 broods. Thus, in total, we collected behavioural data (as described in main text) on 847 juvenile fish (F1 and F2 generations) contained within a pedigree structure having a maximum depth of 3 generations, and 45 sire and 79 dam individuals. Behavioural data were collected on 841 adult fish, comprising parental generation individuals (including those that did not contribute to the F1), as well as all F1 and F2 individuals that survived to the adult data collection period.

Offspring were kept initially in their brood tanks before, at an average of 56 days, being moved as families to larger “grow on” tanks (15L, 18.5cm x 37cm x 22cm). Standard length was measured on each fish on the day of birth and at ages 7, 14, 28, 42, 56, 70 and 84 days, using Vernier callipers. Note, however, that individuals cannot be identified at juvenile stage, precluding individual level analyses of repeated measures data. At an average age of 132 days (range 59-226) all F1 and F2 fish were taken from their brood groups, individually tagged using VIE and placed into mixed-family groups of 16 mature adults (8 males and 8 females). Tagged groups were housed in 15L tanks as described above. Note, that because individuals were not tagged until adulthood we cannot link the identity of those F1 fish that became parents of F2 fish to their juvenile phenotypic

records. However, the family of these fish is known, so for each we added their identity code (as a tagged F1 parent) to the set of dummy codes (for untagged individuals) corresponding to that family. This allowed us to maintain the integrity of known pedigree links between F1 and F2 generations in our animal model analyses.



Visualisation of the three generation (parental, F1 & F2) guppy pedigree structure. Black dots represent individuals, blue lines denote sire-offspring links and red lines denote dam-offspring links.

Supplemental tables 1

Supplemental table 1.1**:** Fixed effect estimates from univariate models of metabolic rate, all behaviours assayed, and standard length (see main text for details).

Trait	Fixed effect	Level	Effect size (SE)	DF	*F*	P
Em	Mean		-0.279 (3.378)	1, 73.9	0.01	0.919
	Temp		0.001 (0.142)	1, 74.1	0.00	0.992
	Group	2	0.076 (0.387)	3, 25.2	0.72	0.550
		3	0.303 (0.401)			
		4	0.484 (0.366)			
	Order caught	2	0.588 (0.372)	7, 87.7	1.06	0.397
		3	0.770 (0.369)			
		4	0.566 (0.368)			
		5	0.828 (0.376)			
		6	0.511 (0.381)			
		7	0.502 (0.424)			
		8	1.026 (0.497)			
	Trial		-0.041 (0.027)	1, 74.6	2.29	0.137
	Weight		0.197 (0.449)	1, 61.9	0.19	0.656
TI	Mean		2.205 (6.664)	1, 71.7	1.57	0.218
	Temp		-0.042 (0.289)	1, 71.7	0.02	0.880
	Group	2	-0.214 (0.352)	3, 23.8	2.18	0.118
		3	-0.214 (0.351)			
		4	0.548 (0.344)			
	Order caught	2	-0.597 (0.362)	7, 87.2	1.21	0.304
		3	-0.738 (0.351)			
		4	-0.055 (0.340)			
		5	-0.194(0.339)			
		6	-0.493 (0.333)			
		7	-0.144 (0.368)			
		8	-0.111(0.419)			
	Trial		0.050 (0.025)	1, 78.2	3.92	0.053
	Weight		1.064 (0.385)	1, 76	7.65	0.008
Act	Mean		1.846 (6.498)	1, 70.8	1.34	0.253
	Temp		-0.019 (0.283)	1, 70.8	0.00	0.941
	Group	2	-0.288 (0.369)	3, 24.1	1.92	0.154
		3	-0.346 (0.369)			
		4	0.435 (0.363)			
	Order caught	2	-0.655 (0.354)	7, 85.2	1.59	0.150
		3	-0.865 (0.345)			
		4	-0.066 (0.335)			
		5	-0.272 (0.334)			
		6	-0.573 (0.327)			
		7	-0.302 (0.363)			
		8	-0.178 (0.412)			
	Trial		0.032 (0.025)	1, 77.3	1.64	0.207
	Weight		0.983 (0.388)	1, 81	6.41	0.014

Trait	Fixed effect	Level	Effect size (SE)	DF	*F*	P
Order caught	Mean		0.198 (0.349)	1, 26.4	0.13	0.726
	Group	2	-0.096 (0.295)	3, 26.9	0.30	0.822
		3	-0.237 (0.297)			
		4	0.017 (0.292)			
	Trial		-0.052 (0.013)	1, 425	17.39	<0.001
	Weight		-0.202 (0.263)	1, 201	0.59	0.443
AC	Mean		17.78 (5.822)	1, 72.2	2.53	0.119
	Temp		-0.74 (0.253)	1, 72.2	8.72	0.004
	Group	2	0.067 (0.375)	3, 27.1	0.41	0.750
		3	-0.298 (0.375)			
		4	0.053 (0.369)			
	Order caught	2	0.043 (0.319)	7, 83.5	3.64	0.002
		3	-0.06 (0.313)			
		4	-0.098 (0.303)			
		5	-0.594 (0.303)			
		6	-0.628 (0.295)			
		7	-1.188 (0.331)			
		8	-1.186 (0.372)			
	Trial		0.081 (0.022)	1, 77.9	13.19	<0.001
	Weight		0.280 (0.366)	1, 89.1	0.59	0.445
TIM	Mean		-0.517 (5.91)	1, 71	1.65	0.205
	Temp		-0.025 (0.257)	1, 71	0.01	0.919
	Group	2	0.616 (0.362)	3, 25.2	1.99	0.142
		3	0.461 (0.362)			
		4	-0.128 (0.356)			
	Order caught	2	0.142 (0.323)	7, 83.8	1.95	0.072
		3	0.307 (0.317)			
		4	-0.449 (0.306)			
		5	-0.287 (0.306)			
		6	-0.085 (0.298)			
		7	-0.570 (0.334)			
		8	-0.729 (0.377)			
	Trial		0.021 (0.227)	1, 77.2	0.89	0.349
	Weight		-0.788 (0.364)	1, 86	4.69	0.035
Length	Mean		-0.722 (0.578)			
	Group	2	-0.112 (0.437)			
		3	0.339 (0.455)			
		4	-0.254 (0.422)			
	Last day		0.012 (0.007)			
	Days since start		0.167 (0.502)			
Mass-spec Metabolic rate	Mean		-0.668 (0.175)	1, 106	18.71	<0.001
	Measuretype		0.758 (0.062)	1, 106	163.85	<0.001
	Group	2	0.049 (0.840)	3, 106	0.22	0.884
		3	0.035 (0.084)			
		4	0.639 (0.082)			
	Weight		0.929 (0.129) -	1, 106	51.17	<0.001
	Order caught	2	0.077 (0.112)	7, 106	1.35	0.233
		3	-0.025 (0.113)			
		4	-0.106 (0.114)			
		5	0.731 (0.116)			

Trait	Fixed effect	Level	Effect size (SE)	DF	*F*	P
		6	-0.163 (0.115)			
		7	0.133 (0.124)			
		8	0.052 (0.127)			
	Trial		-0.005 (0.127)	1, 106	0.18	0.664

Supplemental table 1.2**:** Statistical inference among-individual covariance estimates between behavioural traits.

Among-individual covariance (COV_I) was tested in each case by likelihood ratio test comparison of a bivariate mixed models with COV_I freely estimated to one in which COV_I was constrained to equal zero. We assume twice the difference in model log-likelihood is distributed as χ^2_1.

Trait 1	Trait 2	χ^2_1	P
Track length	Order caught	0.028	0.867
Track length	Area covered	0.530	0.467
Track length	Time in middle	-7.34	0.007
Track length	Activity	8.51	0.004
Activity	Order caught	0.070	0.791
Activity	Area covered	0.781	0.377
Activity	Time in middle	-7.86	0.005
Order caught	Time in middle	0.002	0.964
Order caught	Area covered	0.04	0.841
Time in middle	Area covered	2.59	0.108
Emergence	Track length	0.312	0.576
Emergence	Activity	0.224	0.636
Emergence	Order caught	0.504	0.478
Emergence	Area covered	0.946	0.331

Supplemental table 1.3: Eigen vector decomposition of the among-individual variance-covariance matrix (I) for behavioural traits as estimated from the multivariate mixed model.

	Eigen 1	Eigen 2	Eigen 3	Eigen 4	Eigen 5	Eigen 6
Eigen values	0.983	0.554	0.336	0.199	0.009	0.001
Percentage	47.2	26.6	16.2	9.58	0.409	0.032
ET	0.159	-0.410	0.660	0.607	0.045	0.024
TI	0.546	0.118	-0.139	0.122	-0.688	0.424
Act	0.624	0.154	-0.062	0.033	0.072	-0.760
OC	0.198	0.051	0.651	-0.725	-0.042	0.084
AC	0.093	0.810	0.198	0.269	0.388	0.272
ID	-0.490	0.367	0.281	0.132	-0.606	-0.401

Supplemental table 1.4: Statistical inference of among-individual covariance terms between standard length (SL) and each behavioural trait. Note SL is modelled as a reaction norm with both intercept (size) and slope (growth) terms

Behavioural trait	χ^2_2	P
Em	2.49	0.288
TI	2.26	0.322
Act	1.95	0.377
OC	2.348	0.309
AC	6.053	0.048
ID	1.78	0.411

Among-individual covariance (COV_I) was tested in each case by likelihood ratio test comparison of a bivariate mixed models with COV_I freely estimated between behaviour and both $SL_{intercept}$ and SL_{slope} to one in which both behaviour-SL covariance terms were constrained to equal zero. We assume twice the difference in model log-likelihood is distributed as χ^2_2

Supplemental tables 2

Supplemental table 2.1: Likelihood ratio tests of comparison between full models with both additive genetic and maternal effects fitted vs null models with neither random effects.

Trait	Juvenile		Adult	
	χ^2_2	P	χ^2_2	P
TI	47.40	<0.001	40.23	<0.001
Act	54.44	<0.001	42.12	<0.001
AC	69.90	<0.001	21.42	<0.001
ID	13.82	<0.001	13.56	<0.001
Fr	35.95	<0.001	27.07	<0.001

Supplemental table 2.2: Heritabilities of all traits in full model vs. V_A-only model.

Full model

Trait	V_A	V_M	V_{PE}	V_{Group}	V_R	h^2	m^2	pe^2	$Group^2$
Juvenile									
TI	0.000	0.096 (0.033)	-	-	0.469 (0.028)	0.000	0.170 (0.049)	-	-
Act	0.000	0.134 (0.043)	-	-	0.474 (0.028)	0.000	0.220 (0.057)	-	-
AC	0.000	0.257 (0.077)	-	-	0.756 (0.045)	0.000	0.254 (0.059)	-	-
ID	0.000	0.098 (0.042)	-	-	0.907 (0.053)	0.000	0.097 (0.039)	-	-
Fr	0.000	0.113 (0.040)	-	-	0.634 (0.037)	0.000	0.151 (0.047)	-	-
Adult									
TI	0.056 (0.045)	0.079 (0.037)	0.215 (0.034)	0.043 (0.019)	0.423 (0.014)	0.068 (0.055)	0.097 (0.042)	0.263 (0.042)	0.053 (0.023)
Act	0.164 (0.055)	0.021 (0.023)	0.182 (0.040)	0.023 (0.014)	0.504 (0.017)	0.184 (0.058)	0.023 (0.026)	0.204 (0.046)	0.026 (0.015)
AC	0.167 (0.050)	0.000	0.114 (0.037)	0.155 (0.045)	0.587 (0.020)	0.163 (0.046)	0.000	0.111 (0.038)	0.151 (0.038)
ID	0.158 (0.056)	0.000	0.237 (0.044)	0.026 (0.015)	0.534 (0.018)	0.165 (0.055)	0.000	0.248 (0.048)	0.027 (0.016)
Fr	0.202 (0.054)	0.000	0.093 (0.039)	0.021 (0.013)	0.662 (0.022)	0.206 (0.051)	0.000	0.096 (0.041)	0.022 (0.013)

V_A-only model

Trait	V_A	V_{PE}	V_{Group}	V_R	h^2	pe^2	$Group^2$
Juvenile							
TI	0.252 (0.089)	-	-	0.348 (0.055)	0.420 (0.122)	-	-
Act	0.357 (0.120)	-	-	0.300 (0.069)	0.543 (0.138)	-	-
AC	0.674 (0.208)	-	-	0.422 (0.116)	0.615 (0.136)	-	-
TIM	0.174 (0.087)	-	-	0.829 (0.074)	0.173 (0.081)	-	-
Fr	0.278 (0.104)	-	-	0.499 (0.068)	0.358 (0.114)	-	-
Adult							
TI	0.120 (0.037)	0.186 (0.030)	0.065 (0.024)	0.424 (0.014)	0.151 (0.045)	0.234 (0.039)	0.082 (0.028)
Act	0.178 (0.050)	0.178 (0.038)	0.025 (0.014)	0.504 (0.017)	0.201 (0.052)	0.201 (0.044)	0.028 (0.016)
AC	0.167 (0.050)	0.114 (0.037)	0.155 (0.045)	0.587 (0.020)	0.163 (0.046)	0.111 (0.038)	0.151 (0.038
TIM	0.158 (0.056)	0.237 (0.044)	0.026 (0.015)	0.534 (0.018)	0.165 (0.055)	0.248 (0.048)	0.027 (0.016)
Fr	0.202 (0.054)	0.093 (0.039)	0.021 (0.013)	0.662 (0.022)	0.206 (0.051)	0.096 (0.041)	0.022 (0.013)

Supplemental table 2.3 – Fixed effect estimates and inference for juvenile and adult behavioural traits. All estimates are from “Full models” as described in main text without inclusion of offspring standard length as a covariate

Trait	Fixed effect	Effect size	DF	F	P
Juv TI	Generation 1	0.000	1, 36.3	11.58	0.002
	Generation 2	-0.404 (0.119)			
	Order 1	0.000	25, 587.0	1.26	0.179
	Order 2	0.346 (0.128)			
	Order 3	0.374 (0.132)			
	Order 4	0.372 (0.134)			
	Order 5	0.362 (0.135)			
	Order 6	0.206 (0.138)			
	Order 7	0.417 (0.140)			
	Order 8	0.301 (0.144)			
	Order 9	0.548 (0.151)			
	Order 10	0.378 (0.158)			
	Order 11	0.404 (0.168)			
	Order 12	0.473 (0.168)			
	Order 13	0.305 (0.178)			
	Order 14	0.383 (0.191)			
	Order 15	0.137 (0.200)			
	Order 16	0.545 (0.218)			
	Order 17	0.349 (0.218)			
	Order 18	-0.029 (0.226)			
	Order 19	0.503 (0.244)			
	Order 20	0.404 (0.255)			
	Order 21	0.210 (0.254)			
	Order 22	0.087 (0.302)			
	Order 23	0.424 (0.416)			
	Order 24	0.670 (0.416)			
	Order 25	-0.350 (0.504)			
	Order 26	1.007 (0.707)			
	Age	-0.050 (0.042)	1, 219.2	1.38	0.241
	Temp	0.603 (0.054)	1, 65.5	122.90	<0.001
Juv Act	Generation 1	0.000	1, 35.1	5.53	0.024
	Generation 2	-0.314 (0.134)			
	Order 1	0.000	25, 583.3	1.13	0.306
	Order 2	0.287 (0.129)			
	Order 3	0.347 (0.132)			
	Order 4	0.342 (0.135)			
	Order 5	0.310 (0.136)			
	Order 6	0.167 (0.140)			
	Order 7	0.426 (0.142)			
	Order 8	0.238 (0.145)			
	Order 9	0.556 (0.153)			
	Order 10	0.314 (0.159)			
	Order 11	0.345 (0.169)			
	Order 12	0.453 (0.169)			
	Order 13	0.283 (0.180)			

	Order 14	0.421 (0.193)			
	Order 15	0.163 (0.202)			
	Order 16	0.532 (0.220)			
	Order 17	0.401 (0.220)			
	Order 18	0.087 (0.228)			
	Order 19	0.482 (0.245)			
	Order 20	0.476 (0.257)			
	Order 21	0.301 (0.256)			
	Order 22	0.188 (0.304)			
	Order 23	0.479 (0.419)			
	Order 24	0.601 (0.419)			
	Order 25	-0.236 (0.508)			
	Order 26	1.152 (0.712)			
	Age	0.002 (0.044)	1, 247.6	<0.01	0.962
	Temp	0.604 (0.060)	1, 69.9	102.60	<0.001
Juv AC	Generation 1	0.000	1, 37.8	7.42	0.010
	Generation 2	0.494 (0.181)			
	Order 1	0.000	25, 584.7	1.40	0.097
	Order 2	-0.123 (0.163)			
	Order 3	0.024 (0.167)			
	Order 4	-0.145 (0.170)			
	Order 5	-0.126 (0.173)			
	Order 6	-0.217 (0.176)			
	Order 7	-0.351 (0.179)			
	Order 8	-0.529 (0.183)			
	Order 9	-0.103 (0.193)			
	Order 10	-0.395 (0.202)			
	Order 11	-0.417 (0.214)			
	Order 12	-0.287 (0.214)			
	Order 13	0.154 (0.227)			
	Order 14	-0.115 (0.244)			
	Order 15	-0.382 (0.255)			
	Order 16	0.196 (0.278)			
	Order 17	-0.433 (0.279)			
	Order 18	-0.664 (0.288)			
	Order 19	-0.197 (0.310)			
	Order 20	-0.389 (0.325)			
	Order 21	-0.375 (0.324)			
	Order 22	-0.528 (0.385)			
	Order 23	-0.296 (0.530)			
	Order 24	0.078 (0.530)			
	Order 25	-1.507 (0.641)			
	Order 26	-1.244 (0.900)			
	Age	0.129 (0.057)	1, 282.8	5.14	0.024
	Temp	-0.030 (0.079)	1, 80.4	0.14	0.705
Juv TIM	Generation 1	0.000	1, 32.8	<0.01	0.985
	Generation 2	0.002 (0.127)			
	Order 1	0.000	25, 591.3	1.01	0.457
	Order 2	-0.171 (0.179)			
	Order 3	-0.214 (0.183)			
	Order 4	-0.227 (0.185)			
	Order 5	-0.400 (0.188)			

	Order 6	-0.183 (0.192)			
	Order 7	-0.371 (0.194)			
	Order 8	-0.448 (0.199)			
	Order 9	-0.420 (0.210)			
	Order 10	-0.211 (0.219)			
	Order 11	-0.642 (0.233)			
	Order 12	-0.579 (0.232)			
	Order 13	-0.030 (0.247)			
	Order 14	-0.189 (0.265)			
	Order 15	-0.231 (0.278)			
	Order 16	-0.121 (0.302)			
	Order 17	-0.444 (0.303)			
	Order 18	-0.119 (0.313)			
	Order 19	-0.452 (0.338)			
	Order 20	-0.170 (0.354)			
	Order 21	-0.176 (0.353)			
	Order 22	-0.717 (0.420)			
	Order 23	-0.375 (0.578)			
	Order 24	-0.462 (0.578)			
	Order 25	-1.027 (0.700)			
	Order 26	-2.327 (0.981)			
	Age	0.001 (0.052)	1, 149.5	<0.01	0.980
	Temp	-0.157 (0.061)	1, 51.3	6.57	0.013
Juv Fr	Generation 1	0.000	1, 35.6	6.49	0.426
	Generation 2	0.106 (0.13)			
	Order 1	0.000	25, 587.5	0.91	0.591
	Order2	-0.101 (0.149)			
	Order 3	-0.197 (0.153)			
	Order 4	-0.262 (0.155)			
	Order 5	-0.242 (0.157)			
	Order 6	-0.057 (0.161)			
	Order 7	-0.205 (0.163)			
	Order 8	-0.134 (0.167)			
	Order 9	-0.310 (0.176)			
	Order 10	-0.223 (0.183)			
	Order 11	-0.390 (0.195)			
	Order 12	-0.468 (0.195)			
	Order 13	-0.207 (0.207)			
	Order 14	-0.430 (0.222)			
	Order 15	-0.359 (0.233)			
	Order 16	-0.461 (0.253)			
	Order 17	-0.614 (0.254)			
	Order 18	-0.085 (0.262)			
	Order 19	-0.473 (0.283)			
	Order 20	-0.255 (0.296)			
	Order 21	-0.045 (0.295)			
	Order 22	-0.178 (0.351)			
	Order 23	-0.643 (0.484)			
	Order 24	-0.549 (0.484)			
	Order 25	-0.203 (0.586)			
	Order 26	-1.924 (0.821)			
	Age	-0.038 (0.048)	1, 203.6	0.61	0.429

	Temp	-0.519 (0.061)	1, 62.0	72.60	<0.001
Adult TI	Generation 0	0.000	2, 132.1	5.336	0.006
	Generation 1	0.404 (0.138)			
	Generation 2	0.085 (0.155)			
	Order 1	0.259 (0.118)	17, 2343.4	3.017	<0.001
	Order2	0.404 (0.119)			
	Order 3	0.523 (0.135)			
	Order 4	0.509 (0.135)			
	Order 5	0.523 (0.136)			
	Order 6	0.504 (0.135)			
	Order 7	0.402 (0.136)			
	Order 8	0.429 (0.136)			
	Order 9	0.446 (0.137)			
	Order 10	0.498 (0.138)			
	Order 11	0.487 (0.139)			
	Order 12	0.405 (0.138)			
	Order 13	0.262 (0.140)			
	Order 14	0.332 (0.141)			
	Order 15	0.346 (0.147)			
	Order 16	0.049 (0.152)			
	Order 17	0.290 (0.784)			
	Repeat 0	0.000	4, 1704.1	12.340	<0.001
	Repeat 1	0.598 (0.213)			
	Repeat 2	0.729 (0.215)			
	Repeat 3	0.8432 (0.219)			
	Repeat 4	0.796 (0.220)			
	Age	0.046 (0.049)	1, 132.9	0.866	0.354
	Temp	0.110 (0.029)	1, 1273.0	14.480	<0.001
Adult Act	Generation 0	0.000	2, 111.4	2.083	0.129
	Generation 1	0.0803 (0.143)			
	Generation 2	-0.155 (0.158)			
	Order 1	0.393 (0.131)	17, 2366.4	3.3200	<0.001
	Order2	0.559 (0.132)			
	Order 3	0.664 (0.149)			
	Order 4	0.706 (0.149)			
	Order 5	0.686 (0.149)			
	Order 6	0.682 (0.149)			
	Order 7	0.621 (0.150)			
	Order 8	0.615 (0.150)			
	Order 9	0.679 (0.151)			
	Order 10	0.732 (0.152)			
	Order 11	0.731 (0.153)			
	Order 12	0.630 (0.152)			
	Order 13	0.468 (0.154)			
	Order 14	0.586 (0.155)			
	Order 15	0.588 (0.161)			
	Order 16	0.242 (0.167)			
	Order 17	-0.548 (0.846)			
	Repeat 0	0.000	4, 1696.9	10.890	<0.001
	Repeat 1	0.535 (0.22)			
	Repeat 2	0.683 (0.225)			

	Repeat 3	0.776 (0.228)			
	Repeat 4	0.739 (0.230)			
	Age	0.021 (0.046)	1, 112.6	0.210	0.648
	Temp	0.116 (0.030)	1, 888.7	14.560	<0.001
Adult AC	Generation 0	0.000	2, 103.2	8.124	<0.001
	Generation 1	0.061 (0.157)			
	Generation 2	0.640 (0.180)			
	Order 1	0.077 (0.129)	17, 2423.4	0.6431	0.860
	Order2	0.157 (0.130)			
	Order 3	0.0865 (0.150)			
	Order 4	0.061 (0.150)			
	Order 5	0.109 (0.150)			
	Order 6	0.082 (0.150)			
	Order 7	0.032 (0.150)			
	Order 8	0.025 (0.151)			
	Order 9	0.018 (0.152)			
	Order 10	0.027 (0.153)			
	Order 11	0.103 (0.154)			
	Order 12	0.031 (0.154)			
	Order 13	0.044 (0.155)			
	Order 14	0.063 (0.157)			
	Order 15	0.041 (0.163)			
	Order 16	0.048 (0.169)			
	Order 17	0.886 (0.886)			
	Repeat 0	0.000	4, 1750.8	0.833	0.504
	Repeat 1	0.023 (0.254)			
	Repeat 2	0.0811 (0.256)			
	Repeat 3	0.085 (0.260)			
	Repeat 4	0.095 (0.262)			
	Age	0.098 (0.059)	1, 172.0	2.809	0.096
	Temp	0.002 (0.034)	1, 1538.3	0.003	0.954
Adult TIM	Generation 0	0.000	2, 155.9	16.800	<0.001
	Generation 1	0.483 (0.146)			
	Generation 2	0.906 (0.161)			
	Order 1	-0.043 (0.137)	17, 2365.0	1.741	0.030
	Order2	-0.205 (0.138)			
	Order 3	-0.222 (0.156)			
	Order 4	-0.412 (0.156)			
	Order 5	-0.295 (0.156)			
	Order 6	-0.363 (0.156)			
	Order 7	-0.291 (0.157)			
	Order 8	-0.255 (0.157)			
	Order 9	-0.369 (0.158)			
	Order 10	-0.381 (0.159)			
	Order 11	-0.338 (0.160)			
	Order 12	-0.308 (0.160)			
	Order 13	-0.230 (0.161)			
	Order 14	-0.251 (0.162)			
	Order 15	-0.333 (0.168)			
	Order 16	-0.080 (0.174)			
	Order 17	1.154 (0.878)			
	Repeat 0	0.000	4, 1710.0	5.326	<0.001

	Repeat 1	-0.045 (0.229)			
	Repeat 2	-0.201 (0.230)			
	Repeat 3	-0.192 (0.234)			
	Repeat 4	-0.127 (0.235)			
	Age	-0.145 (0.047)	1, 115.4	9.55	0.003
	Temp	-0.006 (0.031)	1, 853.3	0.043	0.835
Adult Fr	Generation 0	0.000	2, 192.7	4.137	0.017
	Generation 1	0.345 (0.144)			
	Generation 2	0.453 (0.158)			
	Order 1	-0.471 (0.134)	17, 2443.2	3.102	<0.001
	Order2	-0.699 (0.136)			
	Order 3	-0.749 (0.157)			
	Order 4	-0.833 (0.157)			
	Order 5	-0.759 (0.157)			
	Order 6	-0.747 (0.157)			
	Order 7	-0.805 (0.157)			
	Order 8	-0.766 (0.158)			
	Order 9	-0.776 (0.159)			
	Order 10	-0.813 (0.160)			
	Order 11	-0.899 (0.161)			
	Order 12	-0.882 (0.161)			
	Order 13	-0.639 (0.162)			
	Order 14	-0.809 (0.164)			
	Order 15	-0.953 (0.171)			
	Order 16	-0.569 (0.177)			
	Order 17	-0.510 (0.927)			
	Repeat 0	0.000	4, 1742.2	9.857	<0.001
	Repeat 1	-0.040 (0.247)			
	Repeat 2	-0.253 (0.248)			
	Repeat 3	-0.253 (0.252)			
	Repeat 4	-0.297 (0.253)			
	Age	-0.004 (0.044)	1, 111.8	0.009	0.923
	Temp	-0.017 (0.033)	1, 636.4	0.264	0.607

Supplemental tables 3

Supplemental table 3.1: Effect size of sex (male relative to female) from univariate models with the addition of length as a fixed covariate. Effect sizes are in SDU of transformed traits and standard errors in parentheses.

Trait	Effect	Effect size	DF	F	P
Act	sex	-0.039 (0.075)	1, 1055.3	0.28	0.596
	length	0.208 (0.039)	1, 1382.2	28.59	<0.001
AC	sex	-0.170 (0.073)	1, 1026.5	5.39	0.021
	length	-0.013 (0.039)	1, 1291.6	0.12	0.724
TIM	sex	-0.378 (0.075)	1, 1068.8	25.41	<0.001
	length	-0.093 (0.039)	1, 1370.4	5.68	0.018
Fr	sex	0.209 (0.076)	1, 986.2	7.62	0.006
	length	-0.133 (0.040)	1, 1211.9	11.09	<0.001

Supplemental table 3.2: Estimated **I** matrix among OFT traits for a) males and b) females. Variances are on the diagonal (shaded), covariances on lower diagonal and correlations on upper diagonal. Standard errors in parentheses.

a)	Act_m	AC_m	TIM_m	Fr_m	b)	Act_f	AC_f	TIM_f	Fr_f
Act_m	0.311	-0.058	-0.704	-0.797	Act_f	0.338	-0.061	-0.613	-0.791
	(0.043)	(0.111)	(0.050)	(0.043)		(0.034)	(0.076)	(0.047)	(0.031)
AC_m	-0.015	0.207	0.420	-0.176	AC_f	-0.018	0.260	0.619	-0.128
	(0.028)	(0.037)	(0.092)	(0.121)		(0.023)	(0.030)	(0.051)	(0.082)
TIM_m	-0.215	0.105	0.300	0.551	TIM_f	-0.190	0.169	0.285	0.464
	(0.037)	(0.031)	(0.043)	(0.080)		(0.026)	(0.024)	(0.030)	(0.064)
Fr_m	-0.222	-0.040	0.151	0.251	Fr_f	-0.241	-0.034	0.130	0.275
	(0.039)	(0.029)	(0.035)	(0.044)		(0.030)	(0.023)	(0.024)	(0.033)

Supplemental table 3.3: Likelihood ratio tests for among-individual (a) and additive genetic (b) correlations between each OFT behaviour and standard length (modelled as a first order random regression on age). See methods text for details of modelling methods and Table 3 for correlation estimates.

a) Among individual

Behaviour	χ^2_2	P
Act_m	3.800	0.150
AC_m	6.940	0.031
TIM_m	3.34	0.188
Fr_m	3.34	0.188
Act_f	38.014	<0.001
AC_f	4.904	0.086
TIM_f	9.114	0.010
Fr_f	9.466	0.009

b) Additive genetic

Behaviour	χ^2_2	P
Act_m	0.200	0.905
AC_m	2.420	0.298
TIM_m	0.180	0.914
Fr_m	0.200	0.905
Act_f	2.264	0.322
AC_f	1.86	0.395
TIM_f	0.52	0.771
Fr_f	0.32	0.852

Supplemental table 3.4: Likelihood ration comparison of full model with model that assumes a) equality of sex-specific V_A, and b) intersexual additive genetic correlation (r_{mf}) of +1.

Comparison	Trait	χ^2_1	DF	P	
a) $V_{Am}=V_{Af}$	*Act*	0.144	1	0.704	
	AC	0.100	1	0.752	
	TIM	1.276	1	0.259	
	Fr	0.140	1	0.708	
	Length/growth	47.38	3	<0.001*	
					r_{mf}
b) r_{mf} = +1	*Act*	3.894	1	0.048*	0.711 (0.190)
	AC	2.52	1	0.112	0.592 (0.269)
	TIM	11.212	1	<0.001*	0.312 (0.273)
	Fr	0.480	1	0.488	0.910 (0.144)
	Length	14.84	2	<0.001*	-0.113 (0.259)
	Growth	-	-	-	0.682 (0.221)

Supplemental table 3.5: Lower triangle of $\Delta\mathbf{B}$ matrix, calculated as $\mathbf{B}\text{-}\mathbf{B}^{\mathbf{T}}$ (see main text for details). Lower and upper 95% confidence intervals from bootstrap in parentheses.

	Act	*AC*	*TIM*
AC	0.099 (-0.036,0.228)		
TIM	0.124 (0.005,0.245)	0.003 (-0.116,0.12)	
FR	0.003 (-0.085,0.083)	0.028 (-0.098,0.148)	0.031 (-0.101,0.169)

Supplemental tables 4

Supplemental table 4.1: Details of the data collection procedure on all 7 species. Includes the total number of individuals used, number of repeat measures of OFT, the size of the trial tank and the time period over which the repeat trials were performed. Tank size in fish body lengths was calculated using the species average standard length in each case.

Species	Number of individuals	Maximum number of repeats	Experimental period	Maximum group size	Trial tank size (cm)	Trial tank size (fish body lengths)
D. rerio	26	6	5 weeks	4	30 x 20 x20	9.43 x 6.29 x 6.29
L. nigrofasciata	32	4	2 weeks	8	45 x 25 x 25	9.34 x 5.19 x 5.19
P. reticulata	831	4	2 weeks	16	30 x 20 x20	15.41 x 10.27 x 10.27
X.birchmanni	369	9	28 weeks	8	45 x 25 x 25	12.31 x 6.84 x 6.84
X. eiseni	36	4	2 weeks	8	45 x 25 x 25	9.33 x 5.18 x 5.18
X. helleri	78	4	2 weeks	78	45 x 25 x 25	9.52 x 5.29 x 5.29
X. maculatus	107	4	2 weeks	18	45 x 25 x 25	14.79 x 8.22 x 8.22

Supplemental table 4.2: Repeatabilities of OFT for 7 study species. Standard error in parentheses.

Species	Trait	Repeatability $V_I/(V_I+V_R)$	$\chi^2_{0,1}$	P
D. rerio	TI	0.457 (0.097)	40.32	<0.001
	Act	0.157 (0.084)	5.49	0.009
	AC	0.250 (0.091)	14.58	<0.001
	TIM	0.562 (0.089)	63.19	<0.001
L. nigrofasciata	TI	0.460 (0.095)	30.32	<0.001
	Act	0.487 (0.093)	34.13	<0.001
	AC	0.393 (0.097)	22.29	<0.001
	TIM	0.312 (0.098)	14.28	<0.001
P. reticulata	TI	0.543 (0.019)	824.20	<0.001
	Act	0.473 (0.021)	568.99	<0.001
	AC	0.403 (0.022)	414.65	<0.001
	TIM	0.445 (0.021)	531.32	<0.001
X. birchmanni	TI	0.225 (0.030)	79.80	<0.001
	Act	0.266 (0.030)	123.04	<0.001
	AC	0.166 (0.028)	56.65	<0.001
	TIM	0.285 (0.030)	135.16	<0.001
X. eiseni	TI	0.446 (0.090)	31.47	<0.001
	Act	0.422 (0.092)	27.94	<0.001
	AC	0.247 (0.092)	10.14	0.001
	TIM	0.385 (0.093)	22.27	<0.001
X. helleri	TI	0.322 (0.063)	36.55	<0.001
	Act	0.322 (0.063)	36.61	<0.001
	AC	0.319 (0.063)	35.00	<0.001
	TIM	0.246 (0.062)	21.61	<0.001
X. maculatus	TI	0.346 (0.055)	55.38	<0.001
	Act	0.393 (0.054)	70.78	<0.001
	AC	0.326 (0.055)	48.40	<0.001
	TIM	0.395 (0.054)	69.42	<0.001

Supplemental table 4.3: Test of significance of among-individual covariance structure of the 4 OFT traits in each of the 7 study species.

Species	χ^2_6	P
D. rerio	10.46	0.107
L. nigrofasciata	51.24	<0.001
P. reticulata	820.48	<0.001
X. birchmanni	147.79	<0.001
X. eiseni	39.93	<0.001
X. helleri	54.34	<0.001
X. maculatus	103.44	<0.001

Supplemental Table 4.4: OFT **I** matrices for each species (a-g). Variances are in bold on the diagonal, covariances on lower diagonal and correlations on upper diagonal, with standard errors in parentheses. Note, the diagonals are not equivalent to repeatabilities in each species as the values are standardised across all species (see methods).

	TI	Act	AC	TIM		TI	Act	AC	TIM
a) D. rerio					b) L. nigrofasciata				
TI	**0.462** (0.184)	0.444 (0.281)	0.672 (0.169)	-0.328 (0.230)	TI	**0.016** (0.005)	0.996 (0.005)	0.682 (0.141)	-0.264 (0.248)
Act	0.079 (0.075)	**0.069** (0.048)	0.794 (0.170)	0.002 (0.341)	Act	0.036 (0.012)	**0.081** (0.027)	0.634 (0.150)	-0.285 (0.245)
AC	0.234 (0.130)	0.107 (0.068)	**0.262** (0.127)	-0.081 (0.283)	AC	0.038 (0.016)	0.080 (0.036)	**0.194** (0.069)	0.424 (0.227)
TIM	-0.143 (0.119)	<0.001 (0.057)	-0.027 (0.094)	**0.412** (0.138)	TIM	-0.012 (0.012)	-0.029 (0.028)	0.067 (0.046)	**0.131** (0.054)
c) P. reticulata					d) X. birchmanni				
TI	**0.432** (0.028)	0.933 (0.006)	-0.191 (0.048)	-0.356 (0.041)	TI	**0.106** (0.016)	0.868 (0.027)	0.561 (0.081)	0.029 (0.104)
Act	0.289 (0.020)	**0.222** (0.016)	-0.139 (0.051)	-0.466 (0.038)	Act	0.093 (0.015)	**0.109** (0.016)	0.573 (0.077)	0.087 (0.101)
AC	-0.075 (0.019)	-0.039 (0.015)	**0.356** (0.027)	0.474 (0.040)	AC	0.052 (0.013)	0.054 (0.013)	**0.083** (0.016)	0.705 (0.067)
TIM	-0.157 (0.023)	-0.148 (0.018)	0.190 (0.023)	**0.453** (0.032)	TIM	0.003 (0.012)	0.010 (0.012)	0.069 (0.014)	**0.117** (0.017)

	TI	Act	AC	TIM		TI	Act	AC	TIM
e) X. eiseni					f) X. helleri				
TI	**0.040**	0.983	0.650	-0.551		**0.030**	0.988	0.437	-0.396
	(0.013)	(0.008)	(0.198)	(0.182)		(0.008)	(0.005)	(0.152)	(0.184)
Act	0.085	**0.190**	0.656	-0.615		0.061	**0.126**	0.447	-0.454
	(0.029)	(0.064)	(0.202)	(0.171)		(0.016)	(0.033)	(0.151)	(0.177)
AC	0.043	0.095	**0.109**	0.120		0.024	0.050	**0.099**	0.388
	(0.020)	(0.045)	(0.050)	(0.283)		(0.011)	(0.023)	(0.027)	(0.179)
TIM	-0.046	-0.112	0.017	**0.174**		-0.034	-0.081	0.061	**0.249**
	(0.022)	(0.051)	(0.041)	(0.063)		(0.018)	(0.037)	(0.035)	(0.079)
g) X. maculatus									
TI	**0.053**	0.983	0.918	0.574					
	(0.013)	(0.007)	(0.062)	(0.161)					
Act	0.084	**0.136**	0.915	0.592					
	(0.020)	(0.030)	(0.061)	(0.151)					
AC	0.117	0.187	**0.306**	0.805					
	(0.026)	(0.039)	(0.066)	(0.087)					
TIM	0.076	0.126	0.257	**0.332**					
	(0.023)	(0.035)	(0.058)	(0.075)					

Supplemental table 4.5: Pairwise comparison of **I** structure between each species pair, with χ^2 at 10 DF. Species: X. birchmanni (Xb), X. helleri (Xh), X. maculatus (Xm), X. eiseni (Xe), L. nigrofasciata (Ln), P. reticulata (Pr) and D. rerio (Dr).

Species paring	χ^2	P
I_{Xb} - I_{Xh}	64.98	<0.001
I_{Xb} - I_{Xb}	69.94	<0.001
I_{Xh} - I_{Xe}	5.41	0.862
I_{Xb} - I_{Ln}	-	-
I_{Xh} - I_{Ln}	-	-
I_{Xe} - I_{Ln}	-	-
I_{Xb} - I_{Xm}	79.26	<0.001
I_{Xh} - I_{Xm}	40.28	<0.001
I_{Xe} - I_{Xm}	42.91	<0.001
I_{Ln} - I_{Xm}	-	-
I_{Xb} - I_{Pr}	556.48	<0.001
I_{Xh} - I_{Pr}	345.40	<0.001
I_{Xe} - I_{Pr}	172.08	<0.001
I_{Ln} - I_{Pr}	-	-
I_{Xm} - I_{Pr}	388.44	<0.001
I_{Xb} - I_{Dr}	77.12	<0.001
I_{Xh} - I_{Dr}	72.80	<0.001
I_{Xe} - I_{Dr}	79.42	<0.001
I_{Ln} - I_{Dr}	-	-
I_{Xm} - I_{Dr}	71.70	<0.001
I_{Pr} - I_{Dr}	67.8	<0.001