Maternal-by-environment but not genotype-by-environment interactions in a fish without parental care

Regina Vega-Trejo[1*], Megan L. Head[1], Michael D. Jennions[1, 2] and Loeske E.B. Kruuk[1]

[1]Division of Ecology & Evolution, Research School of Biology, The Australian National University, Acton, Canberra, ACT 2601, Australia

[2]Wissenschaftkolleg zu Berlin, Wallotstraße 19 14193 Berlin, Germany

[*]Corresponding author: Regina Vega-Trejo, e-mail: reginavegatrejo@gmail.com

Ph: +61 2 6125 3828

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Abstract

The impact of environmental conditions on the expression of genetic variance and on maternal effects variance remains an important question in evolutionary quantitative genetics. We investigate here the effects of early environment on variation in seven adult life history, morphological, and secondary sexual traits (including sperm characteristics) in a viviparous poeciliid fish, the mosquitofish *Gambusia holbrooki*. Specifically, we manipulated food availability during early development and then assessed additive genetic and maternal effects contributions to the overall phenotypic variance in adults. We found higher heritability for female than male traits, but maternal effects variance for traits in both sexes. An interaction between maternal effects variance and rearing environment affected two adult traits: female age at maturity and male size at maturity. But there was no evidence of trade-offs in maternal effects across environments. Our results illustrate (i) the potential for pre-natal maternal effects to interact with offspring environment during development, potentially affecting traits through to adulthood and (ii) that genotype-by-environment interactions might be overestimated if maternal-by-environment interactions are not accounted for, similar to heritability being overestimated if maternal effects are ignored. We also discuss the potential for dominance genetic variance to contribute to the estimate of maternal effects variance.

Keywords: additive genetic effects, quantitative genetics, heritability, mosquitofish

Introduction

A central tenet of evolutionary ecology is the expectation that environmental conditions affect evolutionary processes. Evolutionary responses to selection on a trait require the trait to have a genetic basis, so an understanding of the genetic components of phenotypic variation is required to predict evolutionary dynamics (McAdam *et al*, 2002; Mousseau and Fox, 1998; Noble *et al*, 2014). Quantitative traits are likely to be determined by a large number of genes each with a small effect (Falconer and Mackay, 1996; Lynch and Walsh, 1998), and the genetic basis of phenotypic variation – or heritability – of a trait can be quantified indirectly from similarities in trait values between relatives (Falconer and Mackay, 1996; Lynch and Walsh, 1998). However, variation in the environmental conditions individuals experience can play an important role in the process of identifying the genetic components that affect traits. First, similarities between relatives might be due to shared environmental effects, such as maternal effects, which therefore need to be accounted for to generate accurate estimates of heritability (Kruuk and Hadfield, 2007; Wolf and Wade, 2016). Second, variation in environmental conditions can affect the expression of genetic variance (Rowiński and Rogell, 2017; Sgrò and Hoffmann, 2004). Third, variation in environmental conditions is also likely to affect the expression of other components of variance, including maternal effects (Mousseau and Fox, 1998; Uller *et al*, 2013).

Changes in the observed genetic variance underlying phenotypic traits in different environmental conditions are known as genotype-by-environment interactions (G×E; Charmantier and Garant, 2005; Hoffmann and Merila, 1999; Rowiński and Rogell, 2017; Wood and Brodie, 2015). There is abundant evidence from laboratory studies on animals of G×E for a range of traits, based on phenotypic responses to manipulation of environmental conditions such as food availability, temperature, and pathogen levels (e.g. Evans *et al*,

2015; Ferguson and Read, 2002; Vieira *et al*, 2000). Plant studies also frequently report evidence for G×E (Des Marais *et al*, 2013): plants of different genotypes or from different populations show marked variation in their phenotypic responses to key environmental variables (e.g. de Leon *et al*, 2016; Donohue *et al*, 2000). Understanding whether the performance of genotypes is correlated across environments is critical to determine the extent to which environmental variation might maintain genetic variance (Barton and Turelli, 1989; Johnson and Barton, 2005). Do genotypes that are successful in one environment also do well in another, or are there 'trade-offs' across environments (Kruuk *et al*, 2008)? Here, we consider how substantial these aspects of G×E might be relative to other determinants of phenotypic variation in key life history and related traits.

An individual's phenotype is shaped by multiple factors in addition to its genotype, one of which is the effect its mother has on it (Pick *et al*, 2016; Wolf and Wade, 2009). 'Maternal effects' occur when a mother's phenotype affects that of her offspring over and above that attributable to the genes it inherits from her (Mousseau and Fox, 1998; Räsänen and Kruuk, 2007). This might involve pre-natal and/or post-natal effects (Lock *et al*, 2007; Pick *et al*, 2016; Wolf *et al*, 2011). The influence of maternal effects on offspring phenotype is often highly dependent on the mother's own environment. For example, mothers experiencing good environmental conditions may produce larger offspring, breed sooner, or provide more food and greater parental care (Marshall and Uller, 2007; Mousseau and Fox, 1998; Reznick and Yang, 1993). In general, the most obvious mechanisms driving variation in maternal effects point towards environmental factors that alter the mother's phenotype (e.g. mothers in poor condition provide less milk; Trivers, 1974). If mothers differ in how they respond to environmental variation, this plasticity can be thought of as "maternal-by-environment interactions" or M×E, akin to genotype-by-environment interactions. Maternal-by-environment (MxE) interactions have been less thoroughly investigated than G×E when

conducting variance component analyses, so in general we have little idea whether 'good' environments generally amplify or depress any differences among mothers, or of the potential for trade-offs across environments such that mothers that are superior in one environment are inferior in another (i.e. negative cross-environment maternal effect correlations).

The timing of environmental variation may also drive important differences. Maternal effects plasticity is typically investigated in the context of variation in the *mother's* environment. M×E could, however, equally plausibly be generated by maternal effects differing in interactions with the environment experienced by the *offspring*. In many cases, the two sources of environmental variation that affect maternal effects are indistinguishable. For example, more stressful environmental conditions reduce variation among mothers in maternal effects for offspring birth weight in wild Soay sheep (Wilson *et al*, 2005), but it is not possible to determine to what extent this is due to effects of the environment on mothers or on their lambs. We therefore know less about how maternal effects vary 'downstream' due to variation in the *offspring*'s environment. More specifically, are there predictable differences between mothers in how their offspring respond to changes in environmental conditions? In particular, do mothers vary in how much they alter the ability of their offspring to withstand environmental stress? Addressing this aspect of M×E requires focusing on changes in the offspring's environment once maternal care has ceased.

Analysis of maternal-by-environment interactions may also be important for methodological reasons. Maternal effects typically make relatives (i.e. siblings) look more similar than they would otherwise. They can therefore be difficult to disentangle from additive genetic effects, which are typically estimated from the degree of similarity between relatives.

Maternal effects have the potential to inflate estimates of genetic variance, unless properly modelled, and there is general acceptance of the need to control for maternal effects when estimating the heritability of a trait (Kruuk and Hadfield, 2007; McAdam *et al*, 2014). However, less attention has been paid to the fact that the same issue applies to tests for G×E interactions: just as the occurrence of maternal effects can inflate estimates of additive genetic variance and heritability, the occurrence of M×E should presumably inflate estimates of G×E. To our knowledge, this possibility remains untested. It implies that, in addition to the fundamental biological question of whether offspring of different mothers are differentially affected by environmental stress due to maternal effects, quantifying M×E may be a critical component of analysis of G×E.

General conclusions about the prevalence of G×E and M×E in a system also require assessment of different traits. Different types of traits typically show different patterns of heritability, of maternal effects and, presumably, of respective interactions with the environment. Traits that are closely associated with fitness often show lower heritability than more weakly-selected traits (Houle, 1992; Postma, 2014; Roff and Mousseau, 1987). For instance, life history traits, such as fecundity and viability, are under strong directional selection and show lower heritability than morphological and physiological traits (Kruuk *et al*, 2000; Roff and Mousseau, 1987). Sexually selected traits may also show different patterns of variation, alongside differences between the sexes in their genetic architecture (Jia *et al*, 2000; Parker and Garant, 2004). However, comparatively little is known about the relative magnitude of G×E, let alone M×E, for different types of traits.

Here, we experimentally manipulated a critical aspect of the environment experienced by offspring during their early development (food availability), to assess the relative contribution of additive genetic versus maternal effects on phenotypic variance, as well as

143 the extent to which each contribution was influenced by the environmental stress of food
144 restriction. We used a multigenerational breeding design of a laboratory population of
145 mosquitofish (*Gambusia holbrooki*) to test for G×E and MxE interactions in seven adult
146 phenotypic traits: size and age at maturity for both males and females, and three sexually
147 selected male traits, namely, relative genital size, sperm number, and sperm velocity. We
148 considered this range of phenotypic traits to investigate the importance of different sources
149 of variance for multiple aspects of adult phenotypes. In many taxa size and age at maturity
150 are key life history traits often linked to fitness (Roff, 1992). Likewise, some sperm traits are
151 strongly positively associated with fitness (Parker and Pizzari, 2010), although this is not
152 always the case (e.g. Simmons *et al*, 2003). We already have clear evidence for maternal
153 effects on growth and development rates that persist until sexual maturity in *G. holbrooki*
154 (Kruuk *et al*, 2015). Here, we assessed the potential for environmental stress (food
155 restriction) during offspring development to generate both G×E and M×E. *Gambusia*
156 *holbrooki* is a live-bearing fish lacking post-natal parental care, so all maternal effects must
157 be mediated by events prior to birth. Our experiment therefore constitutes a test for an
158 interaction between pre-natal maternal effects and post-natal (i.e. offspring alone)
159 environmental conditions. We asked: 1) What is the relative importance of heritable genetic
160 vs maternal effects in contributing to phenotypic trait variance in each sex? 2) Do these
161 effects interact with the environmental conditions experienced by the offspring? And if so,
162 are there (i) consistent differences in the levels of additive genetic and maternal effects
163 variance between good and poor environments and (ii) trade-offs across environments in
164 either genetic or maternal effects?
165
166 **Methods**
167
168 *Study species*

169

170 *Gambusia holbrooki*, a species of viviparous poeciliid fish, is endemic to North America but
171 now introduced worldwide (Pyke, 2005). *G. holbrooki* have internal fertilization, are sexually
172 dimorphic, and males transfer sperm by a modified anal fin ('gonopodium') that acts as an
173 intromittent organ (Pyke, 2005). There is substantial variation in female adult size, which is
174 strongly positively correlated with fecundity (Bisazza *et al*, 1989; Callander *et al*, 2012). Male
175 size also varies considerably, despite their growth ceasing at maturation. Small males have
176 greater manoeuvrability, which seems to increase their propensity to sneak copulations
177 (Pilastro *et al*, 1997), while large males are socially dominant and might transfer more sperm
178 per copulation because they have greater sperm reserves (Bisazza and Marin, 1991; O'Dea *et*
179 *al*, 2014). Recent studies in our lab of free-swimming fish have shown greater paternity
180 success for smaller males (Head *et al*, 2017), no effect of male size (Vega Trejo et al. 2017),
181 and some reproductive advantage to larger males (Booksmythe et al. 2016). Age at
182 maturation in both sexes is highly variable (see Livingston *et al*, 2014; Pyke, 2005; Vega-Trejo
183 *et al*, 2016a). Greater relative gonopodium size is also linked to increased reproductive
184 success (Head *et al,* 2017; Vega-Trejo *et al,* 2017; but see Booksmythe *et al,* 2016). Finally,
185 sperm velocity declines with age (Vega-Trejo *et al*, 2016b), and sperm number has been
186 shown to be condition-dependent and positively related to body length (O'Dea *et al*, 2014).
187

188 *Experimental design*

189

190 Our analyses are based on measurements of seven phenotypic traits from laboratory reared
191 *G. holbrooki* in which we varied the level of food an individual received during development.
192 Our multi-generation breeding design also involved the comparison of fish with different
193 levels of inbreeding, as part of a separate investigation into the effects of inbreeding (Vega-
194 Trejo *et al*, 2016a; Vega-Trejo *et al*, 2017). The base stock (F_0) population consisted of

offspring from 151 gravid wild-caught females collected in Canberra, Australia. F_0 fish were kept in single-sex tanks under a 14:10 photoperiod at 28°C, and fed *ad libitum* with *Artemia* nauplii and commercial flakes (Fig.1). Once they were mature, we randomly paired fish from this base stock to create full-sib families (F_1). Fish from these full-sib families were then used to create an F_2 generation consisting of 58 outbred families (with unrelated parents) and 58 inbred families (with full-sib parents; f=0.25; Fig. 1). To do this, we used 29 pairs of full-sib families (e.g. A and B), which we refer to as "blocks". Within each block, one male from family A and one from family B respectively were paired to females (between 1-4) from the other family, to create outbred full-sibs/half-sibs (AB and BA); and one male from each family was paired to his full-sib sisters (again, 1-4 females per male) to create inbred full-sibs/half-sibs (AA and BB). The same number of females contributed to each of the four cross-types within a block to generate a mix of inbred and outbred half-sib and full-sib families. We then reared a maximum of 10 offspring per female (i.e. full-sib family). See Vega-Trejo *et al* (2015) and Vega-Trejo *et al* (2016a) for a fuller methodological description.

The food manipulation experiment was conducted on the F_2 generation (described below). In the F_2 generation, half of the offspring in each family were raised on a 'control' diet, whereas the other half experienced a 'low food' diet early in life (Fig. 1). Fish on the control diet were fed *ad libitum* with *Artemia* nauplii twice a day from birth until the end of the experiment. Fish on the low diet were fed the control diet until they were one week old, and were then fed 3mg of *Artemia* nauplii once every other day (i.e. < 25% of the control food intake) for 21 days, after which they were returned to the control diet. Fish almost totally suppressed growth while on the low food diet (Livingston et al, 2014; Vega-Trejo et al, 2016a). For completeness, we also included in our analyses measurements on the F_3 generation. The F_3 generation was created by pairing each F_2 female with a stock male, and by using sperm from F_2 males to artificially inseminate stock females (Fig. 1). Thus, all F_1 and

221 F_3 fish were outbred and raised on a control diet, whereas F_2 fish were both outbred or
222 inbred, and raised on either a control or restricted diet. Offspring of all generations were
223 transferred to individual tanks at birth to eliminate the potential for post-natal shared
224 environment effects.
225
226 *Measurements of phenotypic traits*
227
228 For individuals of generations F_2 and F_3, we measured seven adult traits. Table 1 lists the
229 traits measured, with sample sizes and summary statistics. To determine the timing of sexual
230 maturity, we inspected all tanks three times a week. Females were considered to be mature
231 when yolked eggs were evident in the abdomen (Stearns, 1983). Males were considered to
232 be mature when their gonopodium was translucent, with a spine visible at the tip (Stearns,
233 1983; Zulian *et al*, 1993).
234
235 To measure morphological traits, we anaesthetized fish by submersion in ice-cold water for
236 a few seconds to reduce movement. The fish were then photographed alongside a
237 microscopic ruler (0.1 mm gradation). We used Image J software (Abramoff et al. 2004) to
238 measure: *body length* at maturity (snout tip to base of caudal fin, in mm) for both males and
239 females, and male gonopodium length (apical tip to base, in mm). We then calculated
240 *relative gonopodium size* for males as the residuals from a linear regression of (log)
241 gonopodium length on (log) standard length (Horth *et al*, 2010; Vega-Trejo *et al*, 2017).
242
243 We measured two sperm traits in the F_2 generation: *sperm number* and *sperm velocity*.
244 Details on the extraction of ejaculates and the samples are given in the Supplementary
245 Information. In brief, males were anaesthetized in ice-cold water, placed on a glass slide and
246 their gonopodium was swung forward. We then applied gentle pressure to the abdomen to

247 eject all of the available sperm. We counted the sperm and measured sperm velocity.
248 Afterwards, each male was returned to his individual tank. All inspections for maturity and
249 measurements of traits were made blind to food treatment, inbreeding status, and family
250 identity.
251
252 **Statistical analyses**
253
254 We quantified components of variance in the phenotypic traits using an 'animal model', a
255 form of mixed model that uses pedigree information to assess covariance between relatives
256 (Wilson *et al*, 2010), fitted using ASReml-R (Butler *et al*, 2009). All models contained random
257 effects of: an additive genetic effect (with covariance structure defined by relatedness
258 between individuals, as determined by the breeding design's pedigree, and associated
259 additive genetic variance component V_A), a maternal effect (grouping individuals by mother,
260 with associated maternal effects variance component V_M; Kruuk & Hadfield 2007), a block
261 component (V_B, defined above), and residual effect (with associated variance component
262 V_R).
263
264 The animal model estimates the additive genetic variance based on the similarity between
265 the multiple types of relatives, with relatedness defined by our four-generation pedigree (F_0-
266 F_3). The estimate of the maternal effects variance V_M is determined by the increased
267 similarity between offspring of the same mother, beyond that due to additive (direct)
268 genetic effects (Kruuk and Hadfield, 2007). This value will necessarily encompass both
269 maternal environment effects and maternal genetic effects (i.e. effects of the mother's
270 genotype on her offspring, over and above the direct effects of the genes they inherit from
271 her); we do not attempt to separate them. Offspring were reared separately from birth
272 onward (see above), so there is little potential for post-natal common environment effects

to inflate estimates of maternal effects. Offspring of the same mother were, however, always full-siblings, so there is the potential for the estimate of V_M to be inflated by dominance genetic variance (Falconer and Mackay, 1996). We make the standard assumption that dominance variance is small compared to additive genetic variance (Hill *et al*, 2008; Zhu *et al*, 2015), and hence that any impact is also small, but we return to this point in the *Discussion*.

All traits were standardized to unit variance and zero centred prior to analysis, and we analysed the data separately for males and females. In all models, we fitted food treatment (control vs low food diet), inbreeding (inbred vs outbred), and generation (two levels, as phenotypic data were only available for F_2 and F_3) as fixed factors. For sperm number and velocity, generation was not fitted because we only measured F_2 males. However, for the sperm traits we included fixed effects of male age (range 19-125 days post maturity) as age influences sperm number and velocity (Vega-Trejo et al 2016b). The parameter estimates for the fixed effects in a model with only V_A, V_M, V_B, and V_R (see details below) are in Table 1. Parameter estimates for the effects of food treatment are shown in Fig. S1.

Univariate models of interactions with environmental conditions

We first fitted univariate models for each of the seven traits to explore the extent of interactions of both the additive genetic variance and maternal effects variance with environmental conditions (i.e. food treatment). We started with Model 1, a 'null' model with only fixed effects (as described above: food, inbreeding, and generation), block as a random effect and a residual random effect. We then fitted: Model 2, which included variance due to additive genetic effects (V_A); Model 3, a model containing just the maternal effects variance (V_M); and Model 4, a model that included both components of variance (V_A+V_M). We call this

the 'basic model'. We report the trait's heritability (V_A / (V_A+V_M+V_B+V_R)) and maternal effects proportion (V_M /(V_A+V_M+V_B+V_R)) from the basic model in Table 1.

We then investigated whether the variance components changed between the two food treatments (i.e. Genotype × Environment or Maternal effect × Environment) by testing for either a V_A×Food or V_M×Food interaction. To do so, we first included the interactions between each variance component and the food treatment in models with only one main variance component (i.e. Model 5: V_A+V_A×Food; and Model 6: V_M+V_M×Food), and then in models which included both main terms (i.e. Model 7: V_A+V_M+V_A×Food; and Model 8: V_A+V_M+V_M×Food). Finally, we ran a model with all main terms and interactions (Model 9: V_A+V_M+V_A×Food+V_M×Food).

Model comparison

Because we wished to compare several models, many of which were not nested, we used a model comparison approach based on the Akaike Information Criterion AIC (Burnham and Anderson, 2002; see Saastamoinen *et al*, 2013 for a similar model comparison of different animal models). We calculated AIC as -2log(*L*) + 2K, where log(*L*) was the model's log likelihood and K the number of parameters estimated. We only considered the number of parameters associated with estimating variance in the different random effects (other than the residual and block variance estimates), given that the fixed effects were the same in all models for a given trait. Thus, K ranged from 0 (null model: model with only fixed effects and one random effect of block) to 4 (final model with V_A, V_M, V_A×Food and V_M×Food). Akaike weights *w* for each model *i* were calculated as $w_i = \exp(\Delta AIC_i)/\sum\exp(\Delta AIC)$, where ΔAIC is the difference in AIC between model *i* and the model with the lowest AIC (the top model for that trait). Models ranked within two AIC units of the top model were considered to be

325 'reasonable candidate' models providing indistinguishable levels of support (Burnham and
326 Anderson, 2002). The number of reasonable models ranged from one to four across the
327 different traits.
328
329 The informational approach outlined above allowed us to compare differences between
330 models for each trait. We then determined the significance of the interaction parameter
331 estimates in the top model. To do this, we ran likelihood ratio tests (LRT) comparing the top
332 model to one without that parameter. The test statistics and P-values are given in the text of
333 the *Results*. We also tested for the significance of V_A and V_M by LRTs by respectively
334 dropping those terms from the basic model ($V_A+V_M+V_B+V_R$). The P-values from these LRTs
335 are shown in Table 1 adjacent to the heritability (i.e. for V_A) and to the proportion of
336 maternal effects variance (i.e. for V_M).
337
338 For each trait, we report the variance components for the reasonable candidate models in
339 the main *Results*, and for all nine models in the Supplementary Information. However, given
340 that there might be some level of support for multiple models, we also provide model-
341 averaged parameter estimates (Burnham and Anderson, 2002). For each trait, we model
342 averaged the estimates of the different variance components across our 9 models, where
343 the model-averaged variance component V* was calculated by weighting V_i, the variance
344 estimate of model *i* with the weights w_i as calculated above, such that $V^* = \sum (V_i \times w_i)$. We
345 also estimated model-averaged standard error as $SE^* = \sum [w_i \times \sqrt{(SE_i^2 + (V_i - V^*)^2}]$ (Burnham
346 and Anderson, 2002).
347
348 *Bivariate models across environments*
349
350 We found evidence for interactions between food treatment and variance components for

both female age at maturity and male size at maturity (see *Results*). We therefore fitted bivariate models to estimate the variance due to additive genetic effects and maternal effects within each food treatment, by splitting each trait by treatment to define two new "sub-traits". These models contained inbreeding level and generation as fixed effects as above, and maternal and additive genetic effects as random effects in order to generate environment-specific estimates of the variance components in each trait. Block was also included as a random effect for female age at maturity, but was not fitted for male size at maturity given a lack of convergence (note: for other traits, fitting block made little difference to the parameter estimates). Each model also contained estimates of the covariance across environments in additive genetic, maternal effect, and (for female age) block effects. We also ran a model with additive genetic effects only to compare our estimates of V_A when maternal effects were ignored.

Results

Almost all traits were affected by the food treatment during development, but there was no effect of inbreeding on trait means (Table 1). However, there were marked differences among traits as to which of the different combinations of variance components produced the best model. Table 2 shows the reasonable candidate models (<2 AIC units from the top model) for each trait with their corresponding variance estimates, and also the model-averaged variance estimates averaged across all models. For the corresponding details of all 9 models for each trait, see Table S1.

We found evidence of additive genetic and maternal effects variance for almost all traits. However, the interaction with food treatment varied substantially between traits. Below we present results for each trait in turn.

Female size at maturity

Females on the low food treatment were slightly smaller at maturity than those on control diets (Table 1; Figure S1). For the 'basic' model with just V_A+V_M, we found substantial heritability and maternal effects variance (Table 1). Comparing all models, both of the 'reasonable candidate' models contained variance due to additive genetic effects and maternal effects, with the top model containing only V_A+V_M (i.e. the 'basic' model as defined above; Table 1). Although V_M×Food appeared in the second-ranked model, overall there was little indication of support for this interaction (LRT: $X^2_{(1)}$=0.546, P=0.761; Table 2); this model fits the characteristics of a 'hitch-hiking' model, where an additional term generates a very slight improvement in model fit and so falls within 2 AIC units of the top model (Arnold, 2010; Symonds and Moussalli, 2011). Similarly, there was no support for a V_A×Food interaction (LRT: $X^2_{(1)}$<0.001, P=1; Table S1).

Male size at maturity

Males on the low food treatment were also smaller at maturity (Table 1; Figure S1). The estimates of heritability and maternal effects proportions from the basic V_A+V_M model showed little heritability but substantial maternal effects variance in male size (Table 1). Model comparison indicated strong support for a V_M×Food interaction as the term appeared in both of the two reasonable candidate models (Table 2), and the top model contained both V_M and a significant V_M×Food interaction (LRT: $X^2_{(1)}$=18.205, P<0.001). We found little support for a V_A×Food interaction (Table S1). We fitted a bivariate model of environment-specific traits to estimate the variance components for each food treatment. There was significant variance in maternal effects in the control environment but not in the low food

environment (Figure 2a). There was also a negligible covariance (negative) of the maternal effects across environments, most likely because of the lack of variation among mothers in the low food environment (Fig. 2a, Table 3).

Female age at maturity

Females in the low food treatment took on average 21% longer to mature (Table 1; Figure S1). Similar to female size at maturity, the basic V_A+V_M model indicated substantial heritability and maternal effects for female age at maturity. In the model comparison, the only reasonable candidate model was that containing V_A and V_M and a V_M×Food interaction (LRT for V_M×Food: $X^2_{(1)}$ =6.449, P=0.039; Table 2). When we considered female age at maturity in the two environments separately, we found much higher variance in maternal effects in the low food environment than in the control environment, and also a positive covariance of maternal effects across the two environments (Fig. 2b, Table 3).

Male age at maturity

Males in the low food treatment took on average 38% longer to mature than those on the control diet (Table 1, Figure S1). As with male size at maturity, the basic model with only V_A+V_M showed little heritability but substantial maternal effects variance (Table 1). The reasonable candidate model set contained a top model with V_M only and another with V_M and a V_M×Food interaction. However, similar to female size, the second model was consistent with a hitch-hiking model because there was little support for the V_M×Food interaction (LRT: $X^2_{(1)}$ =0.987, P=0.611; Table 2).

Relative gonopodium size

Males in the low food environment had relatively longer gonopodia on average (Table 1, Figure S1). In the basic model with just V_A+V_M, the heritability and the strength of maternal effects were both relatively low, though the latter accounted for nearly 10% of the phenotypic variation and was statistically significant (Table 1). The top model contained only V_M. Although models containing both V_A×Food and V_M×Food interactions were included in the reasonable candidate model set, neither parameter estimates differed significantly from zero (LRT: V_A×Food: $X^2_{(1)}$ =2.019, P=0.364; V_M×Food: $X^2_{(1)}$ =0.097, P=0.953; Table 2).

Sperm traits

Males in the control treatment had sperm with a faster mean velocity, but there was no difference between food treatments in mean sperm number (Table 1; Figure S1). For sperm number, the top model contained only maternal effects variance V_M, and heritability was negligible (Table 2). However, the estimate of V_M in the top model had a large SE and was not significant (LRT: $X^2_{(1)}$ =2.736, P=0.098) and the null model with only fixed and block effects was also a 'reasonable candidate' model (Table 2). For sperm velocity, the top model contained only V_A, with a corresponding significant heritability of 0.215 (0.092 SE), (LRT: $X^2_{(1)}$ = 4.715, P= 0.029), but if we considered the 'basic' model with V_A+V_M, the estimate of heritability was reduced to 0.197 (0.125 SE; Table 1) and was non-significant (LRT: $X^2_{(1)}$ = 2.073, P= 0.149).

Discussion

Understanding the causes of phenotypic variation in traits is fundamental to predict evolutionary responses. Our study of life history and sexual traits in the mosquitofish

455 *Gambusia holbrooki* revealed several patterns. First, we found sex differences in
456 heritabilities. There were significant heritability estimates for female age and size at
457 maturity, but not for the male traits examined. Second, in both sexes there were maternal
458 effects that persisted until sexual maturity for all traits except the sperm traits (i.e. for five of
459 seven traits). Third, there were interactions between maternal effects variance and food
460 treatment for female age at maturity and male size at maturity. Failure to account for
461 maternal by environmental interactions (M×E) led to overestimates of genotype-by-
462 environment interactions (G×E). We discuss each of these points below.
463
464 The relative importance of heritable genetic effects in our study differed between males and
465 females: in general, female traits had higher heritability. Because of the lack of genetic
466 variance in males, we did not attempt to estimate cross-sex genetic correlations, but our
467 results indicate a very different underlying genetic architecture shaping female and male
468 phenotypes. This is in contrast to estimates in many other taxa of strong cross-sex
469 correlations in morphology (Kruuk *et al*, 2008; Poissant *et al*, 2010), and maturation time
470 (e.g. Guntrip *et al*, 1997). The differences between the sexes we observed here could
471 potentially be due to the biology of mosquitofish. Females have indeterminate growth and
472 their fecundity increases with body size (Bisazza *et al*, 1989; Callander *et al*, 2012), while
473 males stop growing upon maturation. Selection pressures on growth and maturation rates
474 are thus likely to be highly sex-specific. Sex differences in heritability of traits might be due
475 to sexual selection acting more strongly on males, thereby depleting the amount of additive
476 genetic variation expressed in males (Van Homrigh *et al*, 2007). Lower heritability of female
477 traits has also been found in other species, for example for morphological traits in house
478 sparrows (Jensen *et al*, 2003). However, the implications for evolutionary dynamics of
479 differences in heritability between the sexes still remain relatively underexplored.
480

Within males, the relative importance of heritable genetic effects also differed among sexual traits. There was a low, non-significant heritability for relative gonopodium size (0.039±0.083 SE; Table 1). Interestingly, this is similar to an estimate of realised heritability based on artificially selection on relative gonopodium length in the same study population (0.028±0.006 SE, Booksmythe *et al,* 2016) – a reminder that a response to selection is possible even when heritability is low. Sperm number showed no evidence of additive genetic variance, but sperm velocity did. The low heritability of sperm number may indicate that sperm quantity is highly condition-dependent (e.g. influenced by diet; see O'Dea *et al*, 2014). Sperm velocity showed significant heritability in the 'top' model (Table 2), which could potentially fit with Y-linked effects as suggested in other poecilids (e.g. Evans, 2011). We note however that the estimate was lower and not significant in the basic model with maternal effects (Table 1), so there was not strong support for significant heritability of sperm velocity across all models.

Maternal effects contributed to trait variance for both males and females. Female mosquitofish invest in their offspring prior to fertilisation by provisioning eggs (lecithotrophy; Fernández-Delgado and Rossomanno, 1997; Pollux *et al*, 2014) and also possibly via subsequent nutrient transfer to embryos (see Marsh-Matthews *et al*, 2005; Marsh-Matthews *et al*, 2010). Our results suggest that differences between mothers in their prenatal allocation of resources to either eggs or embryos have important implications for their offspring's subsequent development: maternal effects were still apparent in traits measured at sexual maturity (see also Kruuk *et al*, 2015). Further, in addition to the overall presence of maternal effects, we observed significant maternal-by-environmental variance (M×E) interactions for female age at maturity and male size at maturity. These interactions were apparent even though the food treatment was applied *after* maternal provisioning ended. They indicate that studies of maternal effects need to consider the potential impact

of environmental heterogeneity: here, we would have reached a very different conclusion as to the importance of maternal effects had we only considered offspring reared under 'control' rather than 'low food' conditions. We found a significant positive covariance of the maternal effects across environments for female age at maturity. That is, mothers with maternal effects that caused their daughters to take longer to mature in the control environment also had daughters that took longer to mature in the low food environment (Fig.2, Table 3). For males, however, there was no support for covariance across the food treatments for maternal effects on male size. This was probably due to the very low maternal effects variance expressed in the low food environment. We therefore found no evidence for trade-offs in maternal effects across environments. Similarly, Charmantier and Garant (2005) also find little evidence of genetic trade-offs via negative cross-environment genetic correlations, which means that the role of environmental heterogeneity in generating life-history trade-offs remains unclear (see also discussion in Kruuk *et al*, 2008). As a final point, we note that estimates of maternal effects variance can be inflated by dominance genetic variance, and the separation of the two remains a challenging issue (Wolak and Keller, 2014). There is therefore a possibility that our estimates of M×E are inflated by dominance genetic-by-environment interactions. We have been unable to find tests of variance components for D×E in the literature (although see Kumar *et al*, 2015 for estimates of V_D×Site interaction variance), but data from different systems indicate that dominance genetic variance itself is typically small relative to additive genetic variance (Hill *et al*, 2008; Wolak and Keller, 2014; Zhu *et al*, 2015). Similarly, in turn, estimates of dominance variance may in turn be inflated by maternal or shared environment effects.

We found striking differences in our results from models incorporating maternal effects and maternal-by-environmental variances (M×E) compared to those without. Estimates of the variance due to additive genetic effects (V_A) were always higher in models where V_M was not

533 estimated, indicating that ignoring maternal effects inflated estimates of V_A (Table S1).
534 Similarly, when we compared estimates of V_A×Food (i.e. GxE) from models without and with
535 V_M×Food (i.e. MxE), we found larger estimates if V_M×Food was not accounted for. For
536 instance, for female age at maturity, V_A×Food was estimated at 0.054±0.065 in the model
537 with $V_A+V_M+V_A$×Food, but as $4\times10^{-8}\pm6\times10^{-9}$ SE in the model with $V_A+V_M+V_A$×Food+V_M×Food.
538 Similarly, for male size at maturity, the model of $V_A+V_M+V_A$×Food provided a GxE interaction
539 estimate of 0.172±0.078 SE, but the model $V_A+V_M+V_A$×Food+V_M×Food provided an estimate
540 of $4\times10^{-7}\pm4\times10^{-8}$ SE. The right-hand panels in Figure 2 also show the much greater change in
541 estimates of V_A across environments in models fitted without maternal effects. Although this
542 is unsurprising, studies of genotype-by-environment interactions rarely also account for
543 maternal-by-environment interactions. It is well established that the presence of maternal
544 effects (or other non-additive causes of covariance between relatives, such as dominance
545 variance) can inflate heritability estimates if not accounted for properly (Falconer and
546 Mackay, 1996; Kruuk and Hadfield, 2007). In the same vein, our results indicate that
547 estimates of G×E can be inflated by the existence of unaccounted-for M×E interactions. It is
548 thus possible that previous studies of other populations have overestimated the role of G×E
549 in driving phenotypic variation in systems where maternal effects (or other causes of
550 increased similarity between relatives such as non-additive genetic effects) are important.
551
552 In sum, our study found sex differences in the genetic architecture underlying important
553 phenotypic traits. Additionally, it suggests that maternal effects can shape phenotypic traits
554 even when there is no postnatal investment and, furthermore, that differences between
555 offspring of different mothers can interact with the offspring's rearing environment to
556 influence their adult phenotypes. Our findings thus illustrate the need to consider maternal-
557 by-environmental interactions in quantitative genetic studies. G×Es have been well studied
558 and there is increasing appreciation of their potential importance in evolutionary ecology

559 (e.g. reviews by Des Marais *et al*, 2013; Hunt and Hosken, 2014 respectively). Our analysis
560 here illustrates the need to also consider other potential contributors to environmental
561 interactions when assessing GxE interactions, as failure to do so could result in an
562 overestimation of the importance of G×E. Including MxE interactions may thus improve our
563 understanding of the factors that contribute to phenotypic variance in different components
564 of individuals' life histories.
565
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578
579 **Conflict of Interest**
580
581 The authors declare no conflict of interest.
582
583 **Data Archiving**
584

585 Data will be deposited in Dryad upon acceptance.

586

587 **References**

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Table 1. Means ± SD (N) for the traits measured, and parameter estimates ± SE for the fixed effects are from analyses of standardised values from our basic model $V_A+V_M+V_B+V_R$. In all models, we fitted food treatment (control vs low food diet), inbreeding (inbred vs outbred), and generation (two levels, F_2-F_3). For sperm number and sperm velocity, generation was not included as a fixed effect because we only had data for F_2 males, but we included the age of the male at measurement (measured in days post maturity). Estimates of fixed effect parameters and proportions are followed by their SEs. Heritabilities (h^2) and the proportion of the maternal effects variance (m^2) ± SE are from the basic model with $V_A+V_M+V_B+V_R$. Significance of h^2 and m^2 is given as *p <0.05, **p <0.01, ***p < 0.001 (see Methods for details).

		Parameter estimates						
Trait	**Trait mean±SD (N)**	**Intercept**	**Food treatment (low)**	**Inbreeding (inbred)**	**Generation (F_3)**	**Adult age**	***h²***	***m²***
Female size at maturity	23.89±2.69 (1035)	0.002±.116	-0.368±0.078***	0.058±0.120	0.142±0.106	—	0.309± 0.121*	0.254± 0.058***
Male size at maturity	22.62±1.93 (1085)	0.325±0.096	-0.192±0.082*	0.076±0.112	-0.507±0.103***	—	0.040± 0.092	0.264± 0.055***
Female age at maturity	94.25±41.42 (1026)	-0.353±0.110	0.635±0.078***	0.185±0.118	0.398±0.104***	—	0.252± 0.113*	0.268± 0.054***
Male age at maturity	77.55±34.78 (1060)	0.018±0.094	0.585±0.079***	0.027±0.110	-0.247±0.101**	—	0.000± 0.000	0.286± 0.039***

Relative gonopodium size	0±0.36 (1053)	-0.481±0.089	0.386±0.089***	0.153±0.102	0.637±0.097***	—	0.039± 0.083	0.094± 0.047*
Sperm number	186.77±94.53 (442)	0.065±0.101	-0.100±0.093	-0.035±0.100	—	0.137±0.049**	0.000± 0.000	0.074± 0.052
Sperm velocity	83.01±16.57 (390)	0.098±0.094	-0.230±0.096*	0.059±0.103	—	-0.302±0.052***	0.197± 0.125	0.016± 0.085

Table 2. The reasonable candidate models for each trait. For each model, the log likelihood (log (L)) is presented. Models shown are the models ranked within two AIC units of the top model in descending order. The difference in AIC value with the model with the lowest AIC value is presented (Δ AIC). Weight = Akaike model weight, relative to all 9 models for each trait (see Methods for definition). V_A = additive genetic variance, V_M = maternal effects variance, V_B = block variance, V_R = residual variance.

Model	log (L)	AIC	Δ AIC	Weight	V_A ± SE	V_M ± SE	V_A × Food ± SE	V_M × Food ± SE	V_B ± SE	V_R ± SE
Female size at maturity										
V_A+V_M	-417.145	842.291	0	0.456	0.310 ± 0.124	0.255 ± 0.060			0.056 ± 0.041	0.382 ± 0.066
$V_A+V_M+V_M\times$Food	-416.872	843.745	1.454	0.221	0.321 ± 0.124	0.210 ± 0.078		0.045 ± 0.060	0.056 ± 0.042	0.368 ± 0.067
Model averaged estimates (across all models)					0.290 ± 0.138	0.243 ± 0.075	5.430×10^{-8} ± 8.791×10^{-8}	0.014 ± 0.031	0.056 ± 0.042	0.377 ± 0.073
Male size at maturity										
$V_M+V_M\times$Food	-466.625	941.249	0	0.574		3.813×10^{-8} ± 2.257×10^{-9}		0.311 ± 0.043	0.019 ± 0.015	0.603 ± 0.036
$V_A+V_M+V_M\times$Food	-466.239	942.478	1.229	0.310	0.060 ± 0.074	4.989×10^{-7} ± 3.839×10^{-8}		0.284 ± 0.051	0.010 ± 0.018	0.574 ± 0.050
Model averaged estimates (across all models)					0.026 ± 0.050	3.188×10^{-4} ± 6.618×10^{-4}	2.238×10^{-4} ± 4.691×10^{-4}	0.299 ± 0.049	0.015 ± 0.017	0.590 ± 0.044
Female age at maturity										
$V_A+V_M+V_M\times$Food	-401.233	812.467	0	0.595	0.255 ± 0.112	0.162 ± 0.071		0.137 ± 0.063	0.044 ± 0.037	0.368 ± 0.062
Model averaged estimates (across all models)					0.240 ± 0.119	0.177 ± 0.079	0.003 ± 0.009	0.116 ± 0.074	0.045 ± 0.038	0.373 ± 0.067
Male age at maturity										
V_M	-436.383	878.765	0	0.4154		0.257 ± 0.257			0.023 ± 0.016	0.618 ± 0.035
$V_M+V_M\times$Food	-435.892	879.783	1.0181	0.2497		0.215 ± 0.061		0.060 ± 0.061	0.023 ± 0.016	0.602 ± 0.037
Model averaged estimates (across all models)					4.620×10^{-4} ± 0.010	0.234 ± 0.142	1.299×10^{-8} ± 2.572×10^{-8}	0.020 ± 0.040	0.023 ± 0.016	0.591 ± 0.044
Relative gonopodium size										

V_M	-501.979	1009.958	0	0.378		0.102 ± 0.035			0.012 ± 0.011	0.839 ± 0.045
$V_A+V_M+V_A\times$Food	-500.879	1011.757	1.799	0.154	0.019 ± 0.062	0.082 ± 0.042	0.083 ± 0.071		$7.749\times10^{-8} \pm 5.965\times10^{-9}$	0.766 ± 0.059
V_A+V_M	-501.888	1011.776	1.818	0.152	0.037 ± 0.079	0.089 ± 0.045			0.005 ± 0.017	0.820 ± 0.060
$V_M+V_M\times$Food	-501.931	1011.861	1.903	0.146		0.094 ± 0.050		0.017 ± 0.059	0.012 ± 0.011	0.831 ± 0.050
Model averaged estimates (across all models)					0.013 ± 0.043	0.083 ± 0.046	0.013 ± 0.034	0.003 ± 0.015	0.007 ± 0.012	0.750 ± 0.088
Male sperm number										
V_M	-215.358	436.716	0	0.323		0.073 ± 0.051			0.085 ± 0.042	0.824 ± 0.068
Null	-216.726	437.452	0.736	0.224					0.099 ± 0.042	0.885 ± 0.062
Model averaged estimates (across all models)					$1.247\times10^{-7} \pm 2.514\times10^{-7}$	0.042 ± 0.054	$4.051\times10^{-9} \pm 1.104\times10^{-8}$	$4.542\times10^{-8} \pm 7.846\times10^{-8}$	0.082 ± 0.044	0.774 ± 0.099
Male sperm velocity										
V_A	-179.474	364.947	0	0.127	0.190 ± 0.085				$7.034\times10^{-8} \pm 9.136\times10^{-9}$	0.695 ± 0.090
V_A+V_M	-179.458	366.915	1.968	0.115	0.175 ± 0.112	0.014 ± 0.075			$7.057\times10^{-8} \pm 9.077\times10^{-9}$	0.697 ± 0.090
Model averaged estimates (across all models)					0.100 ± 0.115	0.014 ± 0.046	$1.389\times10^{-7} \pm 1.928\times10^{-7}$	0.009 ± 0.044	0.008 ± 0.017	0.574 ± 0.172

Table 3. Variance-covariance matrices from the bivariate models of traits expressed in each environment for female age at maturity and male size at maturity. Variances of the parameters for each of the food treatments are shown on the diagonals (shaded), covariances below diagonal (in italics), and correlations above. All SEs are shown in brackets. V_A = additive genetic variance, V_M = maternal effects variance, V_B = block variance (only fitted for female age at maturity due to lack of convergence for male size at maturity), V_R = residual variance. Trait values were standardized to unit variance prior to analyses (see Methods for details).

	Female age at maturity		Male size at maturity	
	Control	Low food	Control	Low food
V_A	0.212 (0.122)	0.800 (0.699)	0.127 (0.102)	0.719 (0.644)
	0.204 (0.160)	0.307 (0.418)	*0.085 (0.068*)	0.110 (0.106)
V_M	0.222 (0.060)	0.711 (0.256)	0.330 (0.072)	-0.712 (1.378)
	0.256 (0.097)	0.585 (0.199)	*-0.062 (0.067*)	0.023 (0.074)
V_B	0.049 (0.036)	0.989 (0.776)	—	—
	0.071 (0.055)	0.106 (0.161)	—	—
V_R	0.363 (0.066)	—	0.570 (0.063)	—
	—	0.418 (0.232)	—	0.437 (0.087)

Titles and legends to figures

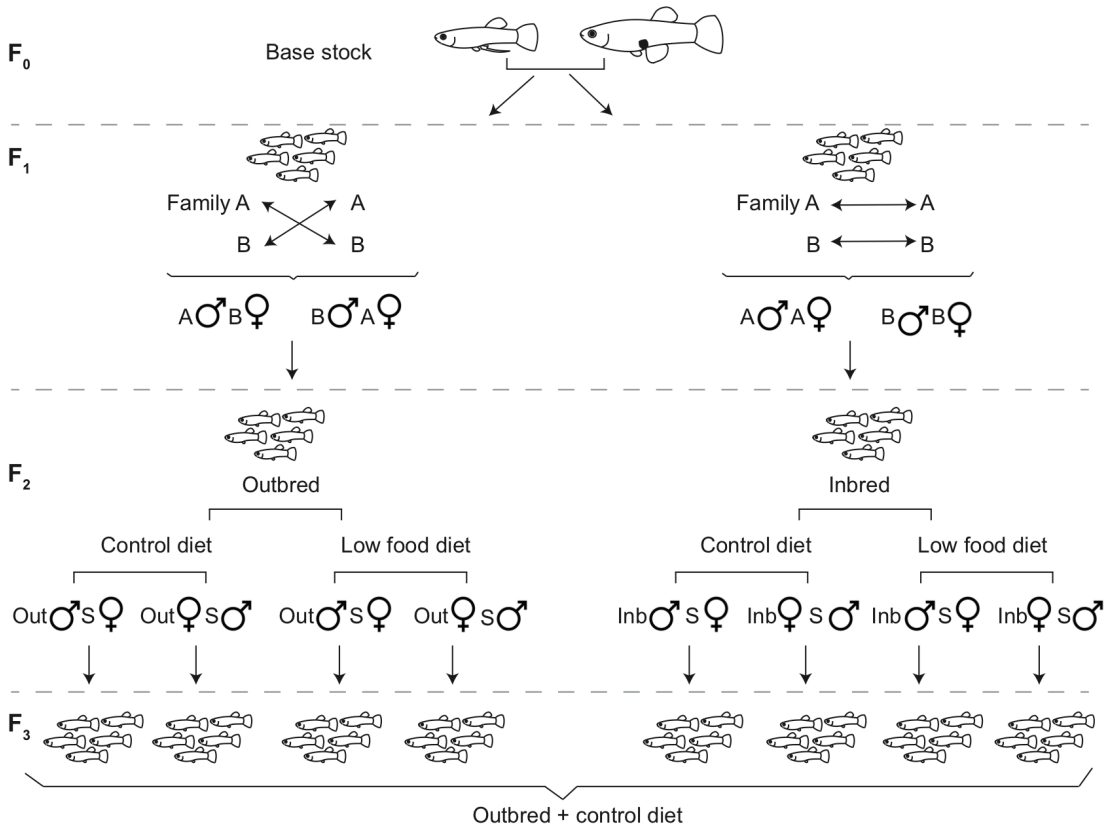

Figure 1. Schematic of the experimental design. S = stock fish. F_0 stock males and females were paired to create F_1 full-sib families (e.g. A and B). We set up 1-4 females per cross-type to create F_2 outbred (AB, BA—Out) and inbred (AA, BB—Inb) fish. These fish were reared on either a control or a low food diet early in life. F_2 females from each treatment were paired with a stock (outbred) male to create F_3 offspring. F_2 males from each treatment artificially inseminated stock (outbred) females to create F_3 offspring. F_3 offspring were classified as outbred and control diet.

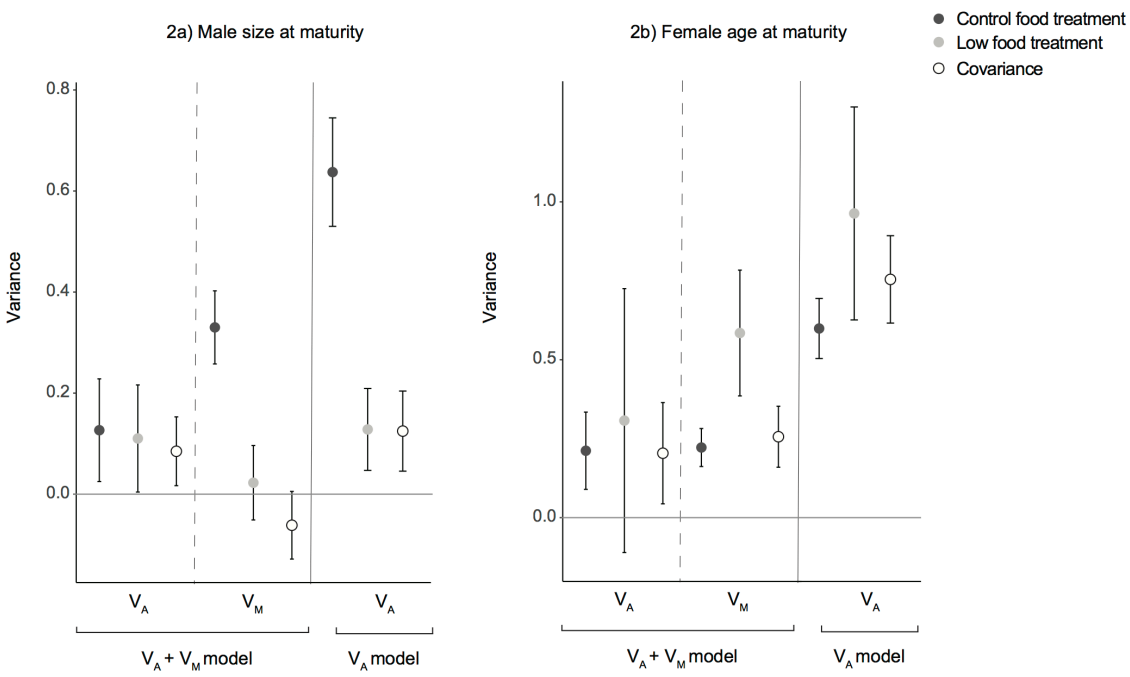

Fig 2. Effect of food treatment on variance components for (a) male size at maturity; (b) female age at maturity: additive genetic effects (V_A) and maternal effects variance (V_M) $\pm$ SE for a bivariate model with $V_A+V_M+V_B+V_R$, and V_A only ($+V_B+V_R$; see Methods for details). Dark symbols represent values for fish in the control food treatment, light symbols represent values for fish in the low food treatment, and white symbols represent the covariance between the traits in the two treatments.