Sex-specific plasticity and genotype x sex interactions for age and size of maturity in the sheepshead swordtail, *Xiphophorus birchmanni*

Running title:

Genotype-by-sex interactions in swordtails

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Abstract

Responses to sexually antagonistic selection are thought to be constrained by the shared genetic architecture of homologous male and female traits. Accordingly, adaptive sexual dimorphism depends on mechanisms such as genotype-by-sex interaction (GxS) and sex-specific plasticity to alleviate this constraint. We tested these mechanisms in a population of *Xiphophorus birchmanni* (sheepshead swordtail), where the intensity of male competition is expected to mediate inter-sexual conflict over age and size at maturity. Combining quantitative genetics with density manipulations and analysis of sex ratio variation, we confirm that maturation traits are dimorphic and heritable, but also subject to large GxS. While cross-sex genetic correlations are close to zero suggesting sex-linked genes with important effects on growth and maturation are likely segregating in this population, we found less evidence of sex-specific adaptive plasticity. At high density there was a weak trend towards later and smaller maturation in both sexes. Effects of sex ratio were stronger and putatively adaptive in males but not females. Males delay maturation in the presence of mature rivals, resulting in larger adult size with subsequent benefit to competitive ability. However, females also delay maturation in male-biased groups, incurring a loss of reproductive lifespan without apparent benefit. Thus, in highly competitive environments female fitness may be limited by the lack of sex-specific plasticity. More generally, assuming that selection does act antagonistically on male and female maturation traits in the wild, our results demonstrate that genetic architecture of homologous traits can ease a major constraint on the evolution of adaptive dimorphism.

Introduction

Sexual dimorphism arises because fitness is limited by different traits in females and males (Bateman, 1948). While fecundity is typically limiting for females, male fitness more often depends on traits that determine mating opportunities within the context of sexual selection imposed by female mate choice and/or male-male competition (Andersson, 1994). An important consequence of this is that homologous traits in males and females can have very different sex-specific optima (i.e. sexually antagonistic selection). In some cases sexual antagonism can be fully resolved over evolutionary time by the evolution of sex-limited traits. However, where this is not the case the degree of sex-specific adaptation depends on constraints arising from genetic architecture that is shared between the sexes (Poissant et al., 2010, Lande, 1980, Fairbairn & Roff, 2006). Here, we describe a study of two key life history traits - age and size at sexual maturation - in a poeciliid fish and evaluate the extent that shared genetic architecture has the potential to limit sex-specific adaptation given an expectation of sexually antagonistic selection in the wild. Additionally, we ask whether sex differences in plastic responses to changing levels of conspecific competition offer an alternative route to sex-specific adaptive phenotypic expression.

Tests of the hypothesis that shared genetic architecture constrains sex-specific adaptation have focussed largely on estimating the cross-sex genetic correlation (subsequently denoted r_{MF}) for homologous traits expressed in males and females (Walling et al., 2014, e.g., Pavitt et al., 2014). Strong cross-sex correlations, whether positive or negative, mean that sex-specific homologous traits are not free to evolve independently of one another. In the situation that directional selection is antagonistic in the two sexes, then the ability of each sex to reach its own optimum will be maximally constrained at $r_{MF} = 1$ (with no constraint when $r_{MF} = 0$). Meta-analysis suggests r_{MF} is usually strongly positive but negatively correlated with the degree of sexual dimorphism, and is lower for traits closely linked to fitness (Poissant et al., 2010). These patterns are consistent with the expectation that sex-specific genetic architecture (and hence reduced r_{MF}) will evolve to at least partially alleviate constraints on adaptive dimorphism (Charlesworth & Charlesworth, 1980).

Nonetheless, sexually antagonistic selection persists despite dimorphism (Cox & Calsbeek, 2009), r_{MF} between homologous traits is typically positive (Poissant et al., 2010), while negative cross-sex genetic correlations have been reported for fitness itself (Brommer et al., 2007). These observations suggest that constraints arising from shared genetic architecture between the sexes have not been fully resolved.

Though the patterns described above are consistent with the constraint hypothesis, the restricted focus on r_{MF} has been criticised as inadequate for understanding the potential for, and limitations to, sex-specific adaption. There are several reasons for this. Firstly, evolutionary responses to sex-specific selection on homologous traits will depend not just on the cross-sex genetic correlation but also on the presence and form of genotype-by-sex (GxS) interactions more generally. Importantly these can be manifest not just as $r_{MF} < 1$, but also as between-sex differences in the levels of additive genetic variance (Wyman & Rowe, 2014, Walling et al., 2014). Secondly, while studies typically focus on the genetic evolution of (mean) sex-specific traits, phenotypic plasticity can also contribute to sexual dimorphism (Stillwell et al., 2010). Not all plasticity is adaptive, and we also note the separation of genetic and environmental effects is not always clear cut (e.g. in the presence of genotype-by-environment interactions). However, where divergence of sex-specific phenotypic optima is sensitive to local environmental parameters, de-coupling of male and female plastic responses could be an important mechanism for allowing adaptive dimorphism (Hallsson & Bjorklund, 2012). For example, where male fitness is limited by intrasexual competition, delayed maturation can be advantageous for males, allowing avoidance of aggression from rivals until their chances of competitive success are improved, e.g., at greater age and/or size (Lyon & Montgomerie, 1986, Studd & Robertson, 1985).

Here we describe a study of sex-specific maturation traits in *Xiphophorus birchmanni,* (Lechner and Radder, 1987, sheepshead swordtail) that examines the potential for cross-sex genetic constraints and sex-specific plasticity. Though quantitative genetic analyses of cross-sex genetic architecture have not previously been conducted in this species, several lines of evidence from other

97 *Xiphophorus* species suggest that sexually antagonistic selection is likely to occur in the wild, and
98 that adaptive dimorphism may be facilitated by both cross-sex decoupling of genetic processes
99 (manifest as GxS) and plastic responses to the social environment. For instance, male-specific
100 genetic variance for life history traits is known to arise from Y-linked loci with major effects on
101 maturation age and size in populations of *X. maculatus* (platyfish; Basolo, 1988, Schreibman &
102 Kallman, 1977) and northern swordtails (*X. nigrensis*, X. montezumae, *X. multilineatus* ; Kallman,
103 1983, Ryan et al., 1992, Lampert et al., 2010). Nonetheless, behavioural and ecological studies
104 indicate inter-sexual conflict is often ongoing and likely to be contingent on the social environment
105 (Walling et al., 2007, Campton & Gall, 1988). Growth in male swordtails is almost determinate (i.e.
106 slows down markedly at maturation) whereas females display indeterminate (continuous) growth
107 (Evans et al., 2011). Size is important for both sexes, predicting dominance in males, who are
108 territorial and compete agonistically over access to females (Wilson et al., 2013, Prenter et al., 2008)
109 and fecundity in females. This can result in sex specific selection in certain environments, with males
110 delaying maturation in the presence of other males to allow increased size (and thus expected
111 dominance) at maturation, while females may even accelerate maturation under such circumstances
112 (Walling et al., 2007, Borowsky, 1973). This illustrates the potential for both ongoing sexual conflict
113 and contrasting sex-specific plastic responses to contribute to resolution.
114 We use quantitative genetic analyses and experimental manipulation of the competitive
115 environment (housing density) to provide the first assessment of the cross-sex genetic architecture
116 for maturation traits in *X. birchmanni* and to examine sex-specific plasticity. To investigate cross-sex
117 genetic architecture we apply pedigree-based animal models to characterise the genetic covariance
118 structure within and across sexes for age and size at maturation. We first estimate the magnitude of
119 genetic variation in sex-specific life history traits before formally testing for GxS interactions. If
120 present, GxS interactions generate sex-specific genetic variance that would facilitate evolution
121 towards divergent phenotypic optima in male and females if sexually antagonistic selection does
122 indeed operate on these traits as thought. If absent, shared genetic architecture will constrain

123 further evolution of sexual dimorphism. To examine sex-specific plasticity we combine a density
124 treatment (low versus high) with analysis of variation in sex ratio among mixed-family groups of
125 juveniles raised to maturation. In general, we expect high rearing density to increase social stress
126 arising from competitive interactions, leading to increased age and/or decreased size at maturity in
127 both sexes. However, we anticipate that maturation traits will respond to male-male competition by
128 being elevated in male biased groups housed at high density. We therefore predict that, if sex-
129 specific adaptive plastic responses are possible, then (conditional on main treatment effect of
130 density) males should mature later and at larger size where male-male competition is high.
131 Conversely, females should not delay maturation, and may accelerate it (Walling et al., 2007).
132
133 **Materials and methods**
134 *Fish husbandry and phenotyping*
135 In the spring of 2010, one hundred adult *Xiphophorus birchmanni* (40 male, 60 female) were
136 sampled by minnow trap from the Arroyo Coacuilco river (near Coacuilco, municipality of San Felipe
137 Orizatlán, Hidalgo, Mexico) and imported to the UK (April 2011). They were housed in breeding
138 groups comprising 1 male:3 females, in 30 L glass aquaria enriched with 3 - 5 mm diameter gravel
139 and live plants. Water was maintained at 21 - 23^{o}C, and a 12:12 hour light:dark cycle provided. Fish
140 were fed twice daily on proprietary flake food (ZM foods, http://www.zmsystems.co.uk/) and
141 previously frozen bloodworm and daphnia. Between August 2010 and May 2011, a captive bred
142 generation was produced (n = 384) comprising 61 full-sibling broods. Mean brood size born was
143 8.72 with a range from 1 - 24. Note that in some cases multiple broods were collected from the
144 same parental pair such that full-sibship sizes represented in the data set are larger (mean = 16.18,
145 range 1 - 51). Given the group housing regime, full-sib families are nested within half-sibships, with
146 a total of 32 female and 19 male parents contributing to the offspring generation.
147 To collect broods, breeding groups were inspected daily and obviously gravid females were
148 removed to isolation tanks enriched with stones and artificial plants to provide refuge for new born

offspring. Isolated females were also checked daily and returned to their breeding group tanks after giving birth. Broods were initially raised in 30 L tanks, partitioned into two equal volumes (using an acrylic frame covered with fine-gauge black nylon net) such that two families were raised in each tank. Tanks were grouped in "stacks", each comprising six 30 L tanks on a common recirculating water supply. This reduces the potential for between-tank variation in water quality to introduce bias in genetic parameter estimation (see below). Large broods were divided across tank partitions (setting a maximum of eight offspring per 15 L volume).

At an average age of 16 weeks (range 12 - 27 weeks), offspring were tagged with visible implant elastomer (http://www.nmt.us/products/vie/vie.shtml) and assigned to mixed family groups (n = 8 fish per group) subject to one of two density treatments. Low density groups (L) were housed in a full 30 L tank, high density groups (H) were housed in a half tank (i.e. 15 L volume partition of a 30 L tank as described above). Six stacks, each comprising four low and four high density groups on a recirculating water supply were sequentially established (Fig. S1). Variation in age of fish entering the experiment was thus unavoidable since a stack could only be set up when 64 fish (eight groups x eight fish per group) reached a size suitable for tagging. Juveniles of this species cannot be sexed from external characters and the sex ratio of mixed family groups was therefore uncontrolled. Fish were fed twice daily with a mixed diet of fresh brine shrimp nauplii and crushed flake from birth until mixing of families, and subsequently on the same diet as the wild caught breeding groups (with L and H groups receiving equal ration).

As part of a wider study involving long-term behavioural and growth phenotyping (see Boulton et al., 2014), all fish in the experiment were measured for standard length (SL) using digital callipers and live weight (WT) using a digital balance at four week intervals, for a period of 28 weeks in total. Age of maturation (AM) was recorded for each individual as age at the first sample date where sexing from external morphology was possible. For males, this was when the first thickening of the anal fin rays associated with gonopodium formation became apparent (i.e. following, (i.e. following Snelson, 1989)). Typically this is sooner than the development of other secondary male

characters such as the nuchal hump, vertical stripes and enlarged and pigmented dorsal fin seen in this species. Female AM was determined from a suite of characters that differentiate juveniles from mature females (abdomen shape, darkening of the "gravid spot" and lateral line). Given the lack of a single objective criterion to discriminate mature females from (unsexable) juveniles, measurement error is likely to be higher for female relative to male traits. However, all designations were made by a single investigator and were blind with respect to pedigree (and previously assigned sex) thus no expectation of bias arises with respect to genetic hypotheses (but see later discussion). Weight and standard length at maturity (WTM, SLM) were simply defined as the corresponding size measurements at AM. Sex could not be determined for a total of ten fish still alive at the end of the 28 week density treatment (n = 368 of the starting 384). However, continued monitoring of individuals for purposes out-with this study (Boulton et al., 2014) meant that maturation trait data was subsequently obtained (one female and nine males).

Analysis and quantitative genetic modelling

Exploratory data analysis was first conducted in R. We used simple linear models (i.e. without random effects) to estimate the relationships between maturation traits, and to test for differences in phenotypic means across sex and density treatment classes. We then used a series of animal models fitted using ASReml (Version 3) to formally test hypothesised plastic and genetic influences simultaneously as follows. First, for each sex-specific maturation trait we fitted a univariate model with the phenotype (y) of each individual (i) specified as:

$$y_i = \mu + \text{Stack} + \text{Density} + GS_i + SR_i + \text{Density:GS} + \text{Density:SR} + a_i + \varepsilon_i \quad \text{(Eqn 1)}$$

where μ is the mean, Stack is a seven level factor included to account for effects of any variation in water chemistry, and Density is a factor denoting treatment (Low (L) = 8 fish in 30 L, High(H) = 8 fish in 15 L). Group size (GS) and sex ratio (SR) experienced were defined as individual, rather than group

level covariates. GS_i is the geometric mean number of fish in i's group, averaged across the monthly assay points up to and including age of maturity (AM_i). Group size (and its interaction with Density) were included to control for any effects of mortality on phenotypes of surviving group mates (though in practice mortality levels were low; see results). SR_i was similarly defined as the geometric mean (over assay points up to an including AM_i) of the proportion of that individual's tank mates that are mature males (i.e. number of males in group excluding self/(number in group -1)). Geometric means across assay points were used to define GS_i and SR_i to better capture cumulative effects of social environment, but both variables were then centred to an (arithmetic) mean of zero across all individuals to aid interpretation of model estimates.

Additive genetic merit (a_i) was included as a random effect, assumed to be normally distributed with a mean of zero, and variance (V_A, the additive genetic variance) to be estimated using the pedigree structure (Wilson et al., 2010). Residuals (ε_i) are assumed to be uncorrelated across observations and normally distributed with a mean of zero and variance (V_R) to be estimated. Inference on fixed effects was based on conditional Wald F-tests implemented by ASReml. Significance of V_A was determined by likelihood ratio tests (LRT) comparing model fit with and without the additive genetic effect. For testing V_A in univariate models we assume the LRT test statistic is distributed as a 50:50 mix of χ^2_1 and χ^2_0 following (Visscher, 2006). Heritability was estimated as V_A/V_P with the phenotypic variance (V_P) determined as V_A+V_R (i.e. conditional on fixed effects).

The univariate model was then extended to the multivariate case to estimate the genetic variance-covariance matrix (**G**) between all six sex-specific traits (AM_F, WTM_F, SLM_F, AM_M, WTM_M, SLM_M), with additive covariance estimates also rescaled to give the corresponding genetic correlations (r_G). Fixed effects on each trait were as specified above. The full estimate of **G** was used to qualitatively assess the presence of GxS interactions. For more formal inference we tested these conditions using a series of bivariate model comparisons applied to each homologous trait pair using likelihood ratio tests (Table 4). These comparisons tested for (A) heterogeneity of total phenotypic

variance (V_P) across sexes and (B) GxS interactions (manifest as r_{MF} <1 and/or $V_{A(F)} \neq V_{A(M)}$). Note that in the absence of GxS, $a_{iF} = a_{iM}$ for any pair of sex-specific homologous traits (e.g. AM_F, AM_M), thus it follows that $V_{A(F)} = V_{A(M)}$ and r_{MF}= 1 (the "No GxS" scenario in table 4). To further explore whether patterns of GxS detected were driven by cross-sex genetic correlations or heterogeneity of V_A we compared (C) the full GxS model to one with freely estimated r_{MF} but with $V_{A(F)}$ constrained to equal $V_{A(M)}$ and (D) the full GxS model to one where $V_{A(F)}$ and $V_{A(M)}$ were free to differ but r_{MF} was constrained to equal +1. Note that for comparisons (B) - (D) all models included heterogeneous residual variance (i.e. $V_{R(F)}$ and $V_{R(M)}$ were free to differ) to prevent differences in environmental variance (or measurement error) generating spurious support for differences in sex-specific additive variance estimates.

Results

Exploratory data analysis

Size at maturity increases with age at maturity as expected (Fig. 1). Regressions of size at maturity (SLM) on age at maturity (AM) are significantly positive in females (β (SE) = 0.034 (0.004) $mm.day^{-1}$, P< 0.001) and males (β (SE) = 0.019 (0.004) $mm.day^{-1}$, P < 0.001). Pooling data and including SEX (male relative to female) and SEX:AM effects (as well as a main effect of AM) in the linear model confirms that the relationship in females is significantly steeper (SEX:AM coefficient (SE) = -0.0153 (0.006), $t = -2.705_{339}$, P = 0.007). The two measures of size at maturity are strongly correlated in both sexes (female, $r_{WTM.SLM}$ = 0.953, P <0.001; male, $r_{WTM.SLM}$ = 0.919, P < 0.001) therefore regressions of weight at maturity (WTM) on AM yield very similar patterns (results not shown). In addition to having steeper regressions of size on AM (Fig. 1), estimated correlations are stronger in females ($r_{AM.WTM}$ = 0.554 (0.057), $r_{AM.SLM}$ = 0.570 (0.056)) than males ($r_{AM.WTM}$ = 0.223 (0.068), $r_{AM.SLM}$ = 0.350 (0.063)). Testing against a null model of equal sex-specific correlations indicates this difference is statistically significant for $r_{AM.WTM}$ (χ^2_1 = 13.1, P < 0.001) and $r_{AM.SLM}$ (χ^2_1 = 6.62, P = 0.010). While suggesting a degree of decoupling of size and age of maturity in males relative to

253 females, we note that this result could also be driven by measurement bias (e.g., if body size
254 unintentionally influences scoring of maturity status in females).
255 Comparison of trait means across sexes and treatment classes shows all traits to be sexually
256 dimorphic but provides little evidence for plastic responses to the density treatment (Fig. 2). Note
257 that since maturity status is assessed from external morphology using sex-specific criteria here we
258 cannot be certain whether similar patterns would be found using physiological assays of maturation.
259 However, based on criteria used, males mature on average 23.7 (5.60) days later than females ($t = 4.22_{341}$, $P < 0.001$), at 0.248 (0.035) g heavier ($t = 7.031_{341}$, $P < 0.001$) and at 2.99 (0.314) mm longer
261 ($t = 9.545_{341}$, $P < 0.001$; results from linear models with sex as categorical predictor). Fish tended to
262 mature later and at smaller size at high density in both sexes but effects were largely non-significant.
263 Overall mean AM is significantly higher at high density (linear model with Density as categorical
264 predictor; difference of +12.4 (5.65) days, $t = 2.196_{341}$, $P = 0.029$). Statistical support for this effect is
265 not robust to addition of the sex effect into the linear model although the effect size is similar (effect
266 of high density = +10.4 (5.55) days, $t = 1.878_{340}$, $P = 0.061$).
267
268 *Genetic variation and GxS interactions*
269 Univariate animal models also provided evidence of genetic variation for maturation traits (Table 2).
270 Heritability estimates from univariate models range from 0.113 - 0.462 and are significant at $\alpha = 0.05$
271 except for male AM ($h^2 = 0.113$ (0.112), $\chi_{0,1}^2 = 2.11$, $P = 0.073$). The corresponding estimates from
272 the full (six trait) multivariate model are similar, though slightly higher (ranging from 0.166 - 0.477;
273 Table 3). In general, genetic correlation estimates are characterised by high uncertainty, although
274 the strong positive estimates between WTM_F and SLM_F, and WTM_M and SLM_M are nominally
275 significant based on $|r_G| > 1.96*SE$ Of particular note are the cross-sex (within-trait) genetic
276 correlation estimates (r_{MF}) of 0.066, -0.291 and -0.108 for AM, WTM and SLM respectively (Table 3).
277 Thus, not only are cross-sex genetic correlations not close to +1 (the expected value in the absence

of GxS), but for size at maturity traits they are actually negative (albeit not significantly less than zero).

More formal comparison of bivariate (cross-sex models) indicated that the null hypothesis of homogeneity in total phenotypic variance could be rejected for WTM (comparison (A) in Table 4). Statistical support for heterogeneous V_P in AM was marginally non-significant. In both cases phenotypic variance conditional on fixed effects is higher in males (as is also qualitatively the case for SLM). The full GxS model (allowing $r_{MF} < +1$ and $V_{A(F)} \neq V_{A(M)}$) was significantly better than the null model for WTM and SLM (comparison (B) in Table 4) though marginally non-significant for AM ($\chi^2_2 = 5.90$, $P = 0.052$). The full GxS model was not significantly better than the more restricted formulation where r_{MF} was free but homogeneity of V_A imposed in any case (comparison (C) in Table 4) for any trait. However, it was preferred in comparison (D) for WTM and SLM. We therefore conclude that, for WTM and SLM there is evidence for significant genotype-by-sex interactions driven primarily by cross-sex genetic correlations of less than 1 (rather than heterogeneity of sex specific genetic variances). For AM (where $r_{MF} = 0.066$ (0.488)) statistical support for GxS is slightly more equivocal since, as noted above, the overall test for GxS was marginally non-significant. However, post hoc comparison between a model with no GxS (such that $V_{A(F)} = V_{A(M)}$ and $r_{MF} = \pm 1$) and one where r_{MF} was allowed to depart form unity (with homogeneity of V_A imposed) suggests the latter is a significantly better fit to the data +1 ($\chi^2_1 = 5.00$, $P = 0.025$; comparison not shown in Table 4).

A graphical representation of these GxS interactions is illustrated in Fig. 3, with the red line denoting the 95% confidence interval for the null distribution of bivariate breeding values (estimated assuming $a_F = a_M$ such that $V_{A(F)} = V_{A(M)}$ and $r_{MF} = +1$). In all cases this line is a very poor fit to the distribution of bivariate breeding values estimated under the unconstrained model allowing GxS, represented by the grey ellipse.

Animal model-based estimates of phenotypic plasticity

Univariate animal models of sex-specific traits confirmed the finding from our exploratory analysis that plastic responses to density were limited (Table 1). In males, a significant density by group size (GS) interaction was found on age of maturation (AM). The positive sign of this coefficient implies that the effect of higher GS (a significant reduction in male maturation age) is less strong at high density than at low (Table 1). For females higher GS was associated with later maturation. No other effects of the density treatment were detected while GS did not significantly influence maturation size traits in either sex. Plastic responses to sex ratio variation were detected in both sexes (Table 1). For focal males, the presence of mature male group mates results in later maturity at larger size. For focal females, AM also increases with sex ratio (SR) but no significant effects on size at maturity were detected. Significant (or marginally non-significant) stack effects were found on all traits except male SLM (Table 1). These likely reflect average plasticity in response to between-stack variation in water conditions and/or uncontrolled temporal patterns in the laboratory environment (since stacks were set up sequentially; see methods). Since they are not relevant to hypotheses being tested here we do not discuss these further.

Discussion

Our results demonstrate that different genetic mechanisms underlie variation in age and size at maturity in males and females. The genetic architecture therefore acts to mitigate intersexual conflict over these traits. This finding is consistent with previous studies in swordtails that show a small number of loci on the Y chromosome can be responsible for much of the among-male variation in these traits. Sexual dimorphism is evident in all traits, with males tending to mature later and at larger size (based on our assessment of maturity status), and in the relationship between age and size at maturity. Though positively correlated in both sexes (as expected from studies of other fish including poeciliids (Snelson, 1984, Rowe & Thorpe, 1990, Morita & Fukuwaka, 2006) AM explains more variation in maturation size traits for females than males. Regressions of size traits on AM also show that absolute juvenile growth is faster in females. However, despite this sexual dimorphism in

mean phenotype, we found only limited support for putatively adaptive sex-specific plastic responses in maturation traits to competition (i.e., density, sex ratio). In what follows we first highlight the evolutionary implications of the GxS effects found before considering the results pertaining to plasticity in more detail.

Genetic (co)variance structure and GxS interactions

Quantitative genetic models revealed the presence of both genetic (co)variation and significant genotype-by-sex interactions. Thus, not only are life histories free to evolve, but to the extent that natural selection acts antagonistically in the wild, there is also potential for adaptive evolution of increased sexual dimorphism. Estimates of heritability were lower in males than for homologous female traits, a pattern driven by higher levels of residual variation rather than differences in additive genetic variance (V_A; discussed further below). Competition in general, and contest competition in particular, is expected to increase variance in resource dependent traits, since winners gain resource at the expense of losers (Wilson, 2014). Thus, higher residual variance in male traits is consistent with the well documented importance of male-male competition in swordtails (Earley, 2006), including *X. birchmanni* (Wilson, 2014). Within-sex genetic correlations (r_G) between SLM and WTM were close to +1 but interestingly we did not find strong genetic correlations between these traits and age at maturity. In females, moderate positive (but non-significant) estimates of r_G were found between traits, while in males these estimates were close to zero. Within both sexes, estimates of r_G between traits were characterised by high uncertainty and therefore should be interpreted cautiously. Nonetheless, while there is perhaps some suggestion that the tighter (positive) phenotypic correlation between age and size of maturity in females relative to males is mirrored at the genetic level, there is no strong evidence for a genetic basis to

353 the widely assumed fitness trade-off between age and size of maturity in either sex (Stearns, 1992,
354 Roff, 2002, Kruuk et al., 2008).
355 Formal testing for GxS interactions provided evidence that the genetic basis of life history
356 variation differs between males and females. While V_A for homologous traits did not differ
357 significantly between the sexes as has been reported elsewhere (Wyman & Rowe, 2014), estimated
358 cross-sex genetic correlations were close to zero, in contrast to the vast majority of empirical studies
359 of r_{MF}. These low genetic correlations between homologous traits in males and females imply a
360 considerable degree of genetic decoupling. Consequently additive variance can be considered
361 largely sex-specific and shared genetic architecture is not expected to be an important constraint on
362 adaptive dimorphism if males and females are subject to antagonistic selection. While our
363 experiment does not provide any information on the detail of this genetic architecture, low
364 estimates of r_{MF} will arise from sex-linkage and/or sex-limited expression of autosomal genes. Both
365 phenomena represent evolutionary solutions to the problem of sexual antagonism (Charlesworth &
366 Charlesworth, 1980) that are known to affect expression of size, growth, colouration and
367 behavioural traits in poeciliids (Postma et al., 2011, Lindholm & Breden, 2002). Y-linked variation
368 with allelic effect sizes sufficiently large to induce phenotypically distinct male morphs are known in
369 some *Xiphophorus* species (Cummings & Gelineau-Kattner, 2009, Ryan et al., 1992, Schreibman &
370 Kallman, 1977). For instance, in *X. nigrensis* and *X. multilineatus* membership of one of three adult
371 size morphs is strongly predicted by copy number variation of the melanocortin 4 receptor (mc4r)
372 gene on the Y chromosome (Lampert et al., 2010). Although there is no evidence of distinct male
373 size morphs, in *X. birchmanni* we note that higher allelic copy numbers (and or variation in copy
374 number) could easily rise to unimodal phenotypic distributions. Thus, we consider mc4r a good
375 candidate for contributing to the male-specific genetic variance found here, although this remains to
376 be tested.
377 Our conclusions with respect to the quantitative genetics of male and female life history
378 traits are contingent on several potentially important caveats. Firstly, parameters are estimated

379 under an additive model and assume absence of maternal and/or other early life common
380 environment effects (since offspring were raised in families until large enough to tag and mix).
381 Maternal effects on offspring traits are known to occur in poeciliid fishes, including *X. birchmanni*
382 (Reznick et al., 1996, Kindsvater et al., 2012), although the extent of their persistence to impact adult
383 traits is variable (Lindholm et al., 2006). Here the failure of some wild caught adults to reproduce
384 under lab conditions meant the size and structure (i.e. limited half-sib structuring) of our progeny
385 data set is not sufficient to effectively disentangle any maternal effects. While upward bias of
386 additive genetic parameters is certainly possible (Falconer & Mackay, 1996), no systematic bias
387 towards finding reduced r_{MF} is expected. Secondly, although we have considered both age and size
388 of maturation in this study, genetic covariance structure may well exist with other components of
389 life history (e.g. fecundity, adult growth, longevity) within and between sexes. Multivariate analysis
390 can identify constraints not apparent from pairwise genetic correlations alone (Walsh & Blows,
391 2009), although we note the converse is also true. Specifically, it has recently been argued that
392 multivariate treatments of sexual dimorphism may actually reveal greater evolutionary potential for
393 dimorphism than previously thought based on r_{MF} estimates (Wyman et al., 2013); see also (Walling
394 et al., 2014, Gosden & Chenoweth, 2014) for related discussion. Thirdly, we have implicitly assumed
395 an absence of GxE such that genetic covariance is modelled as being constant with social
396 environment (i.e., density and/or sex ratio). Available data was insufficient to support modelling of
397 life history traits disaggregated by both sex and environment and thus our genetic estimates should
398 be viewed as averaged across any genotype-by-environment (GxE) effects present. GxE can be of
399 considerable importance for sexually selected traits (Hunt & Hosken, 2014), although explicit studies
400 of GxExS are currently lacking. Despite the formidable empirical challenges, we suggest that
401 experiments to address this gap in our knowledge could offer great insights into the evolution of
402 sexual dimorphism. This is because GxExS implies the presence of sex-limited genetic variance, and
403 thus potential for independent evolution, not just of male and female traits but also of male and
404 female plasticity in those traits.

Sex-specificity of social plasticity

There was evidence of some social plasticity in both sexes, although life history traits were influenced more by sex ratio variation (SR) than by the experimentally applied density treatment. Broadly, responses are consistent with predictions made under the presumption that high density increases competition, and that for males this is exacerbated by a high sex ratio (i.e. presence of more mature rivals). However, while both sexes show a similar trend towards later maturation at smaller size under high density, effects were small and not statistically supported in the mixed models. Nevertheless, to the extent that plastic responses to the treatment are occurring, the trend is consistent with negative density dependence on life history with respect to expected fitness consequences. Although we also found a significant positive effect of group size (GS) on female maturation age (consistent with density dependence), in males the corresponding effect was actually negative (though less so at high density). These latter results are difficult to interpret since GS effects were modelled to control for within-group mortality rather than to test *a priori* hypotheses. It is possible that agonistic interactions between males that have already matured within-group increase their mortality risk, and thus lower GS may indicate that competition has been intense for males. However, since only 16 of 384 fish died before the end of the experiment variation in GS is very low (and non-random with respect to groups). We therefore consider it quite possible that this result is an artefact arising from data structure.

The direct effects of density on life history were thus limited and also similar in males and females. However, we also predicted plasticity in response to sex ratio variation and sex-differences in this response, with males responding to a greater extent at high density. Perhaps unsurprisingly given the lack of main effects, we found no significant interactions between density and sex ratio on either male or female life history to support the second of these predictions. Nevertheless, both male and female traits did respond to sex ratio, albeit in similar directions. Maturation occurs later and at larger size in the presence of more adult males in both sexes. This is consistent with our predictions of adaptive plasticity for males. Increased male-male competition results in sexual

selection that favours larger maturing males in swordtails, even if this comes at the cost of a delayed maturation time (Benson & Basolo, 2006, e.g., Basolo, 1988, Beaugrand et al., 1996).

Delayed female maturation in male-biased groups is counter to our expectations. With increasing numbers of mature males we predicted females should not delay maturation and may even advance it. This prediction was based on an assumption that more available males would increase the fitness benefits of early maturation. It is possible that mature males were socially dominant to females and thus able to monopolize resources (e.g. food) in the experimental conditions. If so, delayed female maturation may be a consequence of resource limitation. Harassment by males could also be a factor since it is energetically costly for both sexes and can disrupt female social structures (Darden et al., 2009, Darden & Watts, 2012). Given the lack of fitness data and presence of artificial conditions, we cannot completely exclude the possibility that this response confers some potential benefits under natural conditions. Nonetheless, while SR effects on female WTM and SLM are positive, they are modest and not significant. Consequently, it seems unlikely that delayed maturation in females can be compensated for by size-related increases in fecundity later. Whatever the explanation, our results suggest that males and females tend towards later and larger maturity in the presence of mature male group mates, the response being larger in males, where we predicted it to be adaptive.

Conclusions

In summary our study sought to test for sex specific genetic and social environment effects on age and size of maturity in the sheepshead swordtail. We found that these traits are sexually dimorphic and responsive to social factors expected to determine the intensity of competition. At high density there was a general tendency towards maturing later and at smaller size in both sexes. Though generally consistent with expected non-sex specific density dependence, these effects were modest and non-significant. Moreover, males were also found to delay maturation in the presence of mature rivals, a putatively adaptive response given that this results in larger adult size (and thus

higher success in male-male competition). Interestingly, females showed a similar pattern (albeit with smaller phenotypic changes) and delayed maturation in response to increased sex ratio. This is contrary to adaptive predictions, suggesting that a lack of sex-specific plasticity could limit expression of (adaptive) sexual dimorphism in social environments where male-male competition is high. Conversely, our quantitative genetic analyses illustrate that life history traits are subject to GxS interactions - age and size at maturity are heritable in both sexes but the cross-sex genetic correlations between homologous traits are close to zero (and significantly less than +1). Thus, to the extent that natural selection on maturation traits does act antagonistically in the wild, our results show that the genetic architecture of homologous traits can ease a major constraint on the evolution of adaptive dimorphism.

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References

Andersson, M. 1994. *Sexual selection, Princeton University Press, Princeton.*

Basolo, A. L. 1988. Female preference for body size, sword length and sword colour in the swordtail, *Xiphophorus helleri*. *Am. Zool.* **28**: A152-A152.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* **2**: 349-368.

Beaugrand, J. P., Payette, D. & Goulet, C. 1996. Conflict outcome in male green swordtail fish dyads (*Xiphophorus helleri*): Interaction of body size, prior dominance/subordination experience, and prior residency. *Behaviour* **133**: 303-319.

Benson, K. E. & Basolo, A. L. 2006. Male-male competition and the sword in male swordtails, *Xiphophorus helleri*. *Anim. Behav.* **71**: 129-134.

Borowsky, R. L. 1973. Social control of adult size in males of *Xiphophorus variatus*. *Nature* **245**: 332-335.

Boulton, K., Grimmer, A. J., Rosenthal, G. G., Walling, C. A. & Wilson, A. J. 2014. How stable are personalities? A multivariate view of behavioural variation over long and short timescales in the sheepshead swordtail, *Xiphophorus birchmanni*. *Behav. Ecol. Sociobiol.* **68**: 791-803.

Brommer, J. E., Kirkpatrick, M., Qvarnstrom, A. & Gustafsson, L. 2007. The intersexual genetic correlation for lifetime fitness in the wild and its implications for sexual selection. *Plos One* **2**: e744.

Campton, D. E. & Gall, G. A. E. 1988. Effect of individual and group rearing on age and size at maturity of male Mosquitofish, *Gambusia affinis*. *J. Fish Biol.* **33**: 203-212.

Charlesworth, D. & Charlesworth, B. 1980. Sex differences in fitness and selesction for centric fusions between sex-chromosomes and autosomes. *Genet. Res.* **35**: 205-214.

Cox, R. M. & Calsbeek, R. 2009. Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *Am. Nat.* **173**: 176-187.

Cummings, M. E. & Gelineau-Kattner, R. 2009. The energetic costs of alternative male reproductive strategies in *Xiphophorus nigrensis*. *J. Comp. Physiol. A.* **195**: 935-946.

Darden, S. K., James, R., Ramnarine, I. W. & Croft, D. P. 2009. Social implications of the battle of the sexes: sexual harassment disrupts female sociality and social recognition. *P. R. Soc. B.* **276**: 2651-2656.

Darden, S. K. & Watts, L. 2012. Male sexual harassment alters female social behaviour towards other females. *Biol. Lett.* **8**: 186-188.

Earley, R. 2006. Xiphophorus: carving a niche towards a broader understanding of aggression and dominance. *Zebrafish* **3**: 283-293.

508 Evans, J. P., Pialstro, A. & Schlupp, I. 2011. *Ecology and evolution of poecillid fishes*. University of
509 Chicago Press, Chicago.
510 Fairbairn, D. J. & Roff, D. A. 2006. The quantitative genetics of sexual dimorphism: assessing the
511 importance of sex-linkage. *Heredity* **97**: 319-328.
512 Falconer, D. S. & Mackay, T. F. C. 1996. *Introduction to quantitative genetics,* Fourth ed. Pearson
513 Education Ltd, Harlow, Essex, England.
514 Gosden, T. P. & Chenoweth, S. F. 2014. The evolutionary stability of cross-sex, cross-trait genetic
515 covariances. *Evolution* **68**: 1687-1697.
516 Hallsson, L. R. & Bjorklund, M. 2012. Selection in a fluctuating environment and the evolution of
517 sexual dimorphism in the seed beetle *Callosobruchus maculatus*. *J. Evol. Biol.* **25**: 1564-1575.
518 Hunt, J. & Hosken, D. J. (Eds.) 2014 *Genotype-by-environmnet interactions and sexual selection,*
519 Chichester, Wiley Blackwell.
520 Kallman, K.D. 1983. The sex determining mechanism of the poeciliid fish, *Xiphophorus montezumae,*
521 and the genetic control of the sexual maturation process and adult size. *Copeia* **1983**: 755-
522 769.
523 Kindsvater, H. K., Rosenthal, G. G. & Alonzo, S. H. 2012. Maternal size and age shape offspring size in
524 a live-bearing fish, *Xiphophorus birchmanni*. *Plos One* **7**.
525 Kruuk, L. E. B., Slate, J. & Wilson, A. J. 2008. New answers for old questions: the evolutionary
526 quantitative genetics of wild animal populations. *Annu. Rev. Ecol. Evol. S.* **39**: 525-548.
527 Lampert, K. P., Schmidt, C., Fischer, P., Volff, J. N., Hoffmann, C., Muck, J., Lohse, M.J., Ryan, M.J. &
528 Schartl, M. 2010. Determination of onset of sexual maturation and mating behavior by
529 melanocortin receptor 4 polymorphisms. *Curr. Biol.* **20**: 1729-1734.
530 Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters.
531 *Evolution* **34**: 292-305.
532 Lindholm, A. & Breden, F. 2002. Sex chromosomes and sexual selection in poeciliid fishes. *Am. Nat.*
533 **160**: S214-S224.

534 Lindholm, A. K., Hunt, J. & Brooks, R. 2006. Where do all the maternal effects go? Variation in
535 offspring body size through ontogeny in the live-bearing fish *Poecilia parae*. *Biol. Lett.* **2**: 586-
536 589.
537 Lyon, B. E. & Montgomerie, R. D. 1986. Delayed plumage maturation in passerine birds: Reliable
538 signalling by subordinate males? *Evolution* **40**: 605-615.
539 Morita, K. & Fukuwaka, M. 2006. Does size matter most? The effect of growth history on
540 probabilistic reaction norm for salmon maturation. *Evolution* **60**: 1516-1521.
541 Pavitt, A. T., Walling, C. A., Pemberton, J. M. & Kruuk, L. E. B. 2014. Heritability and cross-sex genetic
542 correlations of early-life circulating testosterone levels in a wild mammal. *Biol. Lett.* **10**.
543 Poissant, J., Wilson, A. J. & Coltman, D. W. 2010. Sex-specific genetic variance and the evolution of
544 sexual dimorphism: a systematic review of cross-sex genetic correlations. *Evolution* **64**: 97-
545 107.
546 Postma, E., Spyrou, N., Rollins, L. A. & Brooks, R. C. 2011. Sex-dependent selection differentially
547 shapes genetic variation on and off the guppy Y chromosome. *Evolution* **65**: 2145-2156.
548 Prenter, J., Taylor, P. W. & Elwood, R. W. 2008. Large body size for winning and large swords for
549 winning quickly in swordtail males, *Xiphophorus helleri*. *Anim. Behav.* **75**: 1981-1987.
550 Rauchenberger, M., Kallman, K. D. & Morizot, D. C. 1990. Monophyly and geography of the Rio
551 Panuco Basin Mexico swordtails genus *Xiphophorous* with descriptions of four new species.
552 *Am. Mus. Novit.* **2975**: 1-41.
553 Reznick, D., Callahan, H. & Llauredo, R. 1996. Maternal effects on offspring quality in poeciliid fishes.
554 *Am. Zool.* **36**: 147-156.
555 Roff, D. A. 2002. *Life history evolution*. Sinauer Associates, Ltd., Sunderland.
556 Rowe, D. K. & Thorpe, J. E. 1990. Suppression of maturation in male Atlantic salmon (*Salmo salar L*)
557 parr by reduction in feeding and growth during spring months. *Aquaculture* **86**: 291-313.
558 Ryan, M. J., Pease, C. M. & Morris, M. R. 1992. A genetic polymorphism in the swordtail
559 *Xiphophorous nigrensis* - testing the prediction of equal fitnesses. *Am. Nat.* **139**: 21-31.

560 Schreibman, M. P. & Kallman, K. D. 1977. The genetic control of the pituitary-gonadal axis in
561 platyfish, *Xiphophorous maculatus*. *J. Exp. Zool.* **200**: 277-293.
562 Snelson, F. F. 1984. Seasonal maturation and growth of males in a natural population of *Poecilia*
563 *latipinna*. *Copeia* **1984**: 252-255.
564 Snelson, F. F. J. 1989 Social and environmental control of life-history traits in poeciliid fishes. In:
565 *Ecology and evolution of livebearing fishes (Poecillidae)*, (GK, M. & FF, S., eds.). pp. 149-161.
566 Prentice Hall, Englewood Cliffs, New Jersey.
567 Stearns, S. C. 1992. *The evolution of life histories*. Oxford University Press, Oxford.
568 Stillwell, R. C., Blanckenhorn, W. U., Teder, T., Davidowitz, G. & Fox, C. W. 2010. Sex differences in
569 phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to
570 evolution. *Annu. Rev. Entomol.* **55**: 227-245.
571 Studd, M. V. & Robertson, R. J. 1985. Life span, competition, and delayed plumage maturation in
572 malse passerines: the breeding threshold hypothesis. *Am. Nat.* **126**: 101-115.
573 Visscher, P. M. 2006. A note on the asymptotic distribution of likelihood ratio tests to test variance
574 components. *Twin Res. Hum. Genet.* **9**: 490-5.
575 Walling, C. A., Morrisey, M., Foerster, K., Clutton-brock, T., Pemberton, J. M. & Kruuk, L. E. B. 2014. A
576 multivariate analysis of genetic constraints to life history evolution in a wild population of
577 red deer. *Genetics* **198**: 1735-1749.
578 Walling, C. A., Royle, N. J., Metcalfe, N. B. & Lindstrom, J. 2007. Green swordtails alter their age at
579 maturation in response to the population level of male ornamentation. *Biol. Let.* **3**: 144-146.
580 Walsh, B. & Blows, M. W. 2009 Abundant genetic variation plus strong selection = multivariate
581 genetic constraints: a geometric view of adaptation. *Annu. Rev. Ecol. Evol. S.* **40***:* 41-59.
582 Wilson, A. J. 2014. Competition as a source of constraint on life history evolution in natural
583 populations. *Heredity* **112**: 70-78.

584 Wilson, A. J., Grimmer, A. & Rosenthal, G. G. 2013. Causes and consequences of contest outcome:
585 aggressiveness, dominance and growth in the sheepshead swordtail, *Xiphophorus*
586 *birchmanni*. *Behav. Ecol. Sociobiol.* **67**: 1151-1161.
587 Wilson, A. J., Réale, D., Clements, M. N., Morrissey, M. M., Postma, E., Walling, C. A., Kruuk, L.E.B. &
588 Nussey, D. H. 2010. An ecologist's guide to the animal model. *J. Anim. Ecol.* **79**: 13-26.
589 Wyman, M. J. & Rowe, L. 2014. Male bias in distributions of additive genetic, residual and
590 phenotypic variances of shared traits *Am. Nat.* **184**: 326-337.
591 Wyman, M. J., Stinchcombe, J. R. & Rowe, L. 2013. A multivariate view of the evolution of sexual
592 dimorphism. *J. Evol. Biol.* **26**: 2070-2080.

Table 1: Fixed effect results from univariate animal models for age (AM), weight (WTM) and size at maturity (SLM), where GS is group size and SR is sex ratio.

			Female				Male			
Trait	**Effect**	**(Level)**	**Coefficient (SE)**	**DF**	**F**	**P**	**Coefficient (SE)**	**DF**	**F**	**P**
AM	μ		236 (8.89)	1,14.2	1511	<0.001	267 (9.92)	1,11	3191	<0.001
	Stack	(B)	-33.0 (10.3)	5,124.6	8.10	<0.001	-31.3 (12.3)	5,120	4.65	<0.001
		(D)	-57.4 (10.0)				-40.2 (11.8)			
		(E)	-59.3 (11.4)				-34.9 (11.9)			
		(F)	-51.8 (13.3)				-57.0 (12.4)			
		(G)	-34.2 (11.5)				-37.4 (11.5)			
	Density	(High)	8.87 (6.74)	1,126.2	0.77	0.384	-8.89 (6.14)	1,175.1	2.19	0.144
	GS		21.5 (57.3)	1,132.1	5.15	0.026	-159 (29.1)	1,183.6	40.9	<0.001
	SR		70.0 (21.9)	1,135.9	18.5	<0.001	99.7 (21.6)	1,182.4	44.7	<0.001
	Density:GS		-84.7 (61.0)	1,120.3	1.93	0.170	87.7 (32.5)	1,176.8	7.30	0.008
	Density:SR		39.9 (37.0)	1,131.4	1.16	0.285	11.2 (27.3)	1,181.7	0.17	0.676
WTM	μ		1.08 (0.063)	1,13.3	846	<0.001	1.46 (0.082)	1,13.8	1149	<0.001
	Stack	(B)	0.093(0.074)	5,121.8	2.25	0.054	0.020 (0.101)	5,134.2	3.95	0.002
		(D)	-0.067 (0.072)				0.002 (0.097)			
		(E)	-0.099(0.082)				-0.042 (0.098)			
		(F)	0.147 (0.095)				-0.218 (0.102)			
		(G)	0.048 (0.083)				-0.316 (0.094)			
	Density	(High)	-0.029 (0.045)	1,126.6	0.39	0.533	-0.087 (0.048)	1,173.3	3.18	0.078
	GS		-0.186 (0.416)	1,132.6	0.78	0.380	0.285 (0.230)	1,183	1.10	0.298
	SR		0.134 (0.158)	1,136.1	0.93	0.338	0.636 (0.170)	1,179.6	22.3	<0.001
	Density:GS		0.048 (0.443)	1,120.8	0.01	0.908	-0.214 (0.255)	1,176	0.70	0.403
	Density:SR		-0.005 (0.268)	1,132.1	0.00	0.983	-0.088 (0.216)	1,178.6	0.17	0.678
SLM	μ		34.3 (0.664)	1,14.6	7039	<0.001	37.7 (0.678)	1,13.8	11733	<0.001
	Stack	(B)	0.691 (0.765)	5,126.5	2.23	0.056	0.566 (0.838)	5,137.5	1.29	0.272
		(D)	-1.04 (0.746)				0.109 (0.802)			
		(E)	-0.949 (0.842)				-0.151 (0.806)			
		(F)	1.24 (0.981)				-1.10 (0.844)			
		(G)	0.883 (0.852)				-1.14 (0.770)			
	Density	(High)	-0.336 (0.495)	1,125.4	0.75	0.388	-0.668 (0.387)	1,171.6	2.94	0.091
	GS		-0.593 (4.21)	1,131.4	0.65	0.421	2.23 (1.85)	1,181.3	0.11	0.731
	SR		2.20 (1.61)	1,135.5	2.87	0.095	5.41 (1.37)	1,177.6	26.0	<0.001
	Density:GS		-0.875 (4.48)	1,119.4	0.04	0.839	-2.45 (2.05)	1,174.9	1.42	0.237
	Density:SR		0.594 (2.72)	1,130.5	0.05	0.821	-0.531 (1.73)	1,176.4	0.09	0.753

Table 2: Estimated variance components and heritabilities (h^2) from univariate animal models for age (AM), weight (WTM) and size (SLM) at maturity where V_P, V_A, and V_R are the phenotypic, additive genetic and residual variances respectively. Also presented are likelihood ratio tests of V_A with the tests statistic assumed to be distributed as a 50:50 mix of χ^2 on 1 and 0 degrees of freedom are indicated. Standard errors are indicated in parentheses.

Sex	**Trait**	**V_P (SE)**	**V_A (SE)**	**V_R (SE)**	**h^2**	**χ^2**	**P**
Female	AM	1313 (185)	543 (311)	770 (229)	0.413 (0.202)	9.77	0.001
	WTM	0.067 (0.009)	0.024 (0.015)	0.043 (0.012)	0.360 (0.198)	6.20	0.006
	SLM	7.28 (1.05)	3.36 (1.83)	3.92 (1.30)	0.462 (0.208)	9.59	0.001
Male	AM	1764 (189)	200 (204)	1564 (227)	0.113 (0.112)	2.11	0.073
	WTM	0.115 (0.013)	0.028 (0.018)	0.087 (0.016)	0.242 (0.145)	7.40	0.003
	SLM	7.74 (0.931)	2.43 (1.45)	5.31(1.11)	0.314 (0.166)	8.56	0.002

Table 3: Estimated heritabilities (shaded column) and genetic variance-covariance-correlation **(G matrix)** containing additive genetic variances (V_A, shaded diagonal), covariances (cov_A, below diagonal) and correlations (r_G, above diagonal), all with standard errors indicated in parentheses. All parameter estimates are from a multivariate (six trait) model and are conditional on fixed effects fitted as described in main text.

	Heritability	**G matrix**					
		AM_F	**WTM_F**	**SLM_F**	**AM_M**	**WTM_M**	**SLM_M**
AM_F	0.477 (0.207)	643 (341)	0.410 (0.330)	0.361 (0.318)	0.066 (0.488)	-0.137 (0.406)	-0.378 (0.347)
WTM_F	0.368 (0.202)	1.63 (1.79)	0.020 (0.020)	0.987 (0.025)	-0.410 (0.497)	-0.291 (0.436)	-0.084 (0.411)
SLM_F	0.460 (0.208)	16.7 (19.3)	0.282 (0.165)	3.33 (1.82)	-0.526 (0.438)	-0.304 (0.394)	-0.108 (0.381)
AM_M	0.166 (0.132)	28.8 (212)	-1.10 (1.43)	-16.5 (15.5)	294 (247)	-0.027 (0.518)	-0.029 (0.499)
WTM_M	0.274 (0.154)	-0.619 (1.86)	-0.008 (0.012)	-0.099 (0.134)	0.083 (1.58)	0.032 (0.020)	0.892 (0.087)
SLM_M	0.336 (0.165)	-15.5 (16.0)	-0.021 (0.104)	-0.319 (1.13)	-0.796 (13.7)	0.262 (0.147)	2.62 (1.48)

Table 4: Cross-sex tests for (A) heterogeneity of phenotypic variance (V_P) and (B) GxS interactions. Also presented are comparisons of the full GxS model to restricted scenarios where (C) $_{mf}$ is freely estimated but V_A assumed homogeneous and (D) $V_{A(F)}$ are allowed to differ $V_{A(M)}$ but $_{mf}$ is constrained to unity. For each comparison null (H0) and alternate (H1) hypotheses are shown with statistical inference from likelihood ratio tests.

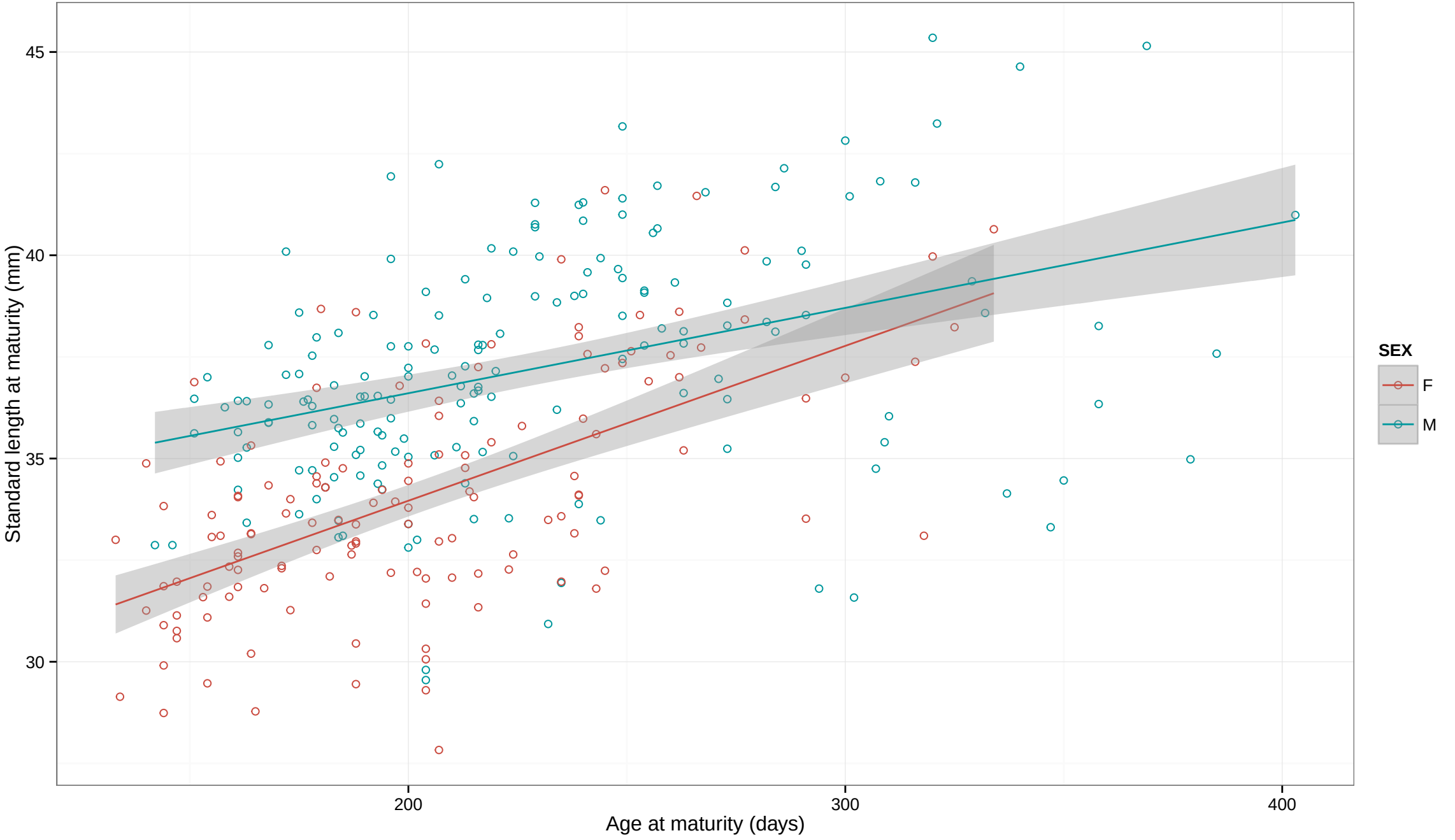
Trait	**Comparison**	**H0**	**H1**	**χ^2**	**DF**	**P**
AM	(A)	Homogeneous V_P	Heterogeneous V_P	3.80	1	0.051
	(B)	No GxS	GxS	5.90	2	0.052
	(C)	GxS	r_{MF} = +1, V_A assumed homogeneous	0.90	1	0.343
	(D)	GxS	V_A heterogeneous, r_{MF} = +1 assumed	1.70	1	0.427
WTM	(A)	Homogeneous V_P	Heterogeneous V_P	11.9	1	<0.001
	(B)	No GxS	GxS	12.5	2	0.002
	(C)	GxS	r_{MF} = +1, V_A assumed homogeneous	0.04	1	0.842
	(D)	GxS	V_A heterogeneous, r_{MF} = +1 assumed	7.10	1	0.029
SLM	(A)	Homogeneous V_P	Heterogeneous V_P	0.222	1	0.638
	(B)	No GxS	GxS	14.9	2	<0.001
	(C)	GxS	r_{MF} = +1, V_A assumed homogeneous	0.122	1	0.727
	(D)	GxS	V_A heterogeneous, r_{MF} = +1 assumed	9.07	1	0.011

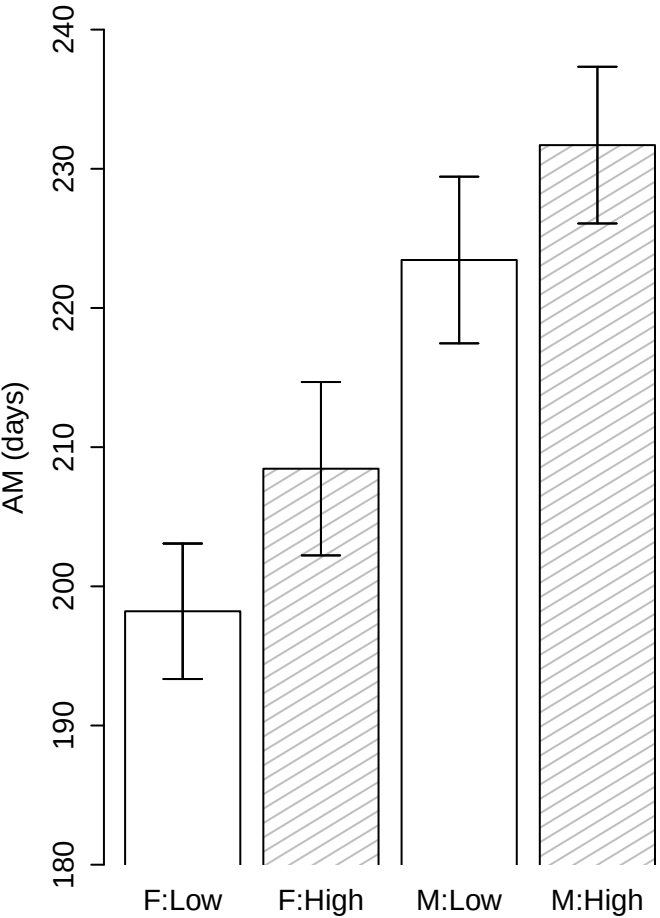
Figure legends

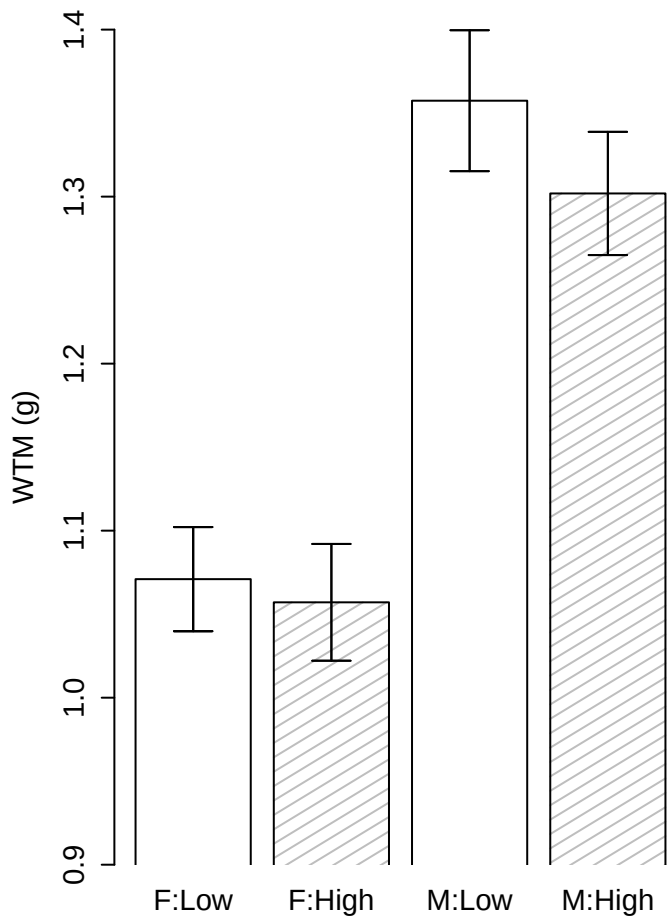
Fig. 1: Observed size (standard length) at maturation as a function of age by sex. Circles denote phenotypic observations (n_F = 148, n_M = 195) while solid lines illustrate predictions from simple linear regressions with shaded areas denoting predicted mean ± SE

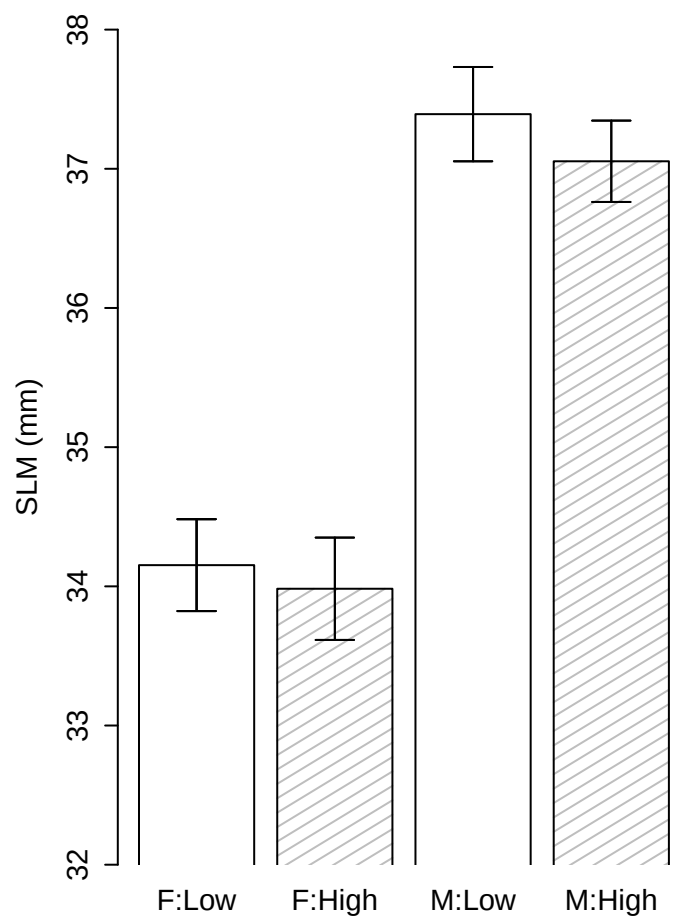
Fig. 2: Mean observed A) maturation age (AM), B) weight (MWT) and C) standard length (MSL) by sex for low and high density treatments. White columns denote low (L) and shaded columns indicate high (H) density treatments for male (M) and female (F) values with bars showing mean ± SE (n_{FL} = 83, n_{ML} = 92, while n_{FH} = 65, n_{MH} = 103)

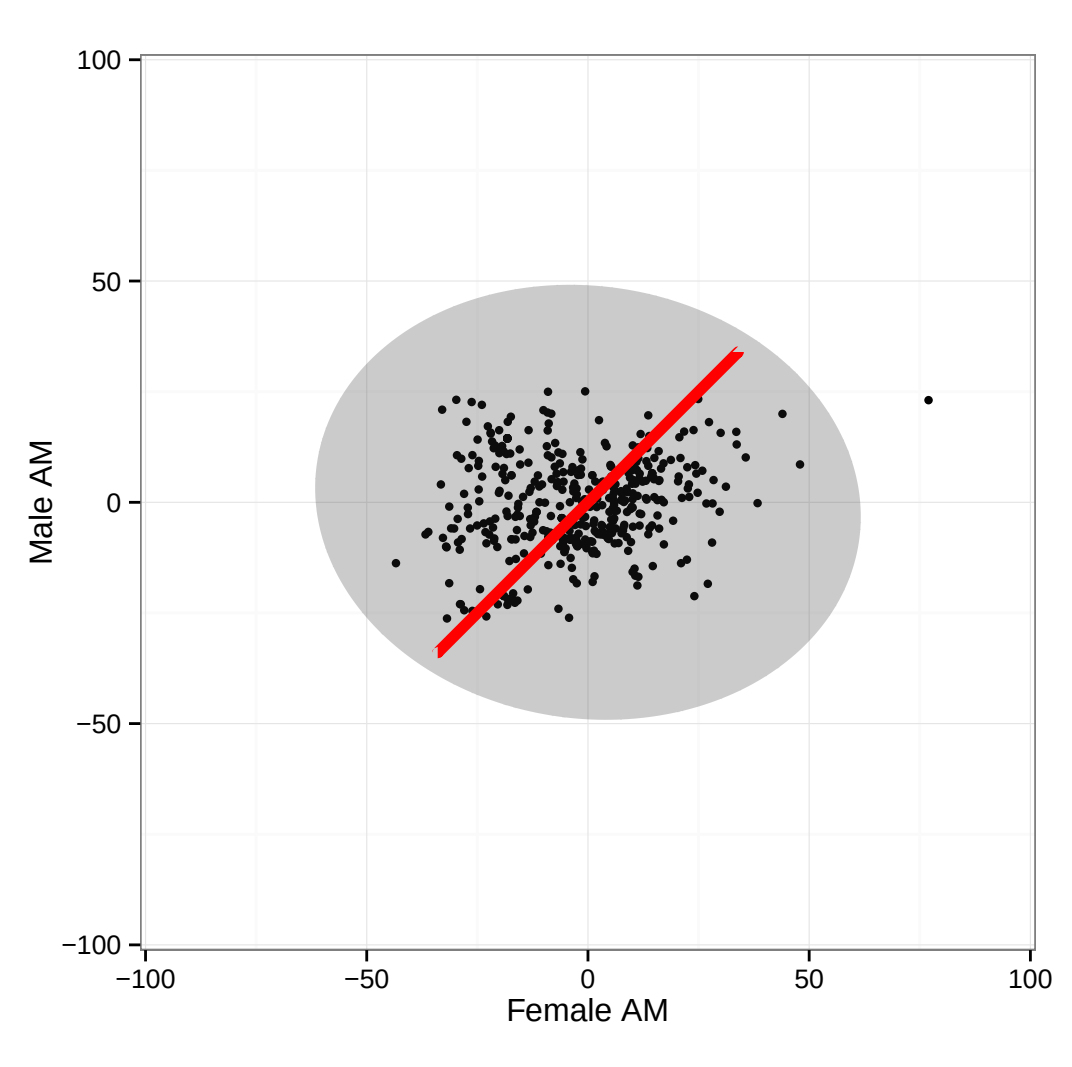
Fig. 3: Cross-sex genetic covariance structures for (A) maturation age (AM), (B) size at maturity (SLM) and (C) weight at maturity (WTM). Shaded ellipses denote the 95% confidence interval for the distribution of (bivariate) genetic merits in the population and solid points indicate BLUP for individuals in the data (both from the full multivariate model). For comparison, red lines indicate the distribution of breeding values estimated under the assumption of no GxS.

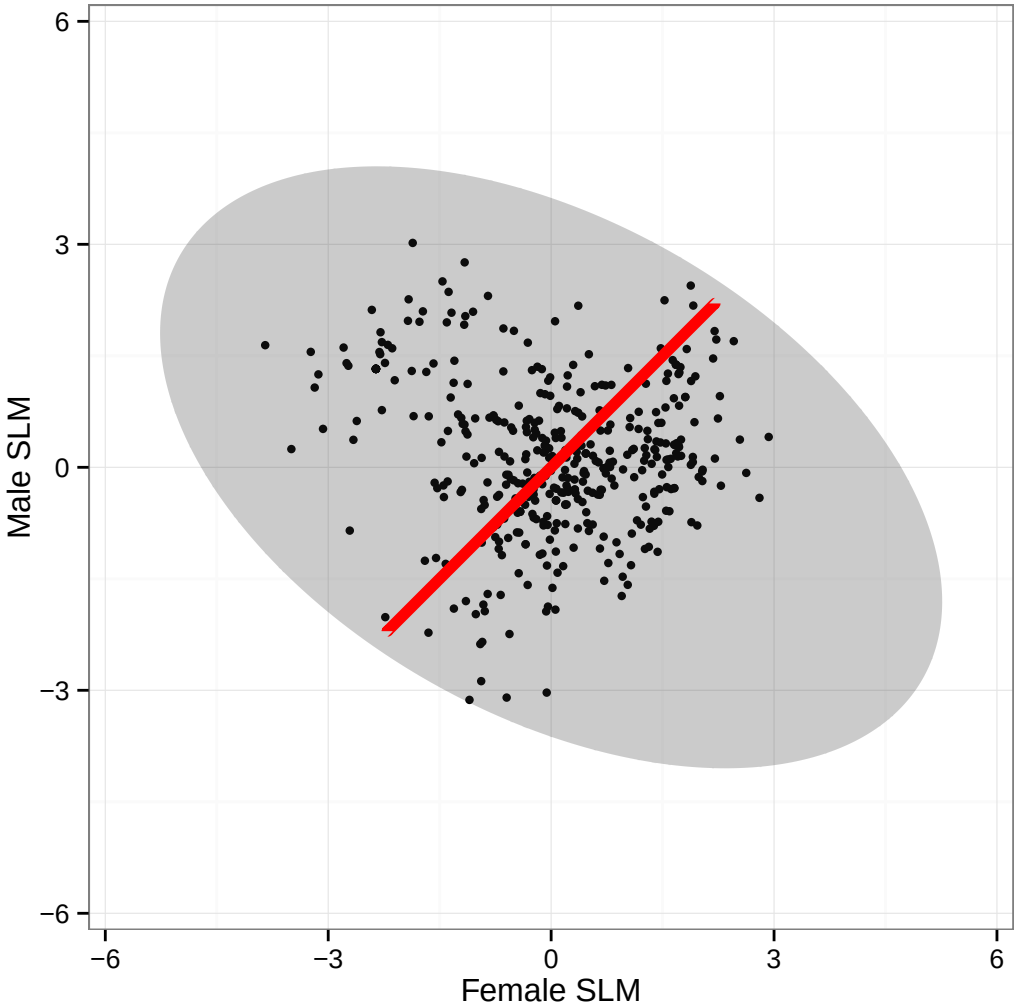

Standard length at maturity (mm)
45
40
35
30
200
300
400
Age at maturity (days)
SEX
F
M

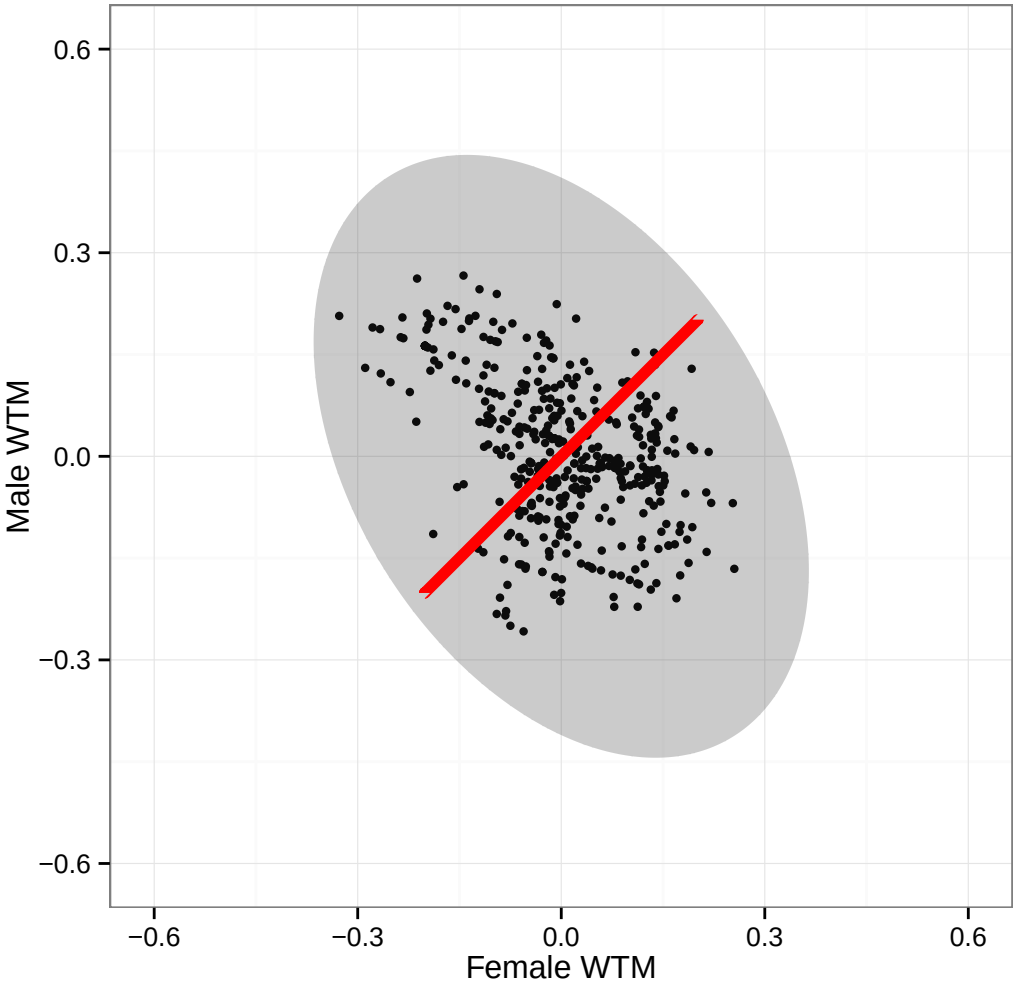

AM (days)
180
190
200
210
220
230
240
F:Low
F:High
M:Low
M:High


WTM (g)
0.9
1.0
1.1
1.2
1.3
1.4
F:Low
F:High
M:Low
M:High


SLM (mm)
32
33
34
35
36
37
38
F:Low
F:High
M:Low
M:High


100
50
0
−50
−100
Male AM
−100
−50
0
50
100
Female AM


6
3
0
−3
−6
Male SLM
−6
−3
0
3
6
Female SLM


0.6
0.3
0.0
−0.3
−0.6
Male WTM
−0.6
−0.3
0.0
0.3
0.6
Female WTM