Variance Components of Sex Determination in the copepod *Tigriopus californicus* estimated from a pedigree analysis.

Jean Richardson[1], Heather Alexander[2], and Bradley Anholt[1]

[1]University of Victoria
[2]Bamfield Marine Sciences Centre

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Abstract

Strong theory exists regarding population sex ratio evolution that predicts equal sex ratio (when parental investment is equal). In most animals, sex chromosomes determine the sex of offspring, and this fixed genotype for sex has made theory difficult to test since genotypic variance for the trait (sex) is lacking. It has long been argued that the genotype has become fixed in most animals due to the strong selection for equal sex ratios. The marine copepod *Tigriopus californicus* has no sex chromosomes, multiple genes affecting female brood sex ratio and a brood sex ratio that responds to selection. The species thus provides an opportune system in which to test established sex ratio theory. In this paper we further our exploration on the possibility that *T. californicus* has polygenic sex determination using an incomplete diallel crossing design and the "animal model" for analysis of the variance components of sex determination in the species. Our data confirm the presence of extra-binomial variance for sex, further confirming that sex is not determined through simple Mendelian trait inheritance. In addition, our crosses and backcrosses of isofemale lines selected for biased brood sex ratios show intermediate phenotypic means, as expected if sex is a threshold trait determined by an underlying "liability" trait controlled by many genes of small effects. Finally, we estimate heritability of an individual to be male or female on the observed binary scale as 0.09 (95% CI: 0.034-0.14). This work furthers our accumulating evidence for polygenic sex determination in T. californicus.

Introduction

Sex determination and sex ratio are intrinsically linked. In organisms where sex is determined solely by sex chromosome, the sex ratio for the offspring of a parent is determined on the basis of Mendelian inheritance and can be predicted simply from a binomial distribution (Krackow et al. 2002). Where multiple sex factor genes or other genes that affect sex tendency are present, sex ratio of offspring may be less straightforward, as has now been observed in a variety of fish species (Vandeputte et al. 2007; Liew et al. 2012; Faggion et al. 2019). In addition, environmental effects, including maternal effects, can modify sex tendency in an individual (Bull et al. 1982; Bull 1985; Sarre et al. 2004; Radder et al. 2008).

Historically, sex determination tended to be labelled as genetic (GSD) or environmental (ESD), but it is increasingly clear that this traditional view of sex determination is unreasonably simple (Uller and Helanterä 2011; Beukeboom and Perrin 2014). A variety of recent studies suggest that in species with sex chromosomes, sex of individuals can be strongly influenced by modifier autosomal genes and/or environmental effects. Notably, this has been observed in a variety of fish when reared in captivity, with aquaculturists often intentionally modifying population sex ratios to increase production of the more profitable sex (Vandeputte and Piferrer 2019; Zhou et al. 2020). In sea bass, Vandeputte (2007) showed that both a polygenic model with environmental variance and a two-locus gene model with environmental variance fit data from a crossing design.

Regardless of the sex determining mechanism, theory predicts that, where male and female offspring are

equally costly to produce, population sex ratio should be stable at 50% males and females (Fisher 1930; Shaw and Mohler 1953; Shaw 1958; Karlin and Lessard 1983) and unstable at biased sex ratios. Further, theory predicts polygenic determination of sex should never be stable (Rice 1986). Yet the harpacticoid copepod, *Tigriopus californicus* , a benthic species inhabiting high-intertidal pools along the west coast of North America, is well documented to have highly variable sex ratios (Ar-Rushdi 1958; Ar-rushdi 1963; Voordouw and Anholt 2002b; Voordouw et al. 2005) that, while affected by environment, are also highly heritable in a manner that suggests polygenic inheritance (Alexander et al. 2014, 2015).

The developmental process of sex determination in *Tigriopus californicus* is not known, but as males engaging in precopulatory mate-guarding will clasp females as early as copepodite stage CII (Burton 1985), sex is presumably determined by this stage. (Sexes cannot be differentiated morphologically until copepodite stage CIV.) Work on delineating sex determination and sex ratio processes in *T. californicus* is ongoing. Foley et al. (2013) crossed isofemale lines from two different populations and found that sex and mitochondrial background are significantly associated with genetic markers in nine of 12 chromosomes**.** In previous studies (Alexander et al. 2014, 2015), we selected on brood sex ratio to create male- and female-biased lines, showing that brood sex ratio responded to artificial truncation selection (Alexander et al. 2014). Using a California population of*T. californicus* with a published linkage map of SNP markers, we conducted crosses between that population (Foley et al. 2011) and a British Columbia population to identify quantitative genetic loci associated with brood sex ratio, finding that at least six QTL for brood sex ratio exist on five different chromosomes (Alexander et al. 2015). Here, we take advantage of the selected lines from the local population to conduct a diallel cross, followed by backcrosses to parental lines, and use an animal model analysis to assess the quantitative genetics of sex determination in *T. californicus* . Using pedigree data for F1 crosses and F2 backcrosses, we look at the heritability of an individual to become male and further explore the nature of polygenic sex determination in *T. californicus* .

Methods

Copepods were collected from high inter-tidal pools at Aguilar Point, British Columbia (48° 51´ 28" N, 125° 09´ 38" W). Samples from five pools were collected in September 2009 and transported directly to the laboratory at nearby Bamfield Marine Sciences Centre, Bamfield, B.C., Canada. From this initial group, two selection lines were established using truncation selection: male-biased and female-biased. For the male-biased line only females that produced the most male-biased brood sex ratios (BSRs) were allowed to breed in the next generation and for female-biased lines only females that produced the most female-biased BSRs were allowed to breed in the next generation (see Alexander et al. 2014 for complete details). At the end of six generations this resulted in a male-biased line with a mean (± SD) proportion male BSR of 0.64 ± 0.029 and female-biased line with a mean (± SD) proportion male BSR of 0.35 ± 0.031 (Alexander et al. 2014). While in the lab, *Tigriopus*were housed in filtered sea water (sea water drawn from 15 m deep in the ocean and filtered through a 1-micron filter), held at room temperature (20 °C) and fed ad libitum a mixture of ground TetraMin® and Spirulina flakes (50:50 by weight) suspended in filtered sea water at a ratio of 0.01 g/mL.

Line crosses were carried out between the two selection lines using offspring produced by the eight females (of >55 sampled) with the most biased brood sex ratio in each of the two selection lines (omitting any families with <6 individuals of either sex). Offspring from these 16 families were mated in an incomplete diallel cross design among four families (within family crosses were not done) from each line, replicated twice (Table 1).

F1 Crosses

Crosses were done by taking individual males and females from the appropriate families and placing them together in the well of a 12-well culture plate filled with 5 mL of filtered sea water (FSW). Males were removed after 7 days, and each female was subsequently checked twice daily for the presence of a mature

(red) egg sac. Females with red egg sacs were placed on moist filter paper and the egg sac gently removed using a fine insect pin (Burton 1985). The egg mass was placed into an empty well of a 6-well culture plate filled with 10 mL of FSW. Females were returned to their home well and continued to be checked for an egg sac; for 50 of the 73 crosses a second ripe egg sac was removed, and sex ratio estimates calculated for the second brood. Females typically have anywhere from 3-6 broods; all eggs are fertilized from sperm stored by the female during a single mating (Burton 1985).

When the brood of egg sacs placed in 6-well plates reached the copepodid stage (10-12 days post-hatching), copepodids were isolated from one another by transferring individuals to an individual well in 24-well plate filled with 2.5 mL FSW. At maturity, the number of males and females in each brood was counted to estimate BSR. A total of 71 (of a possible 112) F1 crosses that produced a minimum of 12 offspring for BSR estimate were analyzed.

Backcrosses

F1 cross offspring from 19 of the 34 families with parents from two different selection lines (FM1, MF1, FM2, MF2; Table 1) were backcrossed to male- and female-biased sex ratio parent lines (using the next generation of parental selection lines, i.e., generation 8). Offspring from all but three of these F1 families were used as both sires and dams, generating reciprocal backcrosses. A total of 223 of 280 possible reciprocal backcrosses for the 19 F1 families were done (Appendix Figure 1).

The first mature egg sac of each female in a backcross was plated into a 6-well plate, as above. For some crosses, we also plated a second egg sac. Offspring of these crosses were allowed to mature in 6-well plates and sex ratio determined when the brood matured by anesthetizing all individuals in the brood using 10% $MgCl_2$ (in FSW) prior to counting males and females.

Statistical Analysis

Phenotypic means and variances of brood sex ratio for each cross were calculated. We then tested whether the observed variance in sex ratio differed significantly (under- or over-dispersion) from that of the expected binomial distribution by generating random numbers of males and females in each observed brood (keeping observed brood size), as recommended by Krackow et al. (2002) and modifying code from Postma et al. (2011).

A pedigree analysis ('animal model'; Wilson*et al.* 2010; Postma *et al.*2011) was used to estimate variance components of sex in *Tigriopus* . We created a pedigree that included all 19,571 individuals sexed and used the R package 'MCMCglmm' (Hadfield 2010) to fit a generalized linear mixed model and generate Bayesian posterior distributions for estimating additive genetic variance, variance due to maternal effects, and heritability for sex. Each individual can only have one measure (it is either male or female) and therefore we cannot estimate residual variance and must instead fix it at some arbitrary value in the model (Postma et al. 2011); we used 1, finding this gave us the most well-behaved model (other values led to increased autocorrelation). We started with a model with no fixed effects and animal and dam as random effects, with the link function set as family = threshold. Six chains were run in parallel, each with a burn-in of 100,000, thinning of 5,000 and one million iterations, for a total sample size of 1080. We estimated effective sample size in all Bayesian models run to confirm validity of models. After ensuring chains were well-mixed, data from all chains were combined and the R Package QGglmm (de Villemereuil et al. 2016; de Villemereuil 2018) used to obtain heritability on the observed scale.

Results

Only 68 of 332 crosses had data for a second brood and we thus used only one brood from each cross in all analyses to avoid unequal sample size issues. Where two broods were available, we used the brood with

the largest size (typically, but not necessarily, the first produced). To avoid imprecise estimates of brood sex ratio, we kept only crosses with brood size >11 for analyses; this led to eight crosses being dropped for a total sample size of 326 crosses (32 parental, 71 F1 and 223 backcrosses).

Phenotypic variance increased substantially within one generation of crosses between selected lines, even within the same selection type and block, and phenotypic variance in F1s was similar regardless of parental selection line types and for both blocks (Table 2). Phenotypic mean values for F1s and backcrosses fell at the midpoints of parental lines and of parental and F1 lines, respectively, as expected for a polygenic inherited trait (Figure 1).

The observed variance in brood sex ratio using all crosses combined was 0.038 and significantly outside the bounds of binomial expected variance (randomization median variance = 0.0055; 95% CI: 0.0046-0.0065); in 5000 simulations a variance as large as that observed did not occur (Figure 2). This result clearly indicates that sex is not inherited as a simple dichotomous trait, as is the case in organisms with a sex chromosome.

In addition, brood sex ratio distribution by generation data show that while variation decreases in brood sex ratio during selection as F1 lines are crossed and backcrossed, observed variance in brood sex ratio far exceeds the range of the expected variance based on a null model of binomial trait inheritance and controlling for observed brood sizes (Figure 2). Similarly, as the selection lines interbreed, while the brood sex ratio distribution goes from bimodal to unimodal, the curve remains flattened with fewer observations of brood sex ratio having 0.4 to 0.6 proportion male than expected and more with proportion male <0.4 and >0.6 than expected under binomial inheritance (Figure 2).

Pedigree Analysis

We first fit a pedigree-based model with the random effects of animal and dam using MCMCglmm. This random effects-only model allowed us to confirm the model fit appropriately and returned the correct overall mean sex ratio. We used a threshold model, as is standard for binary or count trait values, modelling heritability on the underlying liability trait (de Villemereuil 2018) and achieved a model with good mixing of chains and acceptable autocorrelations (the majority <0.10; one of the six replicate chains had high autocorrelations of 0.18 and 0.19; removal of this chain from analysis did not affect analysis outcome). From this model, mean heritability on the liability scale was 0.237 (95% credible interval (CI): 0.00-0.51). Using QGglmm (de Villemereuil et al. 2016) we estimated trait values on the observed scale and found heritability for sex on the observed scale was 0.150 (95% CI: 0.0-0.33).

We next fit a model that included fixed effects of block and parental selection origin (i.e., are maternally and paternally inherited genes from male-biased (M) or female-biased (F) selection lines; four levels were possible: FF, FM, MF, MM, where the first letter indicates maternal gene source, and second letter indicates paternal gene source). No effect of block was present, but paternal by maternal combinations of male-biased or female-biased population origin did differ, with pMCMC <0.006 for each group relative to the reference group of female-biased lines in both paternal and maternal ancestry. We calculated heritability on both scales for this model integrating the variance from fixed effects to ensure our estimate is not inflated by the concomitant decrease in residual variance when fixed effects are partitioned out (de Villemereuil et al. 2018; de Villemereuil 2021). Heritability on the liability scale was 0.272 (95% CI: 0.136-0.437). On the observed scale, heritability for sex was 0.09 (95% CI: 0.034-0.143). Thus, accounting for the variance due to selection line differences did not change the heritability much but did substantially tighten up our credibility interval and provide us with a better estimate of heritability.

Discussion

In this study we have used strongly selected, highly inbred biased sex ratio lines to assess heritability of sex in a marine copepod species, *T. californicus* , without sex chromosomes using an incomplete diallel cross. We show: 1) Substantive extra-binomial variation for sex, that persists through two generations of random crosses in a controlled laboratory environment; 2) Mean phenotypic values for sex ratio in F1 and backcrossed offspring match the midpoint of the parental values as predicted in a normally distributed polygenic trait

with many genes of small effect; 3) Heritability for sex (on the observed scale) of 0.09 and heritability for the underlying threshold trait of 0.271. Heritability estimates for binary traits are necessarily limited to lower values due the nonadditive affects created by the link function and it is unclear what the maximum observable heritability is for sex in

T. californicus.

T. californicus gives every indication of having true polygenic inheritance of sex, with many genes of small effect contributing to the underlying liability trait value for the threshold trait of sex. The data presented in the current study confirms previous work done on sex ratio in this organism using different populations and estimation methods (Voordouw and Anholt 2002b; Voordouw et al. 2005, 2008; Foley et al. 2013; Alexander et al. 2014, 2015). Using a Bayesian pedigree analysis (i.e., the animal model), we show that the tendency for an individual *T. californicus* to become male has a significant genetic component, with a heritability estimate of 0.09, after removing variance due to fixed effects of breeding lines and blocks. Note that in this study, breeding lines were different isofemale lines and thus clearly also included a genetic component; thus, our heritability estimates are likely to be underestimates. By including the fixed effects in our Bayesian model, the credible interval of our estimate of heritability was decreased to one-third the size (Appendix Figure 2). We initially ran the model using a standard uninformative prior distribution, but as we had estimates of heritability from previous studies (Voordouw and Anholt 2002b; Alexander et al. 2014), we were in a position to use an informed prior and Bayesian analysis to update our prior knowledge. Using an informed prior did allow us to minimally reduce credible intervals around our heritability estimates but did not affect analysis results and had far less of an effect than adding in the ancestral selection line information (Appendix Figure 2).

The maintenance of variability, and continued presence of extra-binomial variation, when crossing offspring of two individuals from the same selection line provides strong evidence for many genes of small effect controlling sex determination in *T. californicus* . Further, the increase in phenotypic variation for F1s that occurred when different lines from the same selection type population were crossed suggests selection lines were achieved using different genes among lines. If lines were genetically similar, we would not expect much change between crosses within each selection type by block. This result is further corroborated through consideration of the phenotypic variance in brood sex ratio across generations under selection for biased sex ratios. While the sex ratios responded strongly to selection, the variance in brood sex ratio was essentially unchanging over the seven generations of selection (Figure 3). This also matches observations in the field of extensive variance in brood sex ratio both within and among sites (Voordouw et al. 2008) and models showing polygenic sex determination is maintained indefinitely when combined with seasonal fluctuations of alternating selection (Bateman and Anholt 2017).

Several other aspects of *T. californicus* biology are likely to contribute to maintenance of genetic variance in the species. *Tigriopus* live in supralittoral marine splash pools that are both ephemeral and highly variable environments and form a complex metapopulation, with each splash pool representing a subpopulation and migration occurring between splash pools. Charnov and Bull (1989) demonstrated that in patchy environments, if females do relatively better in one patch, then the primary sex ratio is male-biased (the sex coming from the poorer habitat). In addition, environmental sex determination (ESD) is also known to play role in *Tigriopus* sex determination (Voordouw and Anholt 2002a). In contrast, the failure of *T. californicus* to develop heterogametic sex determination is perhaps surprising given that females have achiasmatic meiosis (Ar-rushdi 1963), and with achiasmata in one sex any sex-determining gene that evolves should quickly lead to differentiated sex chromosomes (Wright et al. 2016).

A recent simulation by Butka and Freedberg (2019) reveals that when environmental sex determination is present and controlled by many loci ([?]10), limited dispersal rates (<0.5) among multiple subpopulations lead to a male-biased sex ratio equilibrium. Population genetic studies do suggest that *Tigriopus* dispersal among rocky outcrops is limited (Burton and Feldman 1981). At least six QTL exist for sex determination (Alexander et al. 2015) and recent research suggests such QTLs likely represent many separate genes each (Walsh and Lynch 2021). In nature, *T. californicus* populations do tend to be male-biased (Voordouw et

al. 2008). The combination of the modelling and field data thus suggest one possible explanation for male-biased sex ratios observed in *T. californicus* and further reinforces the idea that the species has polygenic sex determination. Environmental variance represents only a minor portion of sex ratio variance in *T. californicus* (Voordouw and Anholt 2002a,b) and genetic influence on pivotal temperature has not been considered; it is possible that selection for sex ratio bias is in fact selecting for changes in pivotal temperature (Wright et al. 2016).

Variance for threshold traits on the observed scale contains additional variance and this reduces maximum heritability. For example, while the liability trait, on the latent scale, has a continuous range of values, the observed phenotype has only one of two values, determined by the latent scale breeding value and the threshold value. Thus, if the threshold value is 0.4, whether the individual's breeding value is 0.2 or 0.01 they will be male on the observed scale. This has the effect of increasing the non-additive genetic variance for the trait on the observed scale, thereby limiting the maximum heritability possible. In particular, heritability on the observed scale will always be lower than that on the latent scale (Dempster and Lerner 1950; de Villemereuil et al. 2016; de Villemereuil 2020). This is one reason why the heritability observed here, given on the observed scale, is lower than the realized heritability estimated on the latent scale by Alexander et al. (2014). Nonetheless, a strong response to truncation selection for biased sex ratios clearly indicates some aspect of sex determination in the species is sufficiently heritable to respond to selection. We speculate that epistatic effects may also limit our ability to estimate true heritability.

The difference in estimated heritabilities may also reflect violation of any one of the many assumptions of the threshold model. In particular, the model assumes allelic effects at the many loci contributing to liability are multivariate normal. This is both unlikely to be true and difficult to assess. Benchek and Morris (2013), using simulated data to test heritability estimates when true liability included a common environmental effect that was not normally distributed, found that heritability estimates can be highly biased in this case and that the direction of bias was not consistent. The model also assumes no pleiotropic or epigenetic effects, but environment is known to influence sex determination in *Tigriopus* . Temperature effects on sex may well be influenced by genes and alleles affecting sex determination and temperature effects on sex determination seem likely to interact with each other as well as with the environment. At the heart of the challenge is that selection acts on the multivariate phenotype and any one component in isolation may have low heritability although the combined traits have high heritability (Walsh and Lynch 2018).

Regardless of the underlying genetic mechanism, it seems likely that the complex metapopulation dynamics of *Tigriopus* (Dethier 1980; Burton and Swisher 1984; Powlik 1999; Johnson 2001) may be an important component to understanding the unusual maintenance of polygenic sex determination in the species. The highly unpredictable nature of the splash pools these copepods inhabit may further provide insight into why this species has failed to evolve a single gene of large effect for sex tendency. Pools that are washed out by wave action will cause large-scale mortality unrelated to phenotype, as most individuals washed into the ocean are likely to be consumed by fish (Dethier 1980).

While the presence of multiple genes affecting sex has recently been observed in many animals, particularly in fish species (Martinez et al. 2014), in most of these cases a sex chromosome or gene with large effect on sex is present in the species. The case for polygenic sex determination has perhaps been most strongly made for the model organism zebrafish (Liew et al. 2012), where only two to three (depending on strain) sex determining regions (compared to six in *Tigriopus*) have been identified in domesticated zebrafish (Wilson et al. 2014) and wild zebrafish have a ZZ/ZW sex determining system (Wilson et al. 2014). Similarly in European sea bass, while genetic components for sex determination are present and suggest polygenic sex determination (Vandeputte et al. 2007), sex determination is also strongly influenced by temperature and wild populations do not show the same sex ratio biases seen in farmed populations (Vandeputte et al. 2012). We suggest that *T. californicus* represents a unique polygenic system in that there is no indication that any one gene has a large effect on sex determination nor that such a gene has ever existed in the species. The species thus continues to present an interesting case study that appears to defy theoretical expectations.

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Table 1 . Types of crosses performed; sample size is given in brackets. For F1 crosses, no within-family crosses were done.

	Block 1	Block 1	Block 2	Block 2
Parental Lines*	F1a F1b F1c F1d M1a M1b M1c M1d	F1a F1b F1c F1d M1a M1b M1c M1d	F2a F2b F2c F2d M2a M2b M2c Md	F2a F2b F2c F2d M2a M2b M2c Md
F1 Crosses	FF1 (8) FM1 (8) MF1 (9) MM1 (10)	FF1 (8) FM1 (8) MF1 (9) MM1 (10)	FF2 (9) FM2 (9) MF2 (8) MM2 (10)	FF2 (9) FM2 (9) MF2 (8) MM2 (10)
Back Crosses				
Backcross Dam x F1 Sire	F1.FM1 (12)	F1.FM2 (4)	F2.FM1 (9)	F2.FM2 (3)
	F1.MF1 (17)	F1.MF2 (4)	F2.MF1 (7)	F2.MF2 (5)
	M1.FM1 (12)	M1.FM2 (4)	M2.FM1 (11)	M2.FM2 (3)
	M1.MF1 (10)		M2.MF1 (8)	M2.MF2 (5)
F1 Dam x Backcross Sire	FM1.F1 (9)	FM2.F1 (4)	FM1.F2 (10)	FM2.F2 (4)
	MF1.F1 (14)		MF1.F2 (7)	MF2.F2 (7)
	FM1.M1 (11)	FM2.M1 (4)	FM1.M2 (10)	FM2.M2 (2)
	MF1.M1 (14)		MF1.M2 (5)	MF2.M2 (8)

*16 Lines selected for male-biased (M) or female-biased (F) sex ratios using truncation selection over six generations.

Table 2 . Change in sex ratio variation across one generation of reciprocal crossing of dams and sires between lines and both within and between selection types.

		Parent Generation	F1 Generation
Block 1	Female-biased lines	0.000435	0.0325
			0.0520
			0.0267
	Male-biased lines	0.003228	0.0312
Block 2	Female-biased lines	0.004981	0.04267
			0.06566
			0.03125
	Male-biased lines	0.000470	0.02909

Figure Captions

Figure 1. Mean and standard errors for brood sex ratios for all cross types done. Solid points are parental selection lines (female-biased selection on left-hand side; male-biased selection lines on right-hand side); each point represents block by parent/backcross parent group. Open symbols are results from crosses where $n>$ 5 for each cross type by block combination.

*Figure 2***.** Observed (bars) and expected (points ± SE) distribution of brood sex ratio (left panel) and expected brood sex ratio (BSR) distribution (right panel) for parental, F1 and backcross generations of *Tigriopus californicus* crosses among male- and female-bias selection lines. On right panel median and 95% quantile limits are shown for expected distribution of variance using dashed and dotted vertical lines, respectively; solid lines indicate observed BSR variance.

Figure 3. Change in phenotypic variance for brood sex ratio, measured as standard deviation in qnorm(proportion male) for each generation during truncation selection for biased brood sex ratios. Data from (Alexander et al. 2014). Selection lines are: C = control (no selection), F = female-biased, M = male-biased; standard least-squares lines of best fit are given for each line with gray shading indicating 95% regions. After generation 7, brood sex ratio (proportion male) was 0.75 for M line, 0.35 for F line and 0.45 for C line; all had brood sex ratio = 0.51 at generation 1.

Appendix Figure 1 . Schematic of crossing design. Two blocks were used, each with four female-biased and four male-biased selection lines. Cells filled with a number indicate we have data for a cross between these two lines. Only offspring from crosses between selection line types were carried forward for backcrosses; shaded cells indicate crosses whose offspring were used in backcrosses. Siblings from F1 crosses were used in backcrosses with 3-4 male- and female-biased parental lines in the majority of cases.

Appendix Figure 2 . Result of changing prior distribution used from uninformative (grey line) to informative (black line) (top panel) on posterior distribution of heritability on the observed scale in sex of an individual (bottom panel). The informed prior slightly decreases the width of the posterior distribution (shown in black; grey line is density for uninformative prior). UP = uninformative prior; IP = informative prior.

Data Accessibility Statement

Raw data, R code for cleaning and formatting data, cleaned data and R code for analyses will be made available on Dryad.

Competing Interests Statement

All authors declare they have no competing interests.

Author Contributions

Heather Alexander: conceptualization (equal), methodology (equal), investigation (equal), writing – review and editing (equal)**. Bradley Anholt:** conceptualization (equal), funding acquisition (lead), methodology (equal), writing – review and editing (equal). **Jean Richardson** : conceptualization (equal), methodology (equal), formal analysis (lead), investigation (equal), visualization (lead), writing – original draft preparation (lead), writing – review & editing (equal).

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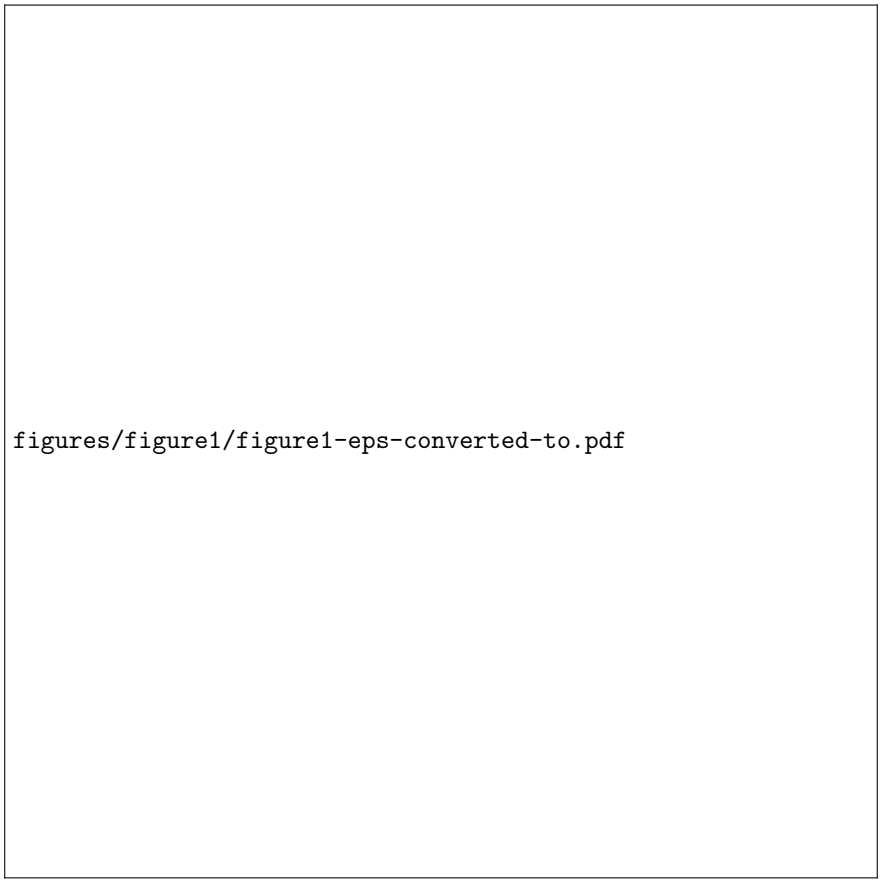

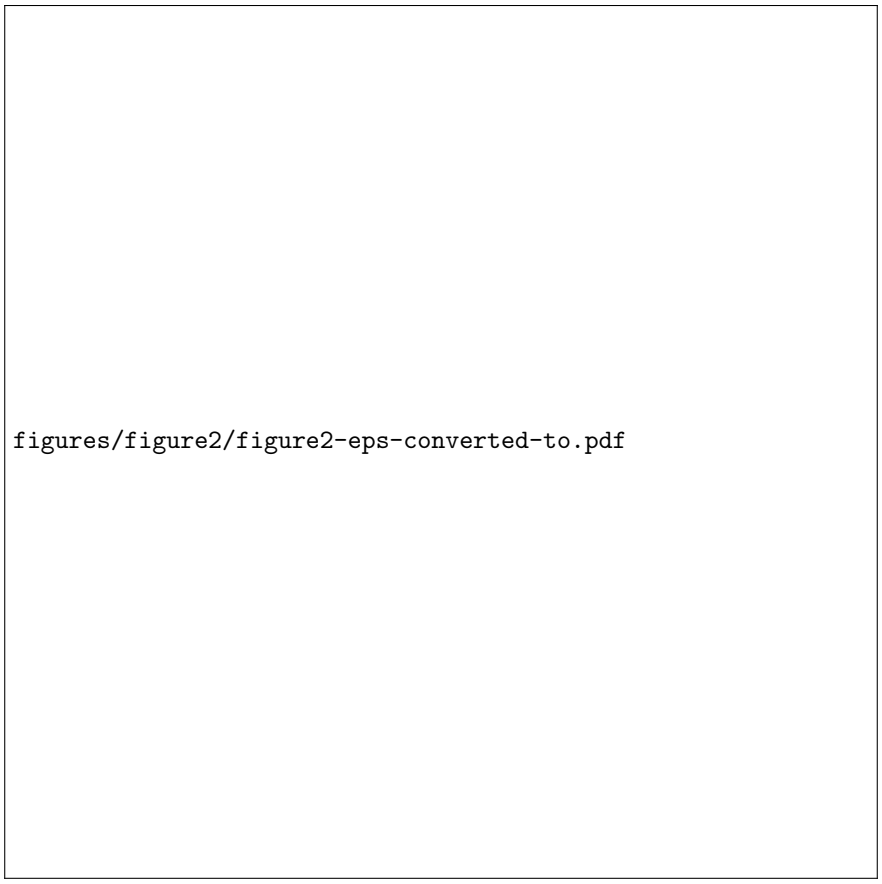

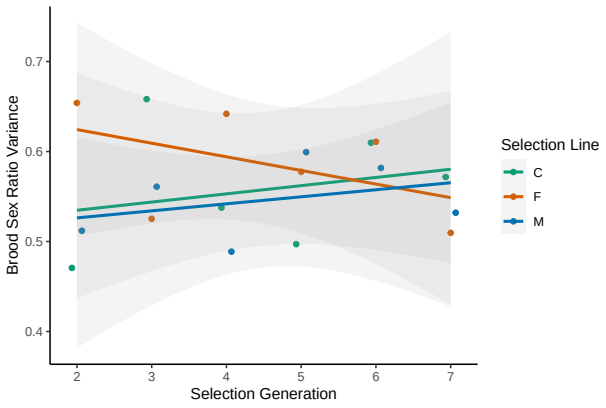

BLOCK 1

		Female Biased Lines				Male Biased Lines			
		Fb1	**Fb2**	**Fb3**	**Fb4**	**Mb1**	**Mb2**	**Mb3**	**Mb4**
Female Biased Lines (F1)	**Fb1**				33		51	52	
	Fb2	34		35	36	53		54	
	Fb3		37		38	55			
	Fb4		39	40		56	57	58	
Male Biased Lines (M1)	**Mb1**		82	83	84		99	100	
	Mb2	85†		86	87*	101		102	103
	Mb3				88	104	105		106
	Mb4	89	90			107	108		

F1 offspring used for Backcrosses with Block 1 parent lines

F1 offspring used for Backcrosses with Block 2 parent lines

† Only daughters used in BX

* Only sons used in BX

BLOCK 2

		Female Biased Lines				Male Biased Lines			
		Fb5	**Fb6**	**Fb7**	**Fb8**	**Mb5**	**Mb6**	**Mb7**	**Mb8**
Female Biased Lines (F2)	**Fb5**		41		42			59	60
	Fb6	43			44			61	62
	Fb7		46		47	63	64		65
	Fb8	48	49	50		66	67		
Male Biased Lines (M2)	**Mb5**		91		92		109	110	
	Mb6	93			94	111		112	113
	Mb7	95	96			114	115		116
	Mb8	97*	98				118	119