A sexually-selected male weapon characterised by strong additive genetic variance and no evidence for sexually antagonistic polyphenic maintenance

Jonathan M Parrett[1], Aleksandra Łukasiewicz[1], Sebastian Chmielewski[1], Agnieszka Szubert-Kruszyńska[1], Paul L. Maurizio[2], Karl Grieshop[3,4], Jacek Radwan[1]

[1]Evolutionary Biology Group, Faculty of Biology, Adam Mickiewicz University, , Poznań, Poland

[2] Department of Medicine, Section of Genetic Medicine, University of Chicago, Chicago, Illinois, United States

[3] Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada

[4]Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden

Corresponding authors: Jonathan M. Parrett and Jacek Radwan email: jonathan.parrett@amu.edu.pl; jacek.radwan@amu.edu.pl

Abstract

Sexual selection and sexual antagonism are important drivers of eco-evolutionary processes. The evolution of traits shaped by these processes depends on their genetic architecture, which remain poorly studied. Here, implementing a quantitative genetics approach using diallel crosses of the bulb mite, *Rhizoglyphus robini*, we investigated the genetic variance that underlies a sexually-selected weapon that is dimorphic among males and female fecundity. Previous studies indicated that a negative genetic correlation between these two traits likely exists. We found male morph showed considerable additive genetic variance, which is unlikely to be explained solely by mutation-selection balance, indicating the likely presence of large-effect loci. However, a significant magnitude of inbreeding depression also indicates that morph expression is likely to be condition-dependent to some degree and that deleterious recessives can simultaneously contribute to morph expression. Female fecundity also showed a high degree of inbreeding depression, but variance in female fecundity was mostly explained by epistatic effects, with very little contribution from additive effects. We found no significant genetic correlation, nor any evidence for dominance reversal, between male morph and female fecundity. The complex genetic architecture underlying male morph and female fecundity in this system has important implications for our understanding of the evolutionary interplay between purifying selection and sexually antagonistic selection.

Keywords: quantitative genetics, diallel, dimorphism, genetic architecture, dominance reversal, condition-dependence, large-effect loci

Introduction

The nature of genetic variation segregating in natural populations is of considerable interest in evolutionary genetics as standing genetic variance constitutes a major source of a population's short-term evolutionary potential (Barton and Keightley 2002; Barrett and Schluter 2008). Understanding the nature of genetic variation has also been of particular importance in the development of sexual selection theory, as it forms the basis of hypotheses explaining indirect genetic benefits of female mating preferences for costly and exaggerated male signalling structures (Andersson 1986; Rowe and Houle 1996), and the phenotypic variation that exists in traits which are used during male contest competition (Berglund et al. 1996). This phenotypic variation may take the shape of the discontinuous or discrete expression of the traits that mediate sexual competition and the evolution of alternative reproductive phenotypes adopting different reproductive strategies (Gross 1996; Sinervo and Lively 1996; Gross and Repka 1998; Brockmann 2001; Shuster and Wade 2003; Tomkins and Hazel 2007). Such alternative reproductive phenotypes are found in a wide diversity of taxa (Oliveira et al. 2008) and are hypothesised to exist due to the costliness of these traits where only males of high 'quality' (Zahavi 1975) or 'status' (Gross 1996) can pay the costs of expression.

One of most likely sources of variation in male genetic quality stems from the continuous influx of deleterious mutations that will segregate in populations at low frequency under mutation-selection balance (Haldane 1937; Lande 1975; Lynch et al. 1999). Such deleterious mutations will be spread across the genome (Andersson 1986), reducing the amount of resources available for an individual to allocate toward fitness related traits, commonly referred to as an individuals' 'condition' (Rowe and Houle 1996). Condition-dependence of exaggerated sexually selected trait (SST) expression can become an evolutionary stable strategy (Maynard Smith and Price 1973), when the survival costs of expressing SSTs in poor-condition individuals exceeds their reproductive benefits (Grafen 1990; Gross and Repka 1998).

The evolution of this condition-dependence thus causes SST expression to be informative of male genetic quality. Furthermore, in at least some taxa it underlies the evolution and expression of alternative reproductive phenotypes, where high-condition males develop into aggressive morphs that engage in contest competition over access to females and express disproportionally large and costly SSTs. In contrast, poor-condition males express disproportionally small or have complete absence of SSTs and adopt non-aggressive, often 'sneaky' mating tactics (Gross 1996). Implicating loci underlying the genetic variance of condition-dependent SSTs has been challenging due to the nature of this variance, i.e. a large number of loci each with individually small effect sizes (Rowe and Houle 1996). Despite challenges, a number of recent examples appear consistent with this scenario and include the polygenic determination of antlers in red deer, *Cervus elaphus* (Peters et al. 2022), mating success in *Drosophila melanogaster* (Dugand et al. 2019) and the discontinuous expression of a sexually selected weapon in the bulb mite, *Rhizoglyphus robini* (Parrett et al. 2022).

However, some systems do not conform to this polygenic condition-dependence model, with SST expression determined by relatively few genes or even a single gene (or supergene) of large effect (Shuster and Wade 1991; Sinervo and Lively 1996; Johnston et al. 2013; Küpper et al. 2016; Lamichhaney et al. 2016; Hendrickx et al. 2022). The maintenance of variation in such systems with large-effect quantitative trait loci (QTLs) is likely a consequence of balancing selection, for example negative frequency-dependence (Gross 1991) or Rock-Paper-Scissor games (Sinervo and Lively 1996) predicted by evolutionary game theory (Maynard Smith 1982), or antagonistic pleiotropy and life-history trade-offs (Johnston et al. 2013; Mérot et al. 2020). One other possible widespread form of balancing selection may stem from sexual antagonism, which could have both large-effect QTLs or polygenic underpinnings, and sexually antagonistic polymorphisms maintained due to alternative alleles having opposite fitness consequences in each of the sexes (Connallon and Clark 2012, 2014). A potentially major source of sexual antagonism may stem from the expression of SSTs, where high-fitness males expressing

elaborated SSTs sire low-fitness daughters (Harano et al. 2010; Plesnar-Bielak et al. 2014; Okada et al. 2021). The likelihood of such polymorphisms being maintained by sexually antagonistic selection increases with epistasis between antagonistic loci (Arnqvist et al. 2014), as well as beneficial reversals of dominance between the antagonistic alleles within a given locus (Barson et al. 2015; Grieshop and Arnqvist 2018; Connallon and Chenoweth 2019) – both of which reduce the fitness costs of carrying any of the 'wrong' alleles for one's sex. Thus, studying the genetic architecture of SSTs and associated sexually antagonistic effects to female fitness (i.e. partitioning total trait variance into that stemming from additivity, dominance and epistasis) will help to clarify whether variation in such traits is maintained by balancing selection, mutation-selection balance, or some combination of the two.

The relative contributions of balancing selection and mutation-selection balance to the genetic variance in SST expression has important implications for sexual selection theory and beyond, but remains largely unresolved. For example, rapid adaptation to altered environments may be facilitated if genetic variation is maintained under balancing selection (Barrett and Schluter 2008), possibly stemming from sexual antagonistic selection (Connallon and Clark 2014). Moreover, sexual selection against deleterious mutations can also improve adaptation rates and/or reduce extinction risk (Lorch et al. 2003; Fricke and Arnqvist 2007; Jarzebowska and Radwan 2010; Plesnar-Bielak et al. 2012; Lumley et al. 2015; Martínez-Ruiz and Knell 2016; Parrett and Knell 2018; Cally et al. 2019; Parrett et al. 2019; Godwin et al. 2020) if condition-dependent expression of SSTs reveals individuals' relative share of the population's mutation load (Grieshop et al. 2021b). On the other hand, by favouring alleles that harm female/population offspring production (Holland 2002; Kokko and Brooks 2003; Rundle et al. 2006; Berger et al. 2016; Grieshop et al. 2017), increasing costs associated with SST expression (Doherty et al. 2003; Bro-Jørgensen 2014; Martins et al. 2018) or by reducing effective population size (Kokko and Brooks 2003; Parrett et al. 2022) adaptation rates may be hindered and extinction risks increased by sexual selection and sexual conflict.

Here, we implemented a quantitative genetic approach using diallel crosses in the bulb mite, *R. robini,* in order to partition genetic variance and investigate dominance relationships of a sexually selected weapon, which earlier work implied has sexually antagonistic effects on female fitness (Plesnar-Bielak et al. 2014; Łukasiewicz et al. 2020). Male *R. robini* comprise of two male morphs distinct in the expression of sexually selected weaponry (Parrett et al. 2022), the aggressive fighters have a thickened third pair of legs and use them while engaging in contest competition, which can be lethal. In contrast, the non-aggressive scramblers have legs with all approximately equal thickness and avoid direct competition (Radwan 1995; Radwan et al. 2000). Previous work has shown that males in poor phenotypic condition tend to express the scrambler phenotype (Radwan 1995; Smallegange 2011), but male morph is nevertheless significantly heritable (Radwan 1995; Smallegange and Coulson 2011), with some previous data suggesting the existence of a large-effect QTL (Radwan 1995). Yet, other evidence suggests that fighters may be associated with a lower load of deleterious mutations (Łukasiewicz et al. 2020; Parrett et al. 2022), such that heritability of morph may result from polygenic condition-dependence. Fighter morphs tend to outcompete scramblers, which incur high mortality in inter-morph competition (Radwan et al. 2000; Radwan and Klimas 2001), yet the scrambler morph persists. It is plausible that sexual antagonism may also contribute to the maintenance of genetic variance in male morph. Previous work showed that selection for morph results in correlated response in female fecundity, such that females from fighter-selected treatments have lower fecundity than females from scrambler-selected treatments (Plesnar-Bielak et al. 2014; Łukasiewicz et al. 2020). The relative contribution of each of these candidate mechanisms remains unknown.

If indeed most of the genetic variance underlying morph is due to deleterious mutations, we can expect additive genetic variance to be moderate and comparable to other life history traits (Mousseau and Roff 1987). Female fecundity is likely a useful benchmark as it is known to be a highly polygenic and therefore a large target for deleterious mutations to act upon (Houle 1992). Furthermore, if additive

genetic variance is determined by deleterious mutations it should also be accompanied by a comparable portion of dominance variation (Crnokrak and Roff 1995; Roff and Emerson 2006), and substantial inbreeding depression (DeRose and Roff 1999), because deleterious mutations segregating in natural populations are typically recessive (Charlesworth and Willis 2009). The potential for sexual antagonism to maintain male morph variation would be much enhanced if there were beneficial dominance reversal, that is, if dominant fighter morph (recessive scrambler morph) allele(s) had recessive (dominant) effects on female fecundity. Under such dominance reversal, heterozygous fighter/scrambler genotypes would express the male fighter morph and have high female fecundity, stabilising the underlying sexually antagonistic polymorphisms (Kidwell et al. 1977; Fry 2010). Therefore, we investigated dominance relationships for male morph and female fecundity. The relative dominance of each inbred lines' genetic variation over the other lines in the diallel was estimated as the covariance between their mean outcrossed values and the 'self-cross' means of the inbred lines they are crossed with (Grieshop and Arnqvist 2018). If these array covariances are positively correlated between male morph and female fecundity it would indicate that the underlying alleles are either dominant or recessive for both traits, whereas a negative correlation indicates that alleles are dominant for one trait but recessive for the other.

Methods

General husbandry

All mites were reared under standard laboratory conditions. Stock cultures were housed in plastic containers (~7 × 10 cm), large colonies (>10 mites) were reared in small plastic containers (~2.2 cm diameter), and small colonies (10 or less) or individual mites were housed in glass vials (~1 cm diameter). All containers and vials had a base of plaster-of-Paris (~1 cm) which was soaked in water prior to transferring any mites. In order to maintain humidity (> 90%), all mite housing was placed on damp

tissue paper and placed within a plastic box containing a ball of soaked tissue paper. Mites were stored in incubators kept at a constant 23°C. Powdered yeast was provided *ad libitum* for feeding. All housing was checked regularly, to ensure mites had access to yeast and humidity remained high.

Establishing inbred lines

In brief, 41 inbred lines of *R. robini,* each founded by a single virgin female and male, were established from mites collected from onions in fields close to Mosina, Poland. The morph of each founding male was recorded and in subsequent generations of inbreeding males of the same morph as the founder male were used to propagate each inbred line (for full details see Łukasiewicz et al. 2020). Inbred lines were developed by full sib × sib mating for ten generations, inbreeding was then relaxed due to logistical constraints and inbred lines allowed to expand for approximately three months (~six generations). Full sib × sib inbreeding was then resumed for a further four generations, thus giving a total of fourteen generations of full sib × sib inbreeding. Inbred lines were again allowed to expand for approximately three months (~six generations) prior to the onset of the current experiment in order to have inbred lines of adequate and stable sizes that could be used for this experiment. During inbreeding protocols, after their initial establishment each inbred line was maintained with back-ups. Each generation we reared 20 larvae (or less if not available) to adulthood for each inbred line and mated each male of the appropriate morph with a randomly selected virgin female. One to five such pairs were formed, giving up to four backups per inbred line per generation. One of these families was randomly selected to found the next generation, but if this family failed to produce offspring, a random backup was selected to replace it. Before the first expansion (i.e. generation 10) we recorded 233 such cases (counts based on two families, per line, each generation), of which 199 were due to infertility, and 34 due to embryonic or larval mortality. Although non-significant (χ^2 = 2.89, *d.f.* = 1, p = 0.089) there was a trend that reproductive failures were observed more often in fighter founded lines compared to those founded by

scramblers, with the average number of observed reproductive failures, per line, in each treatment being 6.42 and 4.40, respectively (Supplementary Figure 1).

Diallel crosses and assays

From those that survived the inbreeding programme we randomly selected 20 inbred lines, 10 founded by a fighter male and 10 from a scrambler male. From each of these inbred lines we transferred 50 females to a new container for egg-laying for five days, after which females were removed from containers. Three inbred lines, one founded by a fighter and two founded by a scrambler, did not have enough females available, and only 20-30 were therefore placed in egg-laying containers. After a further six days we attempted to isolate approximately 200 F_0 larvae and proto-nymphs from each inbred line into individual vials. If on the first day of isolation we did not achieve adequate numbers, we continued isolating individuals on the next two consecutive days. The three inbred lines with low numbers of female parents (from above) were discarded at this stage due to low offspring numbers. In addition, another inbred line (founded by a fighter male) was discarded due to experimenter error. This left us with 16 inbred lines, eight founded by fighter males and eight founded by scrambler males.

From these 16 inbred lines we created a partial diallel in a 'chess-board' design with reciprocal outbred crosses, and all possible inbred crosses. In total, this led to 144 cross combinations: 16 inbred self-crosses (i.e. sires and dams from the same inbred line), and 128 outbred crosses (i.e. sires and dams from different inbred lines) consisting of each inbred line crossed reciprocally with four fighter-founded and four scrambler-founded inbred lines (see Figure 1). For each outbred cross we set up five replicate virgin pairs (P-generation) and for each inbred self-cross we increased this number to ten replicate virgin pairs (for two self-crosses: IN7 and IN14, it was only possible to establish seven and eight pairs, respectively). The mated pairs were left in vials together and females allowed to lay eggs for six days, after which the adults were removed from vials. After a further two days each vial was then checked for

198 larvae. We collected F_1 larvae for two purposes: 1) to gain virgin females for fecundity assays and 2) to
199 estimate male morph proportion. As it was not logistically feasible to isolate every single larva, and in
200 order to spread our efforts as evenly as possible across the entire diallel, we stopped isolating larvae
201 from an outbred cross when we had isolated our target number of larvae from three out of five replicate
202 pairs (or six out of ten for self-crosses). We aimed to collect 70 larvae per replicate pair, with 10 larvae
203 reared individually to obtain virgin females for fecundity assays, and the remaining reared in groups of
204 10 individuals per vial (or less if not available) and used to determine morph proportion. These numbers
205 of mites (10 or less) per vial represent low density rearing conditions. During larvae collection we
206 prioritised fecundity assays and isolated larvae individually first. Until we reached these targets, we
207 continued isolating larvae throughout the following ten days from all replicates of a cross combination.
208 In some cases, we were not able to find enough larvae and on occasions a low level of opportunistic
209 sampling was also performed. Data collected from all replicate pairs were included in our analyses. The
210 number of males used to estimate morph proportion of each replicate is therefore varied, but any
211 analysis (see below) takes this into account.

212 Morph proportion of F_1 adults that eclosed from each group of larvae was determined eight days after
213 larvae were isolated. F_1 adults were sexed and male morph recorded before being removed from vials;
214 any nymphs remaining were left in the vials for future morph scoring. Vials were then checked every
215 other day for any remaining mites to mature, with the morph of any males being recorded. Housing
216 mites in groups for morph proportion assays allowed us to sustainably increase sample size compared to
217 if isolating individual larvae; the method choice is justified as it was previously shown that colony
218 density does not influence morph determination in this species (Radwan 1995). Moreover, this better
219 reflects housing conditions of mites within their pre-experiment conditions compared to individually
220 housed mites. Although in some cases this led to deaths of males, most likely as a consequence of lethal
221 combat, if vials are checked regularly dead mites can be sexed and male morph determined with relative

ease: only six dead mites across the entire experiment were unidentifiable. Splitting mites into vials with low density rearing conditions, rather than rearing all larvae in one pool, allowed us to have similar rearing densities when we did not obtain 60 larvae (the mites were then simply split among fewer groups) or if more offspring were produced and not separated. The numbers of fighters and scramblers were then pooled from these groups of mites for each replicate pair.

For female fecundity, vials containing individually housed F_1 mites were checked every other day and adults sexed. Only females < 3 days post adult-eclosion were used in fecundity assays. From each cross combination we aimed to assay the fecundity of three F_1 females from outbred crosses or six from inbred self-crosses – each from a unique P-generation pair when possible. Isolated F_1 females were paired with a random < 3-day-old (post adult-eclosion) male of either morph from a large outbred stock population, as previous work has shown that male morph does not have direct effects on female fecundity in *R. robini* (Plesnar-Bielak et al. 2014; Parrett et al. 2022). Females were allowed to oviposit for 10 days, transferring the pair to a new vial on day 5 (replacing any dead males; in both first and second vials n = 19 dead males were observed), and removing the pair on day 10. The intermittent removal of a male during fecundity assays does not lead to detectably lower female fecundity for at least two days (Kołodziejczyk and Radwan 2003), so these rare cases of male death were unlikely to affect our results. The eggs from both vials were counted the same day adults were removed from vials. On occasions some eggs had hatched and therefore larvae were also present in the vials, these were included in these counts as eggs. As these were relatively rare and an excess of food remained in tightly plugged vials we do not believe errors were introduced due to some eggs hatching. As female egg-laying rate remains relatively consistent over the first three weeks of their lives, after which females have declining fecundity (Tilszer et al. 2006), and because three weeks make up a significant proportion of an average females lifespan (3-5 weeks depending on study: Kołodziejczyk and Radwan 2003; Parrett et al. 2022), such a measure is likely a good estimate of lifetime fitness. In an attempt to exclude the

246 possibility that females did not lay eggs due to a male effect (for example, male sterility or lack of
247 mating), we provided a new male if there were zero eggs in the first vial. If in the second vial also there
248 were zero eggs, we assumed this to be a consequence of the female, but if females produced eggs in the
249 second vial (i.e. with a new male) we assumed the zero-count in the first vial to be an effect of the first
250 male. We removed those later situations from our dataset (n = 25), but retained the former as zero eggs
251 laid.

252 We performed a partial second block specifically targeting cross combinations in which sample sizes
253 were low or completely missing (n = 48). Sample sizes for each cross combination were considered low if
254 we did not have fecundity data for three females from outbred crosses or six females from inbred-self
255 crosses and/or if we had determined the morph from less than 30 males from each outbred cross
256 combination or 60 males from inbred-self crosses. Although unbalanced sampling effort exists between
257 blocks 1 and 2, by repeating a partial block it allowed us to have estimates from cross combinations with
258 completely missing data or improve estimates of those with low sample size, and it also allowed us to
259 partition some variance due to environment factors (i.e. block). It should be noted that our statistical
260 method for variance partitioning is specifically intended to accommodate imbalanced sampling in the
261 diallel (Lenarcic et al. 2012).

262 *Statistical analysis*

263 We performed diallel analysis for sex-specific data separately. In each case we partitioned the genetic
264 variance of each trait by fitting models using the package *litterDiallel* (Shorter et al. 2019). This package
265 adapted the *BayesDiallel* (Lenarcic et al. 2012) Gibbs sampler to also allow generalised linear mixed
266 models to be fitted using *MCMCglmm* (Hadfield 2010). Using this Bayesian modelling approach we were
267 able to partition the phenotypic variation of male morph and female fecundity (separately) in the F_1
268 offspring of crosses between maternal strain j and paternal strain k into additive effects ($a_j + a_k$),

parental-sex effects ($m_j - m_k$), dominance effects ($\beta_{\text{inbred.overall}} + \beta_j$), symmetric and asymmetric epistatic effects ($v_{jk} + w_{jk}$, respectively), and unexplained variance (noise; ε_i), as follows:

$$\mathbf{d}^{\mathbf{T}}\boldsymbol{\beta} = \{a_j + a_k\} + \{m_j - m_k\} + I_{\text{inbreds}}\left(\beta_{\text{inbred.overall}} + \beta_j\right) + I_{\text{hybrids}}(v_{jk} + w_{jk})$$

Male morph proportion is formulated as a binomial GLMM, with the observed proportion of each male phenotype i (y_i) modelled as: $y_i \sim \text{Binomial}(n_i, \pi_i)$. Here, n_i is the total number of males and π_i is the proportion of fighters. In MCMCglmm, this is specified as a two-outcome "multinomial2" model with $\text{n.fighters}_i = n_i \cdot \pi_i$ and $\text{n.scramblers}_i = n_i \cdot (1 - \pi_i)$. The GLMM uses the inverse logit link to the standard BayesDiallel model, while controlling for overall fixed effects of mean (μ) and block, i.e. $\pi_i = \text{logit}^{-1}\left(\mu + \text{block}_\text{i} \cdot \beta_{\text{block}} + \mathbf{d}^{\mathbf{T}}\boldsymbol{\beta} + \varepsilon_i\right)$**.** Female fecundity is formulated as a Gaussian linear model, with the *Z-scores* (see below) for each individual i modelled as: $y_i = \mu + \text{block}_\text{i} \cdot \beta_{\text{block}} + \mathbf{d}^{\mathbf{T}}\boldsymbol{\beta} + \varepsilon_i$. Models were iterated 1,000,000 times, with a thinning interval of 1,000 and a burn-in of 50,000. Minimally informative priors (V = 1, nu = 0.002) were used during modelling. The variance components from the diallel random effects are modelled as in Shorter et al., (2019). Block was included as a fixed effect in both models.

Counts of male morph from all vials of a given replicate pair were combined and used to determine male morph proportion. In our experience humans may systematically differ in their counts of large numbers of small eggs, therefore for female fecundity data, in order to account for any such observer effects (n = 4 observers) we scaled egg counts for each observer individually and took the sum of *Z-scores* from both egg laying vials. In a number of cases the female died in the first vial (n =14) and therefore no second vial existed for these females. As we wanted to take this into account in our analysis, we scored the 'second vial' as having a count of zero. As a consequence, there was no second observer, so we assigned a *Z-score* equivalent to the mean *Z-score* of all observations with counts of zero. In addition, we ran the same fecundity model but took observer effects into account by taking the residuals from a simple

generalised model fit to fecundity count data with 'observer' as an explanatory variable. Comparison of DIC scores indicate that the model using *Z-scores* (DIC = 1898.2) had a substantially better fit compared to modelling residuals (DIC = 3942.4). We therefore only report the model using *Z-scores*.

To confirm all chains had good mixing we ran Gelman-Rubin analyses for each model: both had acceptable multivariate psrf scores below 1.1 (morph proportion = 1.07, fecundity = 1.06). In addition, we generated null posterior distributions for both models by randomising the unique cross identifier (within inbred and outbred crosses, separately) and repeating the above analyses, with the assumption that true signals of non-zero variance should be absent/zero in the randomized null distributions.

Diallel crosses also allow for the ordination of strains from those carrying the most dominant alleles to those carrying the most recessive alleles at the loci underlying the trait/s in question (Grieshop and Arnqvist 2018). The relative amount of dominant/recessive alleles in a given inbred line is estimated by the covariance of its mean outbred values (*r*; averaged over all outbred combinations of a particular inbred strain) and the mean of the self-cross values of inbred lines to which it was crossed (*P*). This was done separately for all strains, and separately for both male morph ($\sigma_{PM,rM}$) and female fecundity ($\sigma_{PF,rF}$). Positive covariance implies that the alleles a line carries are mostly recessive to alleles carried by the lines it was crossed with (i.e. their outbred values are dependent on the genetic makeup of the inbred line they are crossed with). Contrastingly, if an inbred line's outbred values do not covary with the self-cross values of inbred lines they are crossed with (i.e. values of or close to 0), this is an indication that they harbour more dominant alleles than the inbred lines they were crossed with (i.e. their outbred values are independent of the genetic makeup of the inbred line they are crossed with). This interpretation holds in absence of environmental and epistatic variation (Grieshop and Arnqvist 2018). To fulfil that assumption, we first fit simple general and generalised linear models to fecundity (*Z-score*) and morph data, respectively, which modelled environmental effects by fitting block as a fixed

effect and modelled epistatic effects by fitting the sire x dam combination as a random effect, using the *lme4* package for R (Bates et al. 2015). The residuals from these models were then used to tabulate the mean values for each cross combination, which were used to calculate the array covariances described above (see supplementary S1 of Grieshop et al. 2021a for details). Inbred lines are ordinated according to their array covariances – their relative dominance/recessive relationships to one another. The array covariances were also used to test for dominance reversal between male morph and female fecundity: a negative genetic correlation between the traits would indicate that dominant alleles for one trait tend to be recessive for the other, whereas a positive genetic correlation would indicate that alleles tend to be either dominant or recessive for both traits alike – evidence for and against dominance reversals, respectively.

All statistical analysis was performed in R version 3.6 (R Development Core Team 2020).

Results

In total we recorded the morph of 8905 F_1 males across all diallel crosses: 1550 were from inbred crosses and 7355 were from outbred crosses, and 6572 were scrambler and 2333 were fighter males. We also assayed the fecundity of 548 females, 138 from inbred crosses and 410 from outbred crosses. Across both blocks, morph proportion and female fecundity data was collected from 143 and 144 cross combinations, respectively (Figure 1).

A considerable percentage (86.38%) of the variance in morph proportion was explained by diallel effects, whereas a low percentage (13.62%) of that variance was noise (Figure 2). The majority of variance in morph proportion (62.77%) was explained by additive genetic variance, with symmetric epistatic effects (18.08%) explaining most of the remaining variance. Dominance variance, estimated by strain-specific inbreeding variance, explained only 4.94% of variance, an order of magnitude lower compared to additive variance. Reciprocal crosses that are autosomally identical but have inherited their

cytoplasm and sex-chromosomes from opposite inbred lines revealed almost no influence of parental effects or asymmetric epistasis (0.13% and 0.45% of total variance explained, respectively) on the probability of offspring being a fighter or scrambler. Looking at the highest posterior density (HPD) means and 95% credibility intervals (CIs) of the diallel effects and estimates of inbred line-specific effects provides more detail (Figure 3). The HPD mean for the fixed effect of inbreeding was negative and CIs, close to, but not overlapping zero – indicating effects of inbreeding depression on the expression of the fighter morph. The probability of offspring being either a fighter or scrambler was largely associated with founder morph treatment as seen in their individual additive effects. Seven of the eight fighter-founded inbred lines have positive additive effects (i.e. increased probability of male offspring being fighter) where the HPD interval does not overlap with 0, whereas, five of the eight scrambler-founded inbred lines had negative additive effects (i.e. increased probability of male offspring being a scrambler). A number of specific cross combinations showed symmetric epistatic effects on the probability of male offspring morph beyond their predictive additive effects alone, most notably IN9:IN5, IW1:IN9, IW23:IN8, IW23:IW1 and IW25:IW19 (Figure 3).

In contrast, considerably more of the variance (68.52%) for fecundity was attributed to noise and a lower percentage (31.48%) explained by diallel effects (Figure 4). The majority of this variance was explained by epistatic effects (symmetric = 12.37%, asymmetric = 10.36%), with the remaining explained by dominance variance (6.94%) and additive genetic variance (1.13%). As with the male morph data, there was next to no variance explained by parental effects (0.68%). Again, a detailed look at inbred line-specific HPD means and CIs provides further insight (Figure 5). There was a very pronounced fixed effect of inbreeding, with HPD mean being negative with no overlap with zero – indicating substantial effects of inbreeding depression on female fecundity. Additionally, inspection of how variance in fecundity was explained by symmetric and asymmetric epistatic effects shows three strain-pair specific estimates

where the 95% CIs do not overlap zero (v:IW16:IN3, v:IW24:IW17 and w:IW19:IN9) and many others whose HPD means are far from zero but whose 95% CIs nevertheless do overlap zero (Figure 5).

In both cases, randomisation of data structure showed that the above patterns are unlikely to be a consequence of random chance (random data structure; morph proportion: total explained variance = 5.52%, noise = 94.48%; female fecundity: total explained variance = 4.75%, noise = 95.25%).

The cross-trait/cross-sex additive genetic correlation between female fecundity and morph proportion was negative but non-significant (r = -0.18, p = 0.493; Supplementary Figure 2). For morph proportion, dominance ordination revealed that fighter-founded inbred lines had significantly higher mean array covariances ($\sigma_{PM,rM}$) than scrambler-founded inbred lines (t = 6.56, df = 14, p < 0.001; Figure 6a), indicating that the fighter morph is recessive to the scrambler morph (Figure 1a). In contrast, no dominance effects were observed for female fecundity with covariance ($\sigma_{PF,rF}$) not differing between founder morph treatments (t = 1.60, df = 14, p = 0.131; Figure 6b). Finally, there was no significant genetic correlation between the array covariances for male morph ($\sigma_{PM,rM}$) and female fecundity ($\sigma_{PF,rF}$) among inbred lines (Pearson's r = 0.23, p = 0.393, Spearman's r = 0.23, p = 0.391, Supplementary Figure 3).

Discussion

Here, using diallel crosses of *R. robini* inbred lines, we investigated the genetic variance components underlying both female fecundity and the expression of a sexually selected weapon that is dimorphic among males. Furthermore, we explored the additive genetic correlation between the two traits, as well as the genetic correlation for the dominance ordinations between the two traits. Our results provide evidence for the inheritance of the weapon being largely explained by additive genetic variation, with contributions from symmetric epistatic effects. We detected a significant inbreeding effect on morph expression, indicating that expression of the fighter phenotype is sensitive to the quantity of exposed

384 deleterious recessives. We also found that fighter-morph alleles tend to be recessive to the scrambler-
385 morph alleles. Female fecundity, by contrast, only had a very small percentage of variance explained by
386 additive genetic effects, with much of variance explained by symmetric and asymmetric epistatic effects.
387 Fecundity also showed very high inbreeding depression, indicating that fecundity is determined by the
388 quantity of exposed deleterious mutations. Aside from this (likely) polygenic deleterious recessive basis
389 to both traits, as well as some degree of epistasis underlying both traits, there were no direct
390 associations between male morph and female fecundity, including no additive genetic correlation and
391 no evidence of dominance reversal.

392 The considerable additive genetic variation for morph expression detected here supports earlier work
393 that morph is heritable in *R. robini* (Radwan 1995). Building on this work and, by using inbred lines,
394 knowing the genotype of both males and females in the current study, we provide evidence that both
395 sexes appear to contribute equally to the probability of offspring being a fighter or scrambler.
396 Suggesting that the additive genetic basis to male morph in this species is predominantly due to
397 autosomal genetic variation. This contrasts a previous study of a con-generic, *R. echinopus*, which found
398 evidence for paternal morph effects, but overall weaker evidence for additive genetic effects. It was
399 argued that the former is likely linked to variation on the Y-chromosome or an indirect genetic effect
400 (Buzatto et al. 2012). While seemingly at odds, the lack of parental effect in the current study can be
401 explained by differences in sex determination between these two species. *R. echinopus* has been
402 reported to have XY (Grondziel 1975), whereas *R. robini* has XO (Parrett et al. 2022) sex determination,
403 the latter obviously eliminating the scope for any Y-linked paternal effects. Furthermore, the two
404 species show clear differences in their morph determination mode. In *R. echinopus*, as well as another
405 acarid, *Sancassania berlesei* (Radwan 1993; Michalczyk et al. 2018), fighter morph is suppressed by
406 pheromones emanating from dense colonies (Radwan 2001), whereas no such type of polyphenism is
407 observed in *R. robini* (Radwan 1995). Clearly, there is much variation within acarid mites in genetic and

environmental contributions to male morph determination, even within the genus *Rhizoglyphus*. The significant contribution of additive variance found here for *R. robini* is also in line with some earlier work on this species showing that the proportion of male morph in a population responds to directional selection (Radwan 2003a; Smallegange and Coulson 2011; Plesnar-Bielak et al. 2014; Parrett et al. 2022). Our estimate for additive genetic variance of male morph is over an order of magnitude greater than that for female fecundity – a trait that is often considered an exemplification of a polygenic fitness-related trait in other taxa, with additive variance maintained predominantly via mutation-selection balance (Houle 1992, also see below). The very high proportion of additive variance for male morph, much exceeding dominance variance, suggests that mutation-selection balance is unlikely to be the main contributing mechanism. Accordingly, some estimates of heritability obtained in earlier work were close to or exceeded unity (Radwan 1995), which could be due to segregation of a large effect QTL, although segregation patterns excluded simple Mendelian segregation.

Our estimates of dominance relationships are also inconsistent with morph heritability being mostly due to mutation-selection balance maintaining genetic variance in condition, in turn determining morph expression (Radwan 1995; Smallegange 2011). This is because the majority of segregating deleterious mutations, which reduce condition, are expected to be recessive (Charlesworth and Willis 2009). Thus, the mutation-selection balance hypothesis would not only predict a relatively high proportion of dominance variance that we did not observe, but would also predict the scrambler associated alleles to be recessive – whereas the reverse was observed. Outbred values for fighter-founded inbred lines had considerably higher estimates of covariance with the self-cross value of inbred lines they were outcrossed to, compared to scrambler-founded inbred lines, suggesting that alleles underlying the fighter-morph are recessive. This dominance relationship is consistent with the response to divergent artificial selection of male morph, which led to earlier near-fixation of the fighter-morphs compared to scrambler-morphs (Parrett et al. 2022). This does not exclude the possibility that exposed deleterious

alleles could increase the likelihood of expressing the scrambler phenotype, and indeed, we observed a negative overall general inbreeding effect for fighter expression, suggesting morph expression is in some part sensitive to exposed mutational load. Concordantly, Parrett et al. 2022 observed that populations selected for scramblers males have accumulated a large load of putatively recessive mutations spread across the genome. Yet, Parrett et al. 2022 also found a few genomic regions that contained particularly high density of SNPs that differentiated between fighter and scrambler selected populations, and these regions map to the same linkage group (Chmielewski et al. unpublished data), suggesting the existence of a supergene or inversion associated with male morph determination. Thus, it appears that male morph is determined by two overlying mechanisms: (i) the existence of one or more scrambler-dominant QTL(s) directly influencing the probability (or individuals' 'liability') of morph expression, possibly residing within a supergene or inversion polymorphism (Parrett et al. 2022), and (ii) male condition and condition-dependent weapon expression, where fighter phenotype expression is inversely associated with polygenic deleterious mutation load (Łukasiewicz et al. 2020; Parrett et al. 2022; present study). Consistent with the latter, manipulating phenotypic condition via food availability and temperature have been shown to influence weapon expression (Radwan 1995; Smallegange 2011; Plesnar-Bielak et al. 2018).

In contrast to the estimate for male morph, we found very small additive genetic variance in female fecundity. Earlier work based on daughter-on-mother regression estimated heritability at 27%, but also showed that it is subject to significant inbreeding depression which is not easily purged and leads to inbred line extinction (Radwan 2003b). This was interpreted as evidence that heritability of fecundity is due to a large number of small-effect loci, where it would be hard to purge the deleterious mutation load on fecundity. Here, we also found a large and significant negative general effect of inbreeding, but our estimate of additive genetic variance was considerably lower. It should be noted, however, that additive genetic variance inferred from daughter-on-mother regression ignores epistatic effects (Roff

1997), which we found to have a pronounced effect on fecundity variance (22.73% in total). Thus, our data are consistent with female fecundity being affected by deleterious recessives (with only minor contribution to additive effects) that interact epistatically. Our data do not provide further insight to the basis of this epistatic variance in female fecundity, but we note that significant inbreeding depression as well as large contributions of dominance and epistasis are common features of genetic variance in fitness-related traits (DeRose and Roff 1999; Roff and Emerson 2006). Our results highlight that epistasis may considerably inflate the heritability of life-history traits when they are estimated using methods that ignore it.

Finally, we found little to no support for sexual antagonism contributing to the maintenance of alternative male morphs. We did not find a significant negative additive genetic correlation between male morph and female fecundity, though the estimate was negative ($r = -0.18$), as predicted under sexual antagonism. One possibility that could explain why we have not detected that correlation here despite it being evident in the same inbred lines after four generations of inbreeding (Łukasiewicz et al. 2020) could be that the ten additional generations of inbreeding (see Methods) may have caused the loss of fighter-founded inbred lines with particularly strong fighter-benefit female-detrimental genetic variation (analogous to Grieshop et al. 2017). That is, if sexually antagonistic alleles that decrease female fecundity interacted with recessive deleterious mutations upon inbreeding to cause female infertility and subsequent loss of inbred lines (see Methods), this may have disproportionately purged fighter-benefit/female-detriment sexually antagonistic alleles from our panel of inbred lines. Thus affecting our estimates of sexually antagonistic genetic variance. Indeed, in another system (*Callosobruchus maculatus*), inbred lineage extinction associated with male-benefit/female-detriment sexually antagonistic allelic variation (Grieshop et al. 2017) was likewise accompanied by a reduced additive genetic signal of sexual antagonism in the diallel cross among the extant inbred lines (Grieshop and

Arnqvist 2018) relative the much stronger additive genetic signal of sexual antagonism seen in that population prior to the inbreeding regime (Berger et al. 2014).

Another possibility is that the 16 inbred lines used here simply did not capture a significant proportion of segregating morph-specific sexually antagonistic variation present within an outbred population. How much it is possible extrapolate beyond these 16 inbred lines is therefore in its nature limited, however, we note that the number of inbred lines used in the current study is equal or greater than many comparable experiments using diallel crosses (e.g. Buzatto et al. 2012; Lüpold et al. 2016; Grieshop and Arnqvist 2018; Maurizio et al. 2018; Shorter et al. 2019; Grieshop et al. 2021b), and such limitation did not prevent Grieshop and Arnqvist (2018) from detecting significant sexual antagonism. In the case of our experiment, if sexual antagonism was associated with genetic variants underlying male dimorphism as previously hypothesised (Plesnar-Bielak et al. 2014; Łukasiewicz et al. 2020), these variants should be present in our sample representing equal numbers of inbred lines derived from fighter and scrambler males. We therefore consider the loss of sexually antagonistic variation during inbreeding as a more likely explanation of why we have not observed similar morph-specific sexual antagonism reported by earlier studies.

While we might have underestimated the additive signal of sexual antagonism, we still did not find any evidence for dominance reversal either between male morph and female fecundity, which would help maintain polymorphisms underlying the two traits under sexually antagonistic balancing selection (see Reid 2022 for a common mechanism by which dominance reversal could ensue for both major-effect QTLs and polygenic underpinnings). A presumably polygenic signal of dominance reversal was previously reported for male and female fitness in seed beetles using a similar quantitative genetic approach as that used here (Grieshop and Arnqvist 2018), but other methods have revealed dominance reversals for cases of major-effect QTLs and supergenes (Barson et al. 2015; Pearse et al. 2019). In *R. robini* however,

dominance reversals between male morph and female fecundity do not seem to be contributing to the stable maintenance of polymorphisms underlying genetic variance in male morph.

Overall, our study shows high additive genetic variance for the dimorphic expression of a weapon, which when taken with dominance and inbreeding results strongly suggest two overlaying mechanisms for morph determination exist. We propose that one (or more) large effect scrambler-dominant QTL(s) directly influences male morph expression which is simultaneously affected by polygenic condition. Contrastingly, we did not detect much additive genetic variance for female fecundity, although considerable epistatic effects were found highlighting models not accounting for epistasis may inflate estimates of heritability. Our study revealed that the genetic architecture of male morph is very distinct from that underlying female fecundity, with beneficial dominance reversal unlikely to be contributing to maintaining polymorphisms for male morph. The maintenance of male polyphenism in *R. robini* remains to be fully resolved, with complex genetic and environmental effects yet to be fully teased apart.

Author contributions: JR and KG conceived the idea. JR, JMP, AŁ and SC designed experiment. JMP, AŁ, SC, AS-K and JR performed data collection. JMP, PLM and KG carried out analysis. JMP and JR wrote the manuscript with input from PLM an KG. All authors read and approved the manuscript.

Acknowledgements: We would like to thank Mateusz Konczal for useful discussion. We are also grateful for laboratory assistance from Małgorzata Niśkiewicz, Karolina Przesmycka, Issy Bolitho and Martyna Wilczek. Work from this project was funded by National Science Centre (2017/27/B/NZ8/00077 to JR & 2020/39/D/NZ8/00069 to JMP), Swedish Research Council (2018-06775 to KG) and National Institutes of Health (F32-AG064883 to PLM).

References

Andersson, M. 1986. Evolution of Condition-Dependent Sex Ornaments and Mating Preferences: Sexual Selection Based on Viability Differences. Evolution 40:804–816.

Arnqvist, G., N. Vellnow, and L. Rowe. 2014. The effect of Epistasis on sexually antagonistic genetic variation. Proc. R. Soc. B Biol. Sci. 281.

Barrett, R. D. H., and D. Schluter. 2008. Adaptation from standing genetic variation. Trends Ecol. Evol. 23:38–44.

Barson, N. J., T. Aykanat, K. Hindar, M. Baranski, G. H. Bolstad, P. Fiske, C. Jacq, A. J. Jensen, S. E. Johnston, S. Karlsson, M. Kent, T. Moen, E. Niemelä, T. Nome, T. F. Næsje, P. Orell, A. Romakkaniemi, H. Sægrov, K. Urdal, J. Erkinaro, S. Lien, and C. R. Primmer. 2015. Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. Nature 528:405–408.

Barton, N. H., and P. D. Keightley. 2002. Understanding quantitative genetic variation. Nat. Rev. Genet. 3:11–21.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1–51.

Berger, D., K. Grieshop, M. I. Lind, J. Goenaga, A. A. Maklakov, and G. Arnqvist. 2014. Intralocus sexual conflict and environmental stress. Evolution 68:2184–2196.

Berger, D., I. Martinossi-Allibert, K. Grieshop, M. I. Lind, A. A. Maklakov, and G. Arnqvist. 2016. Intralocus sexual conflict and the tragedy of the commons in seed beetles. Am. Nat. 188:E98–E112.

Berglund, A., A. Bisazza, and A. Pilastro. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. Biol. J. Linn. Soc. 58:385–399.

545 Bro-Jørgensen, J. 2014. Will their armaments be their downfall? Large horn size increases extinction risk
546 in bovids. Anim. Conserv. 17:80–87.

547 Brockmann, H. J. 2001. The evolution of alternative strategies and tactics. Adv. Study Behav. 30:1–51.

548 Buzatto, B. A., L. W. Simmons, and J. L. Tomkins. 2012. Paternal effects on the expression of a male
549 polyphenism. Evolution 66:3167–3178.

550 Cally, J. G., D. Stuart-Fox, and L. Holman. 2019. Meta-analytic evidence that sexual selection improves
551 population fitness. Nat. Commun. 10.

552 Charlesworth, D., and J. H. Willis. 2009. The genetics of inbreeding depression. Nat. Rev. Genet. 10:783–
553 796.

554 Connallon, T., and S. F. Chenoweth. 2019. Dominance reversals and the maintenance of genetic
555 variation for fitness. PLoS Biol. 17:1–11.

556 Connallon, T., and A. G. Clark. 2012. A general population genetic framework for antagonistic selection
557 that accounts for demography and recurrent mutation. Genetics 190:1477–1489.

558 Connallon, T., and A. G. Clark. 2014. Balancing selection in species with separate sexes: Insights from
559 fisher's geometric model. Genetics 197:991–1006.

560 Crnokrak, P., and D. A. Roff. 1995. Dominance variance: Associations with selection and fitness. Heredity
561 75:530–540.

562 DeRose, M. A., and D. A. Roff. 1999. A comparison of inbreeding depression in life-history and
563 morphological traits in animals. Evolution 53:1288–1292.

564 Doherty, P. F., G. Sorci, J. A. Royle, J. E. Hines, J. D. Nichols, and T. Boulinier. 2003. Sexual selection

affects local extinction and turnover in bird communities. Proc. Natl. Acad. Sci. 100:5858–5862.

Dugand, R. J., J. L. Tomkins, and W. J. Kennington. 2019. Molecular evidence supports a genic capture resolution of the lek paradox. Nat. Commun. 10:1359.

Fricke, C., and G. Arnqvist. 2007. Rapid adaptation to a novel host in a seed beetle (*Callosobruchus maculatus*): The role of sexual selection. Evolution 61:440–454.

Fry, J. D. 2010. The genomic location of sexually antagonistic variation: Some cautionary comments. Evolution 64:1510–1516.

Godwin, J. L., A. J. Lumley, Ł. Michalczyk, O. Y. Martin, and M. J. G. Gage. 2020. Mating patterns influence vulnerability to the extinction vortex. Glob. Chang. Biol. 26:4226–4239.

Grafen, A. 1990. Biological signals as handicaps. J. Theor. Biol. 144:517–546.

Grieshop, K., and G. Arnqvist. 2018. Sex-specific dominance reversal of genetic variation for fitness. PLoS Biol. 16:1–21.

Grieshop, K., D. Berger, and G. Arnqvist. 2017. Male-benefit sexually antagonistic genotypes show elevated vulnerability to inbreeding. BMC Evol. Biol. 17:1–10.

Grieshop, K., E. K. . Ho, and K. . Kasimatis. 2021a. Dominance reversals, antagonistic pleiotropy, and the maintenance of genetic variation. arXiv preprint a:34–36.

Grieshop, K., P. L. Maurizio, G. Arnqvist, and D. Berger. 2021b. Selection in males purges the mutation load on female fitness. Evol. Lett. 5:328–343.

Grondziel, E. 1975. Sex chromosomes in *Rhizoglyphus echinopus* (F, & R.) (Acarina, Acaridae). Folia Biol 23:69–72.

585 Gross, M. R. 1996. Alternative reproductive strategies and tactics: diversity within sexes. Trends Ecol.
586 Evol. 11:92–98.

587 Gross, M. R. 1991. Evolution of alternative reproductive strategies: frequency-dependent sexual
588 selection in male bluegill sunfish. Philos. Trans. - R. Soc. London, B 332:59–66.

589 Gross, M. R., and J. Repka. 1998. Stability with inheritance in the conditional strategy. J. Theor. Biol.
590 192:445–453.

591 Hadfield, J. D. 2010. MCMCglmm: MCMC Methods for Multi-Response GLMMs in R. J. Stat. Softw. 33:1–
592 22.

593 Haldane, J. B. S. 1937. The Effect of Variation of Fitness. Am. Nat. 71:337–349.

594 Harano, T., K. Okada, S. Nakayama, T. Miyatake, and D. J. Hosken. 2010. Intralocus sexual conflict
595 unresolved by sex-limited trait expression. Curr. Biol. 20:2036–2039.

596 Hendrickx, F., Z. De Corte, G. Sonet, S. M. Van Belleghem, S. Köstlbacher, and C. Vangestel. 2022. A
597 masculinizing supergene underlies an exaggerated male reproductive morph in a spider. Nat. Ecol.
598 Evol. 6:195–206.

599 Holland, B. 2002. Sexual selection fails to promote adaptation to a new environment. Evolution 56:721–
600 30.

601 Houle, D. 1992. Comparing evolvability and variability. Genetics 130:195–204.

602 Jarzebowska, M., and J. Radwan. 2010. Sexual selection counteracts extinction of small populations of
603 the bulb mites. Evolution 64:1283–1289.

604 Johnston, S. E., J. Gratten, C. Berenos, J. G. Pilkington, T. H. Clutton-Brock, J. M. Pemberton, and J. Slate.

2013. Life history trade-offs at a single locus maintain sexually selected genetic variation. Nature 502:93–95.

Kidwell, J. F., M. T. Clegg, F. M. Stewart, and T. Prout. 1977. Regions of stable equilibria for models of differential selection in the two sexes under random mating. Genetics 85:171–183.

Kokko, H., and R. Brooks. 2003. Sexy to die for? Sexual selection and the risk of extinction. Ann. Zool. Fennici 40:207–219.

Kołodziejczyk, M., and J. Radwan. 2003. The effect of mating frequency on female lifetime fecundity in the bulb mite, *Rhizoglyphus robini* (Acari: Acaridae). Behav. Ecol. Sociobiol. 53:110–115.

Küpper, C., M. Stocks, J. E. Risse, N. Dos Remedios, L. L. Farrell, S. B. McRae, T. C. Morgan, N. Karlionova, P. Pinchuk, Y. I. Verkuil, A. S. Kitaysky, J. C. Wingfield, T. Piersma, K. Zeng, J. Slate, M. Blaxter, D. B. Lank, and T. Burke. 2016. A supergene determines highly divergent male reproductive morphs in the ruff. Nat. Genet. 48:79–83.

Lamichhaney, S., G. Fan, F. Widemo, U. Gunnarsson, D. S. Thalmann, M. P. Hoeppner, S. Kerje, U. Gustafson, C. Shi, H. Zhang, W. Chen, X. Liang, L. Huang, J. Wang, E. Liang, Q. Wu, S. M. Y. Lee, X. Xu, J. Höglund, X. Liu, and L. Andersson. 2016. Structural genomic changes underlie alternative reproductive strategies in the ruff (*Philomachus pugnax*). Nat. Genet. 48:84–88.

Lande, R. 1975. The maintenance of genetic variability by mutation in a polygenic character with linked loci. Genet. Res. 26:221–235.

Lenarcic, A. B., K. L. Svenson, G. A. Churchill, and W. Valdar. 2012. General Bayesian approach to analyzing diallel crosses of inbred strains. Genetics 190:413–435.

Lorch, P. D., S. Proulx, L. Rowe, and T. Day. 2003. Condition dependent sexual selection can accelerate

626 adaptation. Evol. Ecol. Res. 5:867–881.

627 Łukasiewicz, A., M. Niśkiewicz, and J. Radwan. 2020. Sexually selected male weapon is associated with
628 lower inbreeding load but higher sex load in the bulb mite. Evolution 74:1851–1855.

629 Lumley, A. J., Ł. Michalczyk, J. J. N. Kitson, L. G. Spurgin, C. A. Morrison, J. L. Godwin, M. E. Dickinson, O.
630 Y. Martin, B. C. Emerson, T. Chapman, and M. J. G. Gage. 2015. Sexual selection protects against
631 extinction. Nature 522:470–473.

632 Lüpold, S., M. K. Manier, N. Puniamoorthy, C. Schoff, W. T. Starmer, S. H. B. Luepold, J. M. Belote, and S.
633 Pitnick. 2016. How sexual selection can drive the evolution of costly sperm ornamentation. Nature
634 533:535–538.

635 Lynch, M., J. Blanchard, D. Houle, T. Kibota, S. Schultz, L. Vassilieva, and J. Willis. 1999. Perspective:
636 Spontaneous deleterious mutation. Evolution 53:645–663.

637 Martínez-Ruiz, C., and R. J. Knell. 2016. Sexual selection can both increase and decrease extinction
638 probability: reconciling demographic and evolutionary factors. J. Anim. Ecol. 86:117–127.

639 Martins, M. J. F., T. M. Puckett, R. Lockwood, J. P. Swaddle, and G. Hunt. 2018. High male sexual
640 investment as a driver of extinction in fossil ostracods. Nature 556:366–369.

641 Maurizio, P. L., M. T. Ferris, G. R. Keele, D. R. Miller, G. D. Shaw, A. C. Whitmore, A. West, C. R. Morrison,
642 K. E. Noll, K. S. Plante, A. S. Cockrell, D. W. Threadgill, F. P. M. de Villena, R. S. Baric, M. T. Heise,
643 and W. Valdar. 2018. Bayesian Diallel analysis reveals MX1-dependent and MX1-independent
644 effects on response to influenza a virus in mice. G3 Genes, Genomes, Genet. 8:427–445.

645 Maynard Smith, J. 1982. Evolution and the theory of games. Cambridge University Press, Cambridge.

646 Maynard Smith, J., and G. R. Price. 1973. The logic of animal conflict. Nature 246:15–18.

647 Mérot, C., V. Llaurens, E. Normandeau, L. Bernatchez, and M. Wellenreuther. 2020. Balancing selection
648 via life-history trade-offs maintains an inversion polymorphism in a seaweed fly. Nat. Commun. 11.

649 Michalczyk, Ł., M. Dudziak, J. Radwan, and J. L. Tomkins. 2018. Fitness consequences of threshold trait
650 expression subject to environmental cues. Proc. R. Soc. B Biol. Sci. 285.

651 Mousseau, T. A., and D. A. Roff. 1987. Natural selection and the heritability of fitness components.
652 Heredity 59:181–197.

653 Okada, K., M. Katsuki, M. D. Sharma, K. Kiyose, T. Seko, Y. Okada, A. J. Wilson, and D. J. Hosken. 2021.
654 Natural selection increases female fitness by reversing the exaggeration of a male sexually selected
655 trait. Nat. Commun. 12:1–10.

656 Oliveira, R., M. Taborsky, and H. J. Brockmann. 2008. Alternative Reproductive Tactics: An Integrative
657 Approach. Cambridge University Press, Cambridge.

658 Parrett, J. M., S. Chmielewski, E. Aydogdu, A. Łukasiewicz, S. Rombauts, A. Szubert-Kruszyńska, W. Babik,
659 M. Konczal, and J. Radwan. 2022. Genomic evidence that a sexually selected trait captures
660 genome-wide variation and facilitates the purging of genetic load. Nat. Ecol. Evol. 6:1330–1342.

661 Parrett, J. M., and R. J. Knell. 2018. The effect of sexual selection on adaptation and extinction under
662 increasing temperatures. Proc. R. Soc. B Biol. Sci. 285:20180303.

663 Parrett, J. M., D. J. Mann, A. Y. C. Chung, E. M. Slade, and R. J. Knell. 2019. Sexual selection predicts the
664 persistence of populations within altered environments. Ecol. Lett. 22:1629–1637.

665 Pearse, D. E., N. J. Barson, T. Nome, G. Gao, M. A. Campbell, A. Abadía-Cardoso, E. C. Anderson, D. E.
666 Rundio, T. H. Williams, K. A. Naish, T. Moen, S. Liu, M. Kent, M. Moser, D. R. Minkley, E. B.
667 Rondeau, M. S. O. Brieuc, S. R. Sandve, M. R. Miller, L. Cedillo, K. Baruch, A. G. Hernandez, G. Ben-

Zvi, D. Shem-Tov, O. Barad, K. Kuzishchin, J. C. Garza, S. T. Lindley, B. F. Koop, G. H. Thorgaard, Y. Palti, and S. Lien. 2019. Sex-dependent dominance maintains migration supergene in rainbow trout. Nat. Ecol. Evol. 3:1731–1742.

Peters, L., J. Huisman, L. E. B. Kruuk, J. M. Pemberton, and S. E. Johnston. 2022. Genomic analysis reveals a polygenic architecture of antler morphology in wild red deer (*Cervus elaphus*). Mol. Ecol. 31:1281–1298.

Plesnar-Bielak, A., A. M. Skrzynecka, K. Miler, and J. Radwan. 2014. Selection for alternative male reproductive tactics alters intralocus sexual conflict. Evolution 68:2137–2144.

Plesnar-Bielak, A., A. M. Skrzynecka, Z. M. Prokop, and J. Radwan. 2012. Mating system affects population performance and extinction risk under environmental challenge. Proc. R. Soc. B Biol. Sci. 279:4661–4667.

Plesnar-Bielak, A., A. M. Skwierzyńska, K. Hlebowicz, and J. Radwan. 2018. Relative costs and benefits of alternative reproductive phenotypes at different temperatures - Genotype-by-environment interactions in a sexually selected trait. BMC Evol. Biol. 18:1–10.

R Development Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Radwan, J. 2003a. Heritability of male morph in the bulb mite, *Rhizoglyphus robini* (Astigmata, Acaridae). Exp. Appl. Acarol. 29:109–114.

Radwan, J. 2003b. Inbreeding depression in fecundity and inbred line extinction in the bulb mite, *Rhizoglyphus robini*. Heredity 90:371–376.

Radwan, J. 2001. Male morph determination in *Rhizoglyphus echinopus* (Acaridae). Exp. Appl. Acarol.

689 25:143–149.

690 Radwan, J. 1995. Male morph determination in two species of acarid mites. Heredity 74:669–673.

691 Radwan, J. 1993. The adaptive significance of male polymorphism in the acarid mite *Caloglyphus*
692 *berlesei*. Behav. Ecol. Sociobiol. 33:201–208.

693 Radwan, J., M. Czyz, M. Konior, and M. Kołodziejczyk. 2000. Aggressiveness in two male morphs of the
694 bulb mite *Rhizoglyphus robini*. Ethology 106:53–62.

695 Radwan, J., and M. Klimas. 2001. Male dimorphism in the bulb mite, *Rhizoglyphus robini*: Fighters
696 survive better. Ethol. Ecol. Evol. 13:69–79.

697 Reid, J. M. 2022. Intrinsic emergence and modulation of sex-specific dominance reversals in threshold
698 traits. Evolution 76:1924–1941.

699 Roff, D. A. 1997. Evolutionary Quantitative Genetics. Chapman and Hall, New York.

700 Roff, D. A., and K. Emerson. 2006. Epistasis and Dominance: Evidence for Differential Effects in Life-
701 History Versus Morphological Traits. Evolution 60:1981.

702 Rowe, L., and D. Houle. 1996. The Lek Paradox and the Capture of Genetic Variance by Condition
703 Dependent Traits. Proc. R. Soc. B Biol. Sci. 263:1415–1421.

704 Rundle, H. D., S. F. Chenoweth, and M. W. Blows. 2006. The Roles of Natural and Sexual Selection during
705 Adaptation to a Novel Environment. Evolution 60:2218–2225.

706 Shorter, J. R., P. L. Maurizio, T. A. Bell, G. D. Shaw, D. R. Miller, T. J. Gooch, J. S. Spence, L. McMillan, W.
707 Valdar, and F. P. M. de Villena. 2019. A diallel of the mouse Collaborative Cross founders reveals
708 strong strain-specific maternal effects on litter size. G3 Genes, Genomes, Genet. 9:1613–1622.

709 Shuster, S. M., and M. J. Wade. 1991. Equal mating success among male reproductive strategies in a
710 marine isopod. Nature 350:608–610.

711 Shuster, S. M., and M. J. Wade. 2003. Mating Systems and Strategies. Princeton University Press.

712 Sinervo, B., and C. M. Lively. 1996. The rock-paper-scissors game and the evolution of alternative male
713 strategies. Nature 380:240–243.

714 Smallegange, I. M. 2011. Complex environmental effects on the expression of alternative reproductive
715 phenotypes in the bulb mite. Evol. Ecol. 25:857–873.

716 Smallegange, I. M., and T. Coulson. 2011. The stochastic demography of two coexisting male morphs.
717 Ecology 92:755–764.

718 Tilszer, M., K. Antoszczyk, N. Sałek, E. Zajac, and J. Radwan. 2006. Evolution under relaxed sexual conflict
719 in the bulb mite *Rhizoglyphus robini*. Evolution 60:1868–1873.

720 Tomkins, J. L., and W. Hazel. 2007. The status of the conditional evolutionarily stable strategy. Trends
721 Ecol. Evol. 22:522–528.

722 Zahavi, A. 1975. Mate selection-A selection for a handicap. J. Theor. Biol. 53:205–214.

723

Figures

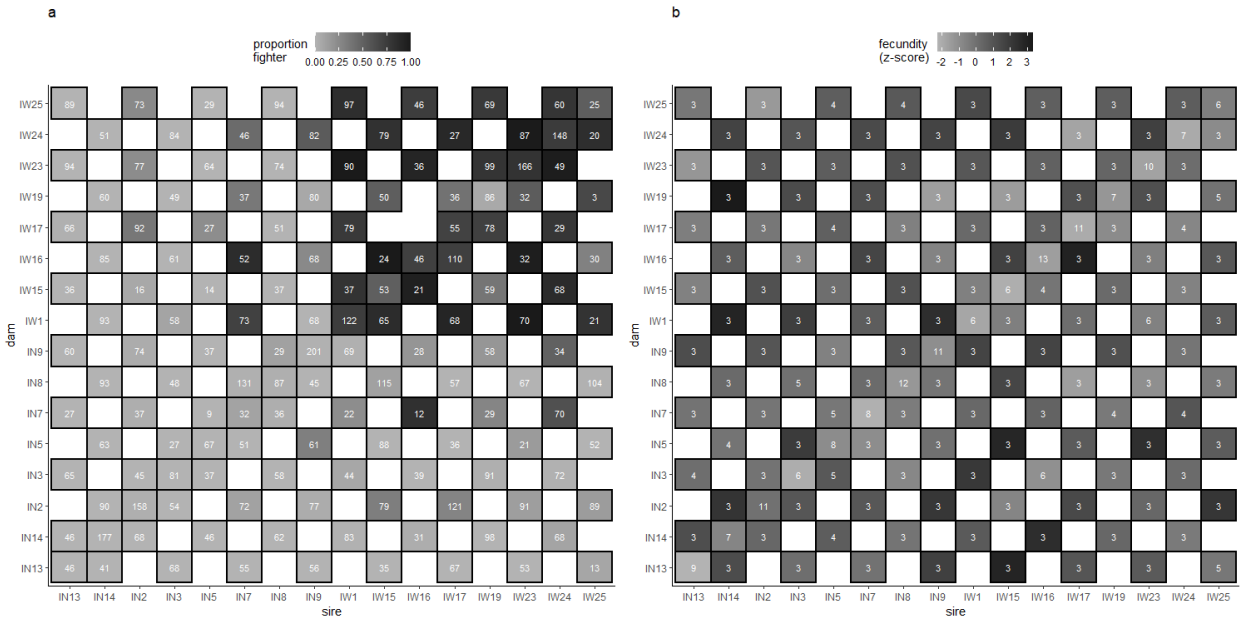
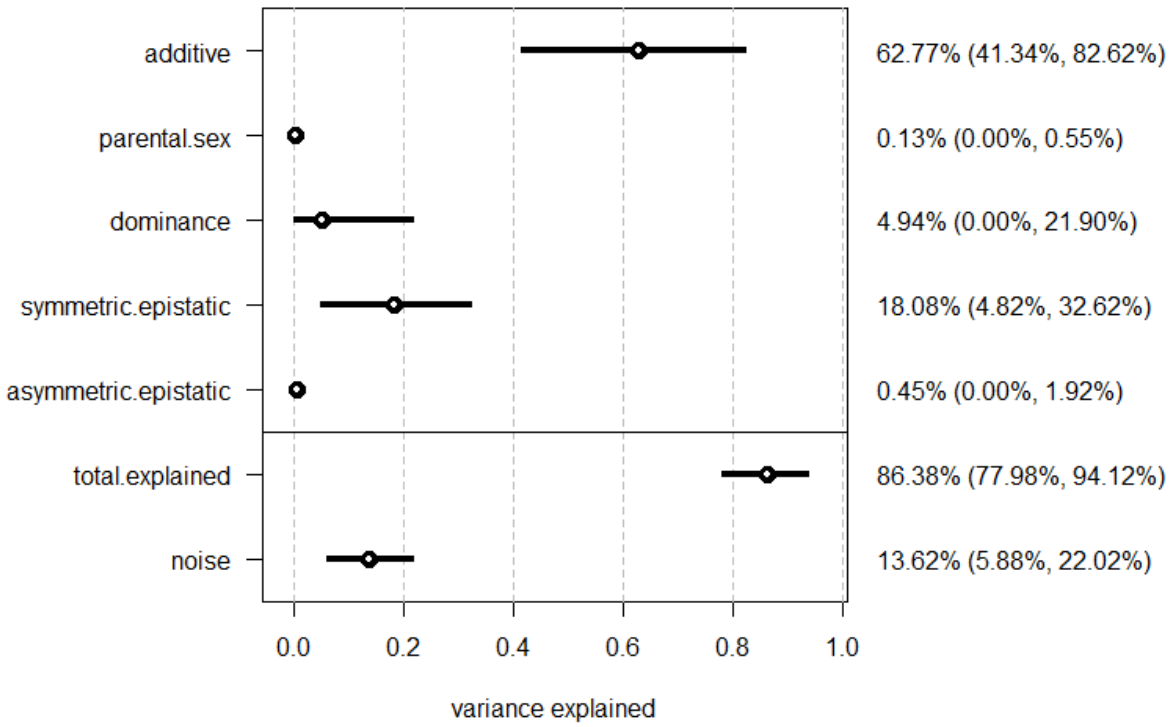


Figure 1. Heatmaps of a) morph proportion and b) female fecundity across the diallel with each sire and dam cross combination. a) Morph proportion (averaged across replicates), where darker grey indicates higher proportion of fighter males in that cross combination. Numbers within each square indicate the total number of males from which morph was recorded, with white squares with no numbers indicating no data was collected. b) Female fecundity (averaged summed *z*-scores across replicates) for each cross combination, where darker grey indicates higher summed *z*-score (i.e. fecundity controlled by observer). Numbers within each square indicate the total number of females which fecundity was recorded, with white squares with no numbers indicating no data was collected. Sire and dam names: IN or IW followed by a number provides inbred lines ID and indicate lines founded by a scrambler or fighter male, respectively (i.e. IN# = scrambler founded inbred line and IW# = fighter founded inbred line).

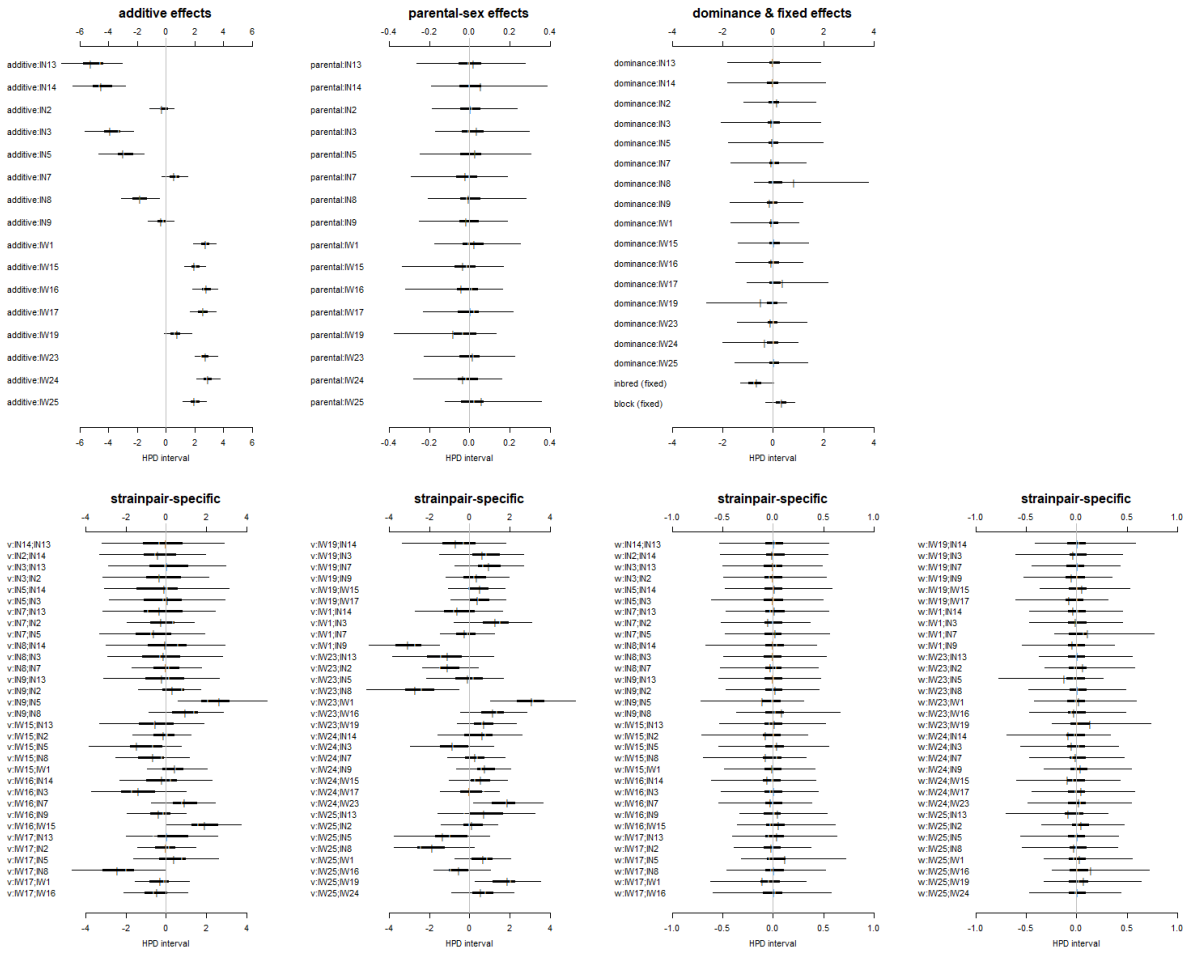


737

738 **Figure 2.** Variance contributions of distinct class effects on morph proportion. Reporting posterior

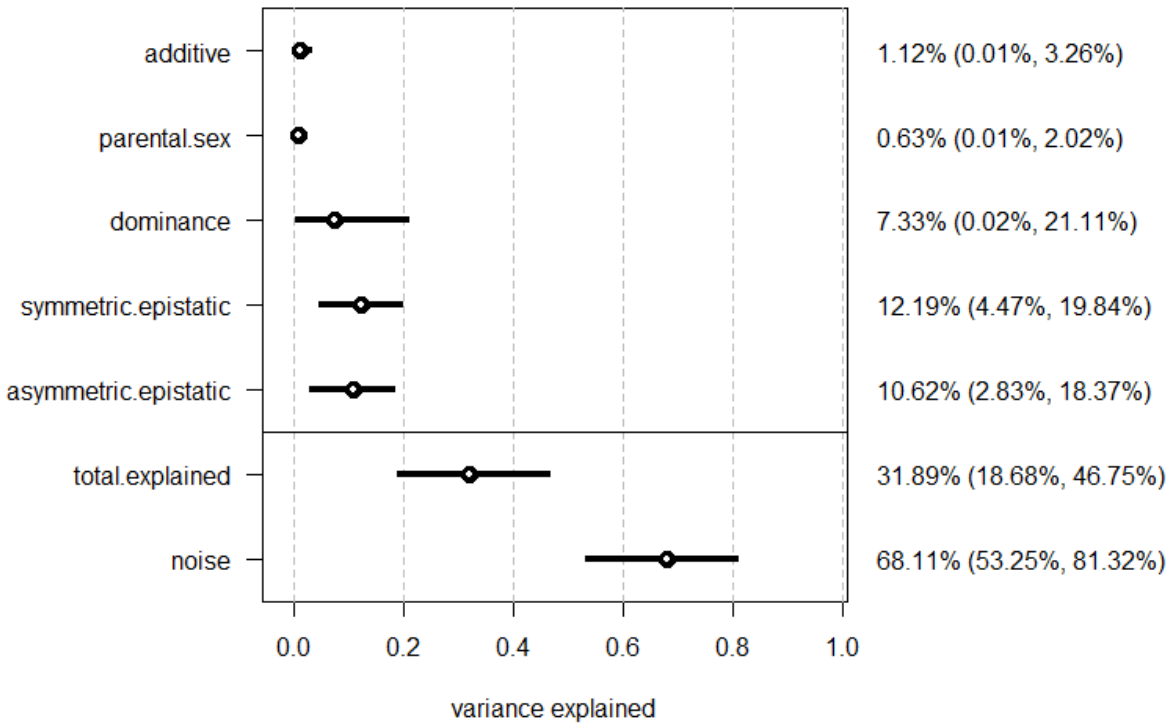
739 means and 95% HPDs of variance projections.

740



741

742 **Figure 3.** Diallel effects on morph proportion of (top row) strain-specific additive, parental sex, and
743 dominance effects, also fixed effects of block and main effect of inbreeding, and (bottom row) epistatic
744 strainpair-specific effects (labels "v" and "w" respectively refer to symmetric and non-symmetric
745 epistatic effects) on morph proportion. Represented for each parameter: thin line: 95% HPD; thick line:
746 50% HPD; vertical break: median HPD; dash: mean HPD. The grey vertical line indicate 0, where intervals
747 that exclude 0 have non-negligible effects on male morph. Positive values indicate increasing
748 contribution to offspring being fighters and negative values there is decreasing contribution of offspring
749 being fighter. IN or IW followed by a number provides inbred lines ID and indicate lines founded by a
750 scrambler or fighter male, respectively (i.e. IN# = scrambler founded inbred line and IW# = fighter
751 founded inbred line).



752

753 **Figure 4.** Variance contributions of distinct class effects on female fecundity (*z*-score). Reporting

754 posterior means and 95% HPDs of variance projections.

755

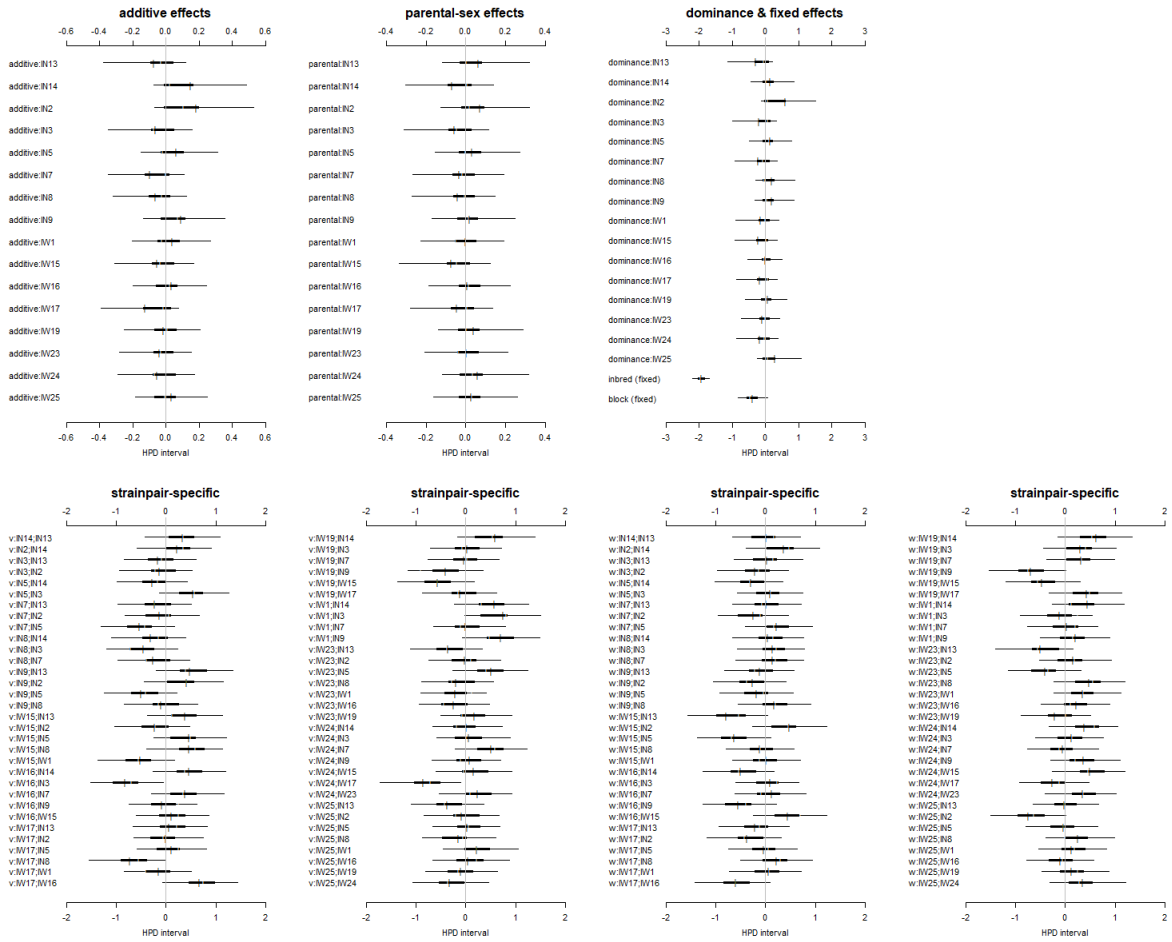
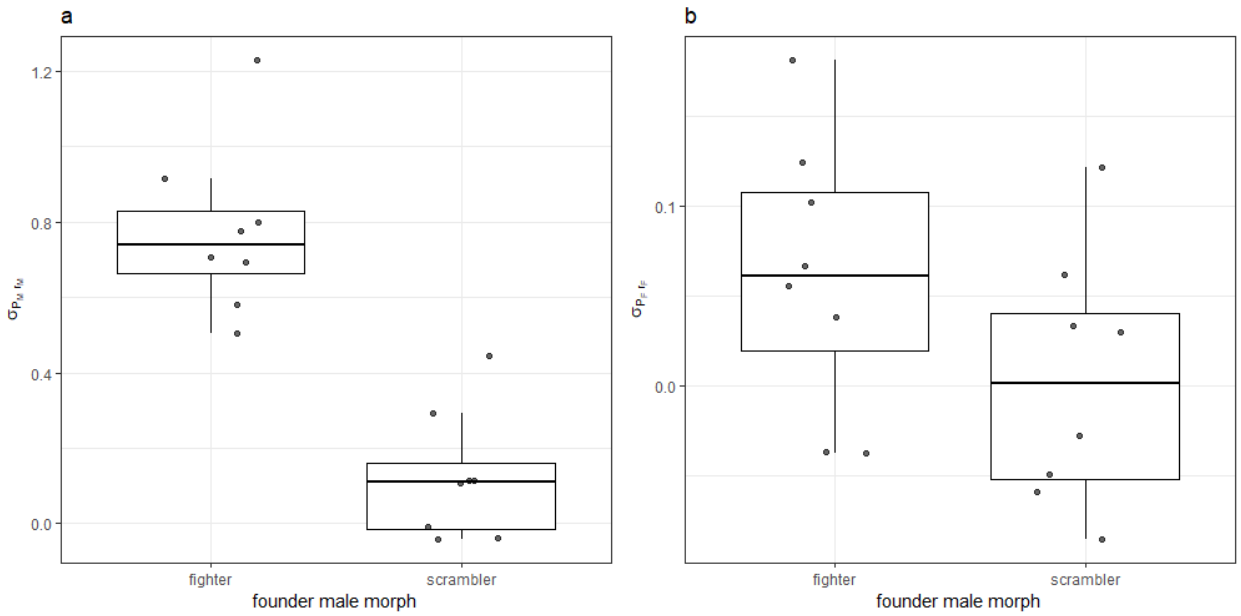


Figure 5. Diallel effects on female fecundity (*z*-score) of (top row) strain-specific additive, parental sex, and dominance effects, also fixed effects of block and main effect of inbreeding, and (bottom row) epistatic strainpair-specific effects (labels "v" and "w" respectively refer to symmetric and non-symmetric epistatic effects) on female fecundity. Represented for each parameter: thin line: 95% HPD; thick line: 50% HPD; vertical break: median HPD; dash: mean HPD. The grey vertical line indicate 0, where intervals that exclude 0 have non-negligible effects on male morph. Positive values indicate increasing contribution to female fecundity and negative values decreasing contribution to female fecundity. IN or IW followed by a number provides inbred lines ID and indicate lines founded by a scrambler or fighter male, respectively (i.e. IN# = scrambler founded inbred line and IW# = fighter founded inbred line).



767

768 **Figure 6.** Estimates of the relative amount of recessive allelic variation for a) morph ($\sigma_{PM,rM}$) and b)
769 fecundity ($\sigma_{PF,rF}$) between founder morph treatments. Boxes are composed of the median and hinge
770 values (25th and 75th percentiles), with whiskers ± interquartile range * 1.5. Individual points denote
771 each inbred lines mean array covariance for male morph and female fecundity (a: $\sigma_{PM,rM,}$ b: $\sigma_{PF,rF}$).