The role of testosterone in structuring genetic covariances and the role of parasites in structuring variation in fitness and natural selection

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Abstract

In a quantitative genetics framework, evolutionary change in quantitative phenotypes depends upon both selection, the change in the fitness weighted distribution of phenotypes within a generation, and on transmission across generations, which is shaped by the pattern of additive genetic variances and covariances among phenotypes (modeled as the **G** matrix). Due to their evolutionary importance there has been much work to estimate these parameters in natural populations and to understand what factors shape them. However, manipulative experiments to establish causality are relatively rare, many aspects of the environment that likely shape selection remain unexplored, and the effects of internal physiological mechanisms on **G** are largely unknown. In this dissertation, I investigate the role of testosterone in structuring **G**, and the role of parasites in structuring selection on their hosts using both experimental and comparative methods. In Chapter 1, I test the idea that hormones, through their pleiotropic effects on multiple phenotypes, are important in shaping patterns of additive genetic covariance. To this end, I performed a large-scale breeding study in brown anole lizards (*Anolis sagrei*), paired with the experimental manipulation of testosterone during development. I show that the hormone testosterone structures patterns of additive genetic covariance. Females given testosterone exhibit a **G** matrix that is statistically indistinguishable from that of control males and statistically distinct from that of control females. This demonstrates that the hormonal environment in which genes are expressed is important for shaping patterns of additive genetic variance and covariance, which themselves are important for the short term response to selection.

In Chapters 2-4, I turn my attention to the role of parasites in structuring variance in fitness and shaping patterns of natural selection in host populations. In Chapter 2, I perform a meta-analysis to determine how costly parasites are to their hosts in terms of survival, and to test whether the survival cost of parasitism is mediated by host mating system and sex. Across a phylogenetically and ecologically diverse set of hosts and parasites, I show that on average, parasitized hosts have 3.5 times greater odds of mortality than unparasitized hosts. By increasing the odds of mortality, parasites increase the opportunity for selection. Further, within promiscuous and polygynous species, males have a greater survival cost of parasitism than females, while in monogamous systems, females suffer greater costs than males. In Chapter 3, I test for costs of parasites in terms of growth, performance, survival and mating success in *A. sagrei* hosts. I experimentally removed the nematode parasites from *A. sagrei* using a custom, extended-release formulation of the antiparasite drug ivermectin that I developed. I demonstrate that parasites in this system impose costs to the growth, performance, and mating success of their hosts. In Chapter 4, I expand on the work from Chapter 3 and perform a larger experiment, again using wild *A. sagrei*, to investigate the impact of parasites on phenotypic selection. I show that parasites decrease survival for adults across the breeding season, thus increasing the variance in relative fitness. Additionally, parasites shape selection in juveniles by changing the correlation between some phenotypes and fitness. The results in adults are ambiguous but suggest that parasites may affect selection both by increasing the opportunity for selection and by altering the correlation between phenotype and fitness.

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Introduction

The evolution of a quantitative phenotype can be modeled through the breeder's equation by decomposing the evolutionary response into a term for the relationship between phenotype and relative fitness (i.e., selection), and one for the resemblance between offspring and parents. (Lush, 1937; Lande & Arnold, 1983; Walsh & Lynch, 2018). The breeder's equation is given by

$$\Delta\bar{z}' = Cov(w, z) * V_A/V_P \qquad (Eqn.\ 1)$$

where z is a quantitative phenotype, w is relative fitness (an individual's absolute fitness divided by the population mean absolute fitness), $\Delta\bar{z}'$ is the change in the mean phenotypic value across a generation, $Cov(w, z)$ gives the difference in the mean phenotypic value before selection and the fitness-weighed mean phenotypic value after selection, V_A is the additive genetic variance of phenotype z, V_P is the phenotypic variance of phenotype z, and V_A/V_P is the heritability (h^2) of phenotype z. This term for heritability describes how change within a generation is transmitted across generations. The term $Cov(w, z)$ is known as the selection differential, s. When investigating the evolution of multiple phenotypes, the multivariate extension of the breeder's equation is used (Lande, 1979; Lande & Arnold, 1983; Walsh & Lynch 2018). The multivariate breeder's equation is given by

$$\Delta\bar{\mathbf{z}}' = \mathbf{GP^{-1}s} \qquad (Eqn.\ 2a)$$

$$\Delta\bar{\mathbf{z}}' = \mathbf{G\beta} \qquad (Eqn.\ 2b)$$

where $\Delta\bar{\mathbf{z}}'$ is now a vector of changes in phenotypic means across generations, **s** is a vector of selection differentials, **P** is the phenotypic covariance matrix, **G** is the additive genetic covariance matrix, and **β** is the vector of selection gradients. The multivariate form reveals the importance of phenotypic and genetic covariances among traits for determining the response of a population to selection (*Eqn.* 2ab). It can be seen from (*Eqn.* 2b) that **G** acts as a prism to transform the vector of selection gradients into a vector of evolutionary response. The additive genetic variances contained on the diagonal of **G** in conjunction with the strength and direction of selection determine the magnitude of the response vector, while the genetic covariances determine the rotation of **β** into $\Delta\bar{\mathbf{Z}}'$ in multivariate space. This dissertation addresses the questions 1) is the structure of **G** moderated by the hormonal environment of the population? and 2) do parasites structure the magnitude and direction of selection acting on their hosts? In Chapter 1, I test whether the hormone testosterone is important in structuring patterns of additive genetic variance and covariance in **G**. In Chapters 2-4 I test whether and how parasites impact various aspects of host populations that jointly determine the magnitude and direction of selection.

The **G** *matrix*

Given its importance in the multivariate breeder's equation the **G** matrix has been extensively studied. Studies have explored how **G** evolves (Sgró & Hoffmann, 2004; Arnold et al., 2008; Careau et al., 2015), how the environment influences **G** (de Jong, 1990; Holloway et al., 1990; Sgró & Hoffmann, 2004; Wood & Brodie, 2015), and how selection shapes the evolution of genetic covariances (Holloway et al., 1990; Sinervo &

Svensson, 2002; Delph et al., 2011; Steven et al., 2019). In populations with two sexes, the evolutionary response to selection within each sex depends on the relative independence of genetic variation between sexes (Lande, 1980; McGlothlin et al., 2019; Cheng & Houle, 2020). Evolutionary change in populations with two sexes can be modeled with the breeder's equation by the inclusion of sex specific measures of selection and the addition of a **B** matrix, which describes between-sex genetic covariance among traits. The pattern of genetic covariances represented in **B** can constrain or facilitate the evolution of sexual dimorphism.

Given the importance of genetic covariances represented in **G** and **B,** we know surprisingly little of the physiological mechanisms structuring them. Hormones may play an important role in structuring genetic covariances. Hormones can structure phenotypic integration by linking the expression of disparate traits to a common signal, such as a hormone (McGlothlin & Ketterson, 2008, Cox et al., 2016, Dantzer & Swanson, 2017). This effect of a single hormone on multiple phenotypes, termed hormonal pleiotropy, is analogous to genetic pleiotropy where one gene affects multiple phenotypes. Hormones have been shown to have large effects on patterns of gene expression, and thus in addition to structuring patterns of phenotypic covariance, hormonal pleiotropy may structure patterns of genetic covariance (Cox et al. 2017, Cox, 2020). Males and females within a population share much of the same genome, while sex differences in circulating levels of hormones such as testosterone create different hormonal environments in which these genotypes are translated into phenotypes. In Chapter 1, I test whether hormonal pleiotropy structures patterns of genetic covariance for **G** and **B**. To do this, I quantify the

effect of the hormone testosterone on the quantitative genetic architecture of the dewlap, a secondary sexual phenotype, in *Anolis sagrei*. I perform a half-sib breeding study paired with the experimental manipulation of testosterone during juvenile growth, the period when sexual dimorphism begins to develop, in males and females. I find that with natural sex differences in testosterone the **G** matrix of males is significantly different from that of females and between-sex genetic correlations are weak for most traits. However, the **G** matrix of females treated with testosterone is statistically indistinguishable from that of control males or males treated with testosterone, while it differs significantly from that of control females. Further, the between-sex genetic correlations between testosterone treated females and control males are significantly stronger than those between control females and control males. This demonstrates that the hormonal environment in which genes express themselves is important for shaping patterns of additive genetic variance and covariance. Thus, the distribution of the internal hormonal states of individuals during development can influence the short-term evolutionary response of a population, much like the external environment can.

Selection

The selection component of the breeder's equation, $Cov(w, z)$, gives the difference between the phenotypic mean and the fitness weighted phenotypic mean within a generation (*Eqn.* 1). The variance in relative fitness sets the upper bound to the strength of selection and is given by

$$I = V_w = V_W/\bar{W}^2 \qquad (Eqn.\ 3)$$

where V_W is variance in absolute fitness, and $\bar{W}^2$ is mean absolute fitness squared. The variance in relative fitness (V_w) (*Eqn.* 3), termed the opportunity for selection (*I*), defines the maximum amount of change in fitness possible within a generation, and selection differentials must be less than or equal to the square root of the opportunity for selection (Crow, 1958; Arnold & Wade, 1984). When phenotypes are standardized to unit variance, selection can be further decomposed into the product of the square root of the opportunity for selection and the correlation between phenotype and fitness (or the partial correlation in the multivariate case). Taken together, equations 1-3 show that the direction and magnitude of selection are jointly determined by the multivariate phenotypic distribution, the variance in relative fitness, and the correlations between phenotypes and fitness. While much work has been done to empirically estimate patterns of selection in natural populations (Cox & Calsbeek, 2009; Kingsolver *et al.*, 2012), there remains much to learn about the identity of ecological agents of selection and through what of the previously mentioned mechanisms they work through (Wade & Kalisz, 1990; Calsbeek & Cox, 2010; MacColl, 2011; Cox *et al.*, 2022).

Due to the ubiquity of host-parasite associations and the effect of parasites on host phenotypes and fitness, parasites have the potential to be important drivers of selection in natural populations (Poulin & Thomas, 1999; Robar *et al.,* 2010). The co-evolutionary dynamics between host resistance and parasite infectivity and the role of parasites in driving sexual selection for host resistance and signaling traits have been well studied, but the role parasites may play in shaping selection for host traits not directly involved in

host-parasite interactions has received little attention (Dybdahl & Lively, 1998; Moller *et al*., 1999; Kerstes *et al.*, 2012; Gibson *et al*., 2020).

One way in which parasites can affect selection on traits not directly involved in host-parasite interactions is through their effect on the opportunity for selection. When fitness differences are measured as or solely due to differences in survival, the opportunity for selection is equal to the odds of mortality divided by the odds of survival (Crow, 1958). By reducing survival, parasites influence the opportunity for selection and thus may affect the magnitude of selection a population experiences (*Eqn.* 3). If the correlation between a phenotype and relative fitness is the same in two hypothetical populations, one parasitized and one parasite free, then selection is expected to be stronger in the parasitized population.

The sexes are expected to differ in their optimal allocation between reproduction and survival, because of this the survival cost of parasitism may differ between sexes (Trivers, 1972; Rolff, 2002; Cox 2014). The mating system of a species, by differentially effecting the intensity of sexual selection acting on either sex, is also expected to structure the sex difference in optimal allocation between life-history processes (Promislow, 1992; Weatherhead & Teether, 1994; Innocenti *et al.,* 2014). Thus, by decreasing survival in a sex specific manner, parasites can potentially mediate the magnitude of sex differences in selection. Males and females often differ in their parasite burden, and such evidence of sex-biased parasitism is often assumed to translate into a sex bias in the cost of parasitism. However, because males and females can differ in their tolerance to parasites sex-biased parasitism does not necessarily translate into a sex

difference in the fitness costs of parasitism. In Chapter 2, I perform a meta-analysis to estimate the survival cost of parasitism across a phylogenetically and ecologically diverse group of hosts and parasites. I further test whether there is a sex difference in the survival cost of parasitism, and whether host mating system moderates sex differences in the survival cost of parasitism. I include studies that used natural variation, experimental infection, or experimental parasite removal to generate variation in parasitism among groups of individuals. I calculate the survival cost of parasitism as the ratio of the odds of mortality in the parasitized group to the odds of mortality in the unparasitized group. As described above, this is equivalent to the opportunity for selection in the parasitized group divided by the opportunity for selection in the unparasitized group. I found that parasites impose a large survival cost to their hosts. On average across species, the odds of mortality is 3.5 times greater for parasitized groups compared to unparasitized groups. On average, males have a greater survival cost of parasitism than females, but sex bias in this cost is structured by the host mating system. Males have a greater cost in promiscuous and polygynous species and females have a greater cost in monogamous species. These results show that parasites may be a potent force moderating the strength of selection on traits in host populations, and they may generate sex differences in the magnitude of selection.

Parasites are known to have a variety of phenotypic costs on their hosts, including decreased host growth, performance, metabolism, and changed behavior (Lafferty & Morris, 1996; Forbes et al., 2002; Careau et al., 2009; Binning et al., 2017; Finnerty et al., 2017; Kelehear et al., 2019). Ultimately these phenotypic costs are expected to

decrease fitness, and parasites have frequently been shown to reduce the survival and reproductive success of their hosts (Robar et al., 2010; Patterson et al., 2013; Albery et al., 2021, Chapter 2). Through their effects on fitness, parasites can regulate host populations and may influence their evolutionary dynamics (Anderson & May, 1978; Pedersen & Fenton, 2015). Historically, studies of the costs of parasitism in natural populations were observational, and their inferences relied on correlations between parasite burden and host phenotypes and fitness (Minchella & Scott, 1991; Rousset et al. 1996). However, establishing the causal effect of parasites on host phenotypes and fitness requires experimental manipulation (Pedersen & Fenton, 2015). The experimental removal of parasites in natural populations provides a powerful approach to study the individual and population effects of parasites (Pedersen & Antonovics, 2013; Pedersen & Fenton, 2015; Budischak et al., 2016; Binning et al., 2017). The aim of this approach is to experimentally reduce the within-population variance in parasitism for one treatment group, while maintaining the natural variation in parasitism for the other group. When performed in a longitudinal framework, this approach provides a powerful method for measuring the costs of parasitism and their effect on population-level processes such as selection. However, the required dosing schedule of anti-parasite drugs is difficult to meet in longitudinal studies of natural populations (Barragry, 1987; Soll 1989). While extended-release formulations of anti-parasite drugs are commercially available, they are generally unsuitable for the small-bodied organisms that are often used in ecological and evolutionary research (Soll et al., 1990; González Canga et al., 2009).

In Chapter 3 I test for costs of parasitism in terms of growth, performance, survival and mating success in *Anolis sagrei*. I use the experimental removal of parasites both in the laboratory and combined with a capture-mark-recapture field study. To this end, I developed a custom extended-release formulation of the antiparasite drug Ivermectin. I demonstrate that this formulation is safe and effective at removing nematode parasites from *A. sagrei* both in the lab and field. I find that nematode parasites consistently reduce the growth of juvenile *A. sagrei* and decrease the sprinting performance and mating success of adult males. However, they do not affect the survival of juveniles or adults of either sex. The growth cost of parasitism may have important consequences for lifetime fitness, given that *A. sagrei* body size at the beginning of the first breeding season is consistently positively correlated with reproductive success within that year (Unpublished data: R.M. Cox, R.S. Bhave, A.F. Kahrl, A.M. Reedy, H.A. Sears, & T.N. Wittman).

While most work has focused on the effects of parasites on the means of host phenotypes and fitness, their influence on the eco-evolutionary dynamics of host populations is likely also due to their effects on the variance in host phenotypes and fitness (Poulin & Thomas, 1999). Parasites can potentially influence selection by 1) influencing the variance and covariance of phenotypes, 2) by increasing the variance in relative fitness (the opportunity for selection), and 3) by shaping the correlation between phenotypes and fitness (*Eqns*. 1-3).

The variance of and covariance among phenotypes is the substrate that selection acts upon, determines what selection can “see”, and gives rise to indirect selection. The

impact of parasites on the phenotypic distribution of their hosts due to within individual effects depends on the prevalence of parasites in the population and their effect on individual phenotypes. With a non-zero phenotypic effect and moderate prevalence, parasites are expected to increase the variance of the phenotype. Further, depending on how parasites jointly affect the expression of multiple phenotypes, they could increase or decrease phenotypic covariance.

By definition, parasites should reduce the fitness of their hosts, and this has been shown for multiple components of fitness (Marzal *et al.*, 2005; Robar *et al.*, 2010; Hillegass *et al*., 2010; Worden *et al.*, 2010; Holand *et al*., 2015). For binary measures of fitness such as survival or mating success, the variance in relative fitness is calculated as the probability of failure divided by the probability of success, thus a reduction in mean survival or mating success necessarily increases the variance in relative fitness. The variance in relative fitness sets an upper limit to the strength of selection. For a given correlation between phenotype and fitness, the strength of selection will increase with the variance in relative fitness. Thus, parasites may be an important factor in determining the magnitude of selection in natural populations. Additionally, as random processes can generate phenotype fitness correlations in finite populations parasites can influence the strength of drift that population experiences (Rice, 2004).

Finally, parasites can potentially change the correlation between phenotypes and fitness. A correlation between phenotype and fitness could be generated by parasites if their effect on fitness differed with host phenotypic values. For example, positive directional selection would result if larger hosts are better able to tolerate the detrimental

effects of parasites. By interacting with other agents of selection parasites could influence the magnitude and direction of phenotype fitness correlations.

In Chapter 4 I explore how parasites influence the eco-evolutionary dynamics of their host populations by testing for their effects on patterns of phenotypic covariance, survival and thus variance in relative fitness, the correlation between phenotypes and fitness, and the direction and magnitude of selection. To this end, I perform a capture-mark-recapture study paired with the experimental removal of parasites using the drug formulation I developed in Chapter 3. I follow a cohort of *A. sagrei* in an island population across their first year of life, censusing the island four times and measuring a suite of phenotypes. This is a large proportion of their total lifespan, as only 17% of females and 7% of males in this population survive to a second year. This is a powerful system for studies of selection because nearly the entire population can be sampled and included in the experiment, and the recapture probability of lizards is high in each census (female > 85%, males > 95%). I found that parasite removal did not affect the phenotypic variances for all but one phenotype nor did it influence patterns of phenotypic covariance. This suggests that parasites are unlikely to influence selection through their effect on phenotypes. Consistent with the results from Chapter 3, parasite removal did not increase survival for juveniles or adults early in the breeding season, and thus did not affect the opportunity for selection during those periods. However, parasite removal greatly increased survival across the entire breeding season, showing that parasites increase the opportunity for selection during that period. This increase in the opportunity for selection did not result in a significant increase in the strength of selection measured across the

breeding season. For the overwinter period in juveniles, parasite removal significantly changed the form and strength of nonlinear selection for both males and females. Because the opportunity for selection did not differ between treatment groups during this period, the effect of parasite removal on selection came about due to an effect on the correlation between phenotype and fitness. Contrary to my prediction, parasite removal increased the strength of multivariate linear selection acting on adult males early in the breeding season. Again, this was due to changes in the correlations between phenotypes and fitness because the opportunity for selection did not differ between treatment groups for this period. Parasite removal did decrease the magnitude and variance in randomly generated covariances between phenotype and fitness, suggesting that parasites increase the action of drift acting in finite populations. Overall, while parasites have a large effect on the opportunity for selection (Chapter 2) this is not borne out though an increase in the strength of realized selection. My results suggest that the effect of parasites on selection are likely context specific and more often due to changes in the correlation between phenotypes and fitness rather than an increase in the opportunity for selection.

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Chapter One:

Hormonal pleiotropy structures genetic covariance[1]

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Abstract

Quantitative genetic theory proposes that phenotypic evolution is shaped by **G**, the matrix of genetic variances and covariances among traits. In species with separate sexes, the evolution of sexual dimorphism is also shaped by **B**, the matrix of between-sex genetic variances and covariances. Despite considerable focus on estimating these matrices, their underlying biological mechanisms are largely speculative. We experimentally tested the hypothesis that **G** and **B** are structured by hormonal pleiotropy, which occurs when one hormone influences multiple phenotypes. Using juvenile brown anole lizards (*Anolis sagrei*) bred in a paternal half-sibling design, we elevated the steroid hormone testosterone with slow-release implants while administering empty implants to siblings as a control. We quantified effects of this manipulation on the genetic architecture of a suite of sexually dimorphic traits, including body size (males are larger than females) and the area, hue, saturation, and brightness of the dewlap (a colorful ornament that is larger in males than in females). Testosterone masculinized females by increasing body size and dewlap area, hue, and saturation, while reducing dewlap brightness. Control females and males differed significantly in **G**, but treatment of females with testosterone rendered **G** statistically indistinguishable from males. Whereas **B** was characterized by low between-sex genetic correlations when estimated between control females and control males, these same correlations increased significantly when estimated between testosterone females and either control or testosterone males. The full **G** matrix (including **B**) for testosterone females and either control or testosterone males was significantly less permissive of sexually dimorphic evolution than was **G** estimated

between control females and control males, suggesting that natural sex differences in testosterone help decouple genetic variance between the sexes. Our results confirm that hormonal pleiotropy structures genetic covariance, implying that hormones play an important yet overlooked role in mediating evolutionary responses to selection.

Introduction

When natural selection acts on phenotypes, the evolutionary response of a population depends on the extent to which these phenotypes are heritable and genetically correlated with one another. In quantitative genetics, these properties are often represented by the genetic variance-covariance matrix, **G** (Lande 1979; Lande and Arnold 1983; Eqns. 1-2, Supplemental Material). In addition to its importance for evolutionary theory (Steppan et al. 2002; Jones et al. 2007; Roff 2007), **G** can inform studies of adaptation and reveal properties of the genotype-phenotype map (Grant and Grant 1995; Wilson et al. 2010; Milocco and Salazar-Ciudad 2020). Accordingly, estimates of **G** have been obtained for a variety of species (Arnold et al. 2008; Pitchers et al. 2014; Wood and Brodie 2015), comparative studies have explored its evolution (Chenoweth et al. 2010; McGlothlin et al. 2018; Walter et al. 2018), and experiments have characterized its sensitivity to the environment (Sgro and Hoffmann 2004; Charmantier and Garant 2005; Wood and Brodie 2015) and to mutation (Camara and Pigliucci 1999; Estes et al. 2005). By contrast, no experiment has explored how **G** is structured by internal physiological mechanisms that mediate the translation of genotype to phenotype, such as hormones.

In species with separate sexes, phenotypic evolution also depends on patterns of genetic covariance between females and males, as represented by the sub-matrix **B** within

G (Lande 1980; Eqn. 3, Supplemental Material). Between-sex genetic covariance represents a short-term constraint on the evolution of sexual dimorphism, but it is also predicted to break down over time in response to sexually antagonistic selection (Lande 1980, 1987; Fairbairn and Roff 2006). Sexually antagonistic selection may not always reduce between-sex covariance in the short term (McGlothlin et al. 2019), but selection experiments confirm that it can do so rapidly in some circumstances (Delph et al. 2011), and comparative studies indicate that the evolution of sexual dimorphism is generally associated with a reduction in between-sex genetic covariance (Poissant et al. 2010). Although recent work has emphasized the importance of **B** in shaping the evolution of sexual dimorphism (Gosden et al. 2012; Wyman et al. 2013; Cheng and Houle 2020) and studies on a variety of species have empirically characterized **B** (Steven et al. 2007; Campbell et al. 2011; Lewis et al. 2011; Ingleby et al. 2014; Cox et al. 2017a; White et al. 2019), we know relatively little about the physiological mechanisms that orchestrate the breakdown of between-sex genetic covariance to facilitate the evolution of sexual dimorphism (Cox et al. 2017b).

In this study, we experimentally test the hypothesis that hormonal pleiotropy structures **G** and **B**. Hormonal pleiotropy (one hormone influencing multiple phenotypes) is analogous to genetic pleiotropy (one gene influencing multiple phenotypes) with the substitution of a hormone and its receptor in place of a gene in the literal sense (Ketterson and Nolan 1999; Lema 2014; Cox 2020). Hormonal pleiotropy has served as an important conceptual framework for evolutionary biology (Finch and Rose 1995; Flatt et al. 2005; Bourg et al. 2019), but only a handful of studies have formally integrated this concept with quantitative genetics (McGlothlin and Ketterson 2008; Ketterson et al. 2009; Cox et

al. 2016; Dantzer and Swanson 2017; Cox 2020). To test whether hormonal pleiotropy structures **G** and **B**, we focus on the steroid hormone testosterone, which naturally circulates at higher levels in adult males than in females. In vertebrate genomes, hundreds to thousands of genes contain response elements that bind the androgen receptor, such that testosterone can exert massively pleiotropic effects (reviewed by Cox 2020). Consequently, sex differences in circulating testosterone lead to sex differences in the transcription and translation of shared genes into dimorphic phenotypes, which is predicted to produce sex-specific patterns in **G** and break down between-sex covariance in **B**.

We test these predictions in the brown anole (*Anolis sagrei*), a sexually dimorphic lizard in which males are larger than females and possess a large and colorful ornament (dewlap) that is much smaller in females (Cox and Calsbeek 2010; Cox et al. 2017a). These sex differences are regulated in part by maturational divergence in testosterone, which enhances growth and dewlap development when administered to juveniles and restores these phenotypes in castrated adult males (Cox et al. 2009a; 2009b; 2015). Testosterone also alters the female transcriptome in ways that parallel natural sex differences in gene expression that emerge during maturation (Cox et al. 2016; 2017b; Cox 2020). Females and males differ in **G** for dewlap phenotypes, most of which are also characterized by relatively weak between-sex genetic covariance in **B** (Cox et al. 2017a). Between-sex genetic covariance for body size is high during early ontogeny, but it breaks down as sexual dimorphism develops, coincident with maturational increases in testosterone and sex-biased gene expression (Cox et al. 2017b). Collectively, these studies suggest that females and males share a similar genetic architecture for body size

and dewlap morphology, from which the sex-specific expression of genetic variance and covariance are coordinated by maturational divergence in testosterone (Cox 2020). We provide the first experimental support for this hypothesis, and for the more general hypothesis that hormonal pleiotropy structures genetic covariance, by demonstrating pronounced changes in **G** and **B** in response to testosterone manipulation in a pedigreed breeding population of anoles.

Materials and Methods

Breeding and Experimental Design

We bred anoles in a paternal half-sibling design with two dams per sire (n = 120 dams, 60 sires) following published protocols (Cox et al. 2016; Cox et al. 2017a,b; Logan et al. 2018; see Supplemental Materials). Sample sizes and family sizes are summarized in Table S1. Dams and sires were F_2 descendants of stock from Great Exuma in the Commonwealth of the Bahamas (23°29'N, 75°45'W; imported under permits from the Bahamas Environment, Science and Technology Commission, the Bahamas Ministry of Agriculture, and the United States Fish and Wildlife Service). Breeding was conducted in captivity with all F_1 and F_2 crosses set to avoid inbreeding. All procedures were reviewed and approved by the University of Virginia's Animal Care and Use Committee (protocol 3896).

We raised F_3 progeny to 3 months of age and then administered one of two treatments: (1) a slow-release implant containing 100 μg testosterone, or (2) an empty implant as a control. Implant design and surgical procedures followed previous studies (Cox et al. 2015; Cox et al. 2017b; see below, in which identical implants elevated

testosterone levels of juvenile males and females approximately 5-fold relative to controls, while remaining within the natural physiological range for adult males. Because anoles lay a single egg every 7-14 days, progeny were produced continuously over 10 months (August 2017 to June 2018). To balance treatments within maternal families, we haphazardly determined whether the first offspring of each sex would receive a testosterone or a control implant for a given family, then alternated between treatment groups for all subsequent progeny of each sex. At 8 months of age, we measured each individual for snout-vent length (SVL) and photographed its dewlap to measure area, hue, saturation, and brightness following Cox et al. (2017a, see below). We used these five traits to estimate **G** and **B**. Dewlap area and SVL are metric traits in which variance increases with the mean, so we ln-transformed these traits, rendering values proportional and preventing sex and treatment differences in size from influencing total genetic variance.

Implant Construction and Testosterone Manipulation

We made implants by dissolving testosterone (T-1500, Sigma-Aldrich Inc., St. Louis. MO, USA) in dimethyl sulfoxide (DMSO) at a concentration of 100 µg testosterone per µL DMSO, then injecting 1 µl of this solution into a 4-mm section of Silastic tubing (Dow Corning, Midland, MI, USA: 1.47 mm I.D. x 1.96 mm O.D.) which we had previously sealed on one end using 100% silicone gel. After loading the hormone solution, we sealed the open end of the implant with silicone gel, then waited 48-72 h for the silicone to cure and for the DMSO to diffuse out of the implant, leaving 100 µg of crystalized testosterone within the lumen of the sealed Silastic implant. As an experimental control, we produced empty implants by following the same procedure with

1 µl pure DMSO in place of the testosterone solution. This hormone dose and implant size were selected because they have been shown to increase circulating testosterone in juvenile anoles of comparable size while maintaining plasma levels within the natural physiological range for adult males (Cox et al. 2015).

We fasted lizards for 24 h prior to surgery, then gave each animal a 2-µl intra-peritoneal injection of bupivacaine (2.5 mg mL^{-1}) at the site of incision as an anesthetic and analgesic. We then cooled lizards at -20°C for 4-5 min and immobilized them atop a partially thawed chemical ice pack. We sanitized the incision site with alternating wipes of 70% isopropyl alcohol and 4% chlorhexidine gluconate solution. Surgical instruments were heat-sterilized in a Germinator 500 bead sterilizer (DS-501, Roboz Surgical Instrument Co., Gaithersbur, MD, USA) before each surgery. We made a 3-mm incision in the ventral abdomen and inserted an ethanol-sterilized implant (testosterone or empty control) into the coelomic cavity and closed the incision with cyanoacrylate adhesive (VetClose®, Butler Schein Animal Health, Dublin, OH, USA).

Measurement of Dewlap Phenotypes

We manually extended the dewlap by pulling out the second ceratobranchial cartilage with forceps, then took a digital photograph (Canon EOS Rebel T3i with 100-mm macro lens) from a set distance against a standardized background (graph paper, 5-mm grids) under standardized lighting (FE30050-10 28W fluorescent photography bulb in reflecting hood at set distance and angle) next to a color standard (Kodak Gray Scale and Color Control patches). We measured dewlap traits using the Fiji distribution (Schindelin et al. 2012) of ImageJ (Schneider et al. 2012). We uploaded images into ImageJ and set the scale of measurement using the 5-mm grids of the graph paper. We

measured area (mm^2) by outlining the dewlap from its anterior projection from chin to its posterior attachment to the venter using the "polygon" tool. To quantify the color of the dewlap, we used the "oval" tool to define a circle in the center of the dewlap, with diameter of the circle equal to 1/3 the width of the dewlap, providing a consistent measure of the "center" of each dewlap despite variation in its absolute size. We used the "color histogram" function to extract the mean red, green, and blue values for the selected area, then transformed these values into hue (primary color reflected, measured on a 360° color wheel), saturation (purity of the hue, 0% = achromatic, 100% = pure color), and brightness (relative to maximum possible for color of the same hue and saturation, 0% = black, 100% = white-tint-pure color) using the rgb2hsv function of the package gDevices within R3.6.2 (R Core Team 2019).

Estimation of Genetic Covariance Matrices

We estimated **G** using the program WOMBAT (Meyer 2007) and a restricted error maximum likelihood (REML, animal model) framework that allowed us to incorporate three generations of pedigree information describing relationships among F_1 grandparents, F_2 parents, and F_3 experimental progeny. We estimated separate within-sex **G** matrices for each of the four experimental groups (control females, control males, testosterone females, testosterone males). For pairs of male and female treatments, we estimated full **G** matrices including both within-sex matrices ($\mathbf{G_F}$, $\mathbf{G_M}$) and the between-sex matrix (**B**). When estimated for control females and control males, **B** describes natural patterns of between-sex covariance. When estimated for testosterone females and either control or testosterone males, **B** describes experimentally induced patterns of between-sex covariance that occur when both sexes translate genotype to phenotype in

the presence of testosterone. For all estimates, we included month of hatching as a random effect to account for any inadvertent shifts in husbandry (e.g., size and number of crickets fed per individual) that may have occurred despite our best efforts at standardization. Inclusion of Dam ID as a random (maternal) effect did not significantly improve fit for any model, so it was not included in our final matrix estimates. We estimated **G** and **B** using penalized estimation with shrinkage of genetic partial autocorrelations toward zero by setting a mild penalty (sample size of beta distribution = 3.0) using the PACORR function in WOMBAT (Meyer 2011, 2016). To confirm significant genetic variance and covariance, we used likelihood ratio tests to compare models estimating the full **G** for each group (or the full **G** and **B** for each combination of female and male groups) with simpler models setting covariances to zero or excluding additive genetic effects entirely (see Supplemental Material and Tables S5 and S12).

In addition to **G**, we used WOMBAT to estimate phenotypic covariance matrices (**P**) describing overall patterns of trait variance and covariance across individuals without taking genetic relationships into account (Table S9). We also used WOMBAT to estimate both phenotypic and genetic correlation matrices (Tables S8, S9). Prior to analysis, we variance-standardized our estimates of **G** and **B** by dividing genetic variances by phenotypic variances (narrow-sense heritability, h^2) and dividing genetic covariances by mean phenotypic variances (Tables S6, S13) (Hansen and Houle 2008). This standardization ensures that traits measured on different phenotypic scales (e.g., mm, degrees, percentages) can still contribute equal genetic variance to the matrix. Unstandardized matrices are presented (Tables S7, S14) and gave qualitatively identical results when compared among groups (Tables S10, S16).

Statistical Analyses and Matrix Comparisons

All statistical analyses were performed in R 3.5.3 (R Core Team 2019). To test for phenotypic effects of sex and testosterone, we individually analyzed each phenotype as the dependent variable in a linear mixed effects model with sex and treatment as fixed effects with interaction, and month of hatching, sire, and dam (nested within sire) as random effects. We conducted these analyses at 3 months of age to describe patterns of sexual dimorphism just prior to treatment, and at 8 months of age to describe the development of sexual dimorphism and the effects of testosterone. To clarify statistical interactions, we conducted similar analyses of treatment effects within each sex, as well as analyses of sex effects within each treatment. For analyses within each sex, we included ln SVL as a covariate to assess treatment effects on dewlap phenotypes independent of effects on size. We also conducted PCA analyses to compare multivariate sex and treatment effects in reduced phenotypic space (Supplemental Materials).

To test whether testosterone shapes **G**, we conducted pairwise matrix comparisons between all experimental groups using random skewers (Cheverud 1996; Cheverud and Marroig 2007). We generated 10,000 random skewers by drawing each gradient in each vector from a normal distribution with a mean of 0 and standard deviation of 1 (Marroig et al. 2011), then standardizing each vector to a norm of 1. These vectors represent **b** in Eqn. 2 (Supplemental Material). We multiplied each skewer by each **G** matrix to derive 10,000 vectors of evolutionary response for each matrix ($\boldsymbol{\Delta \bar{z}}$ in Eqn. 2), then calculated the mean correlation between response vectors as an estimate of similarity between any two matrices. If testosterone structures **G**, the matrix of control females should exhibit low correlations with those of all other groups, and treatment of

females with testosterone should produce a matrix more highly correlated with those of males. In the hypothetical absence of sex differences and treatment effects, the null hypothesis is that each estimate of **G** should be identical ($r = 1$) aside from sampling error. To test for sex and treatment effects while incorporating error in **G**, we simulated a sampling distribution for each matrix using the REML-MVN method in WOMBAT (Meyer and Houle 2013; Houle and Meyer 2015), with 10,000 samples per matrix. We used random skewers to produce a null distribution of 10,000 mean vector correlations between our best estimate of **G** from each group and each of the 10,000 simulated matrices in its own sampling distribution. This null distribution describes how correlated each matrix is expected to be with itself, given sampling error. We then asked whether the best estimate of **G** from each of the other experimental groups produced a mean vector correlation that fell below the lower 5% bound of this null distribution when compared to the best estimate of **G** from the reference group. We compared correlation matrices using modified versions of the Mantel test and the T method (see Supplemental Materials).

To test whether natural sex differences in testosterone contribute to the breakdown of between-sex genetic correlations, we estimated full **G** matrices (including **B**) for (1) control females and control males, (2) testosterone females and control males, and (3) testosterone females and testosterone males. We then converted the five diagonal elements in **B** to genetic correlations (r_{MF}) and used paired (by trait) *t*-tests to assess whether these correlations are weaker in the correlation matrix for control females and control males than in either of the matrices including testosterone females. To incorporate uncertainty in matrix estimation, we also obtained r_{MF} values for each of the simulations

in the REML-MVN error distribution for each matrix and calculated the mean difference in r_{MF} values (paired by trait) between 10,000 pairs of matrices from each distribution. We then tested whether the lower 5% bound of this distribution fell above zero when subtracting r_{MF} values in the control female and control male matrix from r_{MF} values in either of the matrices including testosterone females.

To test whether natural sex differences in testosterone structure both **G** and **B** in ways that could potentially influence the evolution of sexual dimorphism, we compared the full **G** matrices (including $\mathbf{G_F}$, $\mathbf{G_M}$ and **B**) using sexually antagonistic skewers (Cox et al. 2017a). In this modification of random skewers, the magnitude of each selection gradient is drawn from a normal distribution and vectors are standardized to a norm of 1, but gradients for each homologous trait are constrained to be opposite in sign between sexes. We passed 10,000 sexually antagonistic skewers through each matrix and calculated the mean vector correlations between response vectors of (1) control females and control males, (2) testosterone females and control males, and (3) testosterone females and testosterone males. We compared these mean vector correlations to null distributions created by applying the same sexually antagonistic skewers to each of the 10,000 simulated matrices in the REML-MVN distribution for each matrix. Our *a priori* prediction was that natural sex differences in testosterone shape **G** and **B** in ways that should facilitate the evolution of sexual dimorphism, such that the mean vector correlation between responses of testosterone females and either control males or testosterone males should be higher than that between control females and control males. Therefore, we tested whether the mean vector correlation for testosterone females and either male group fell above the upper 5% bound of the simulated distribution for control

females and control males, and whether the mean vector correlation for control females and control males fell below the lower 5% bound of the simulated distribution for testosterone females and either male group.

Results and Discussion

Sexual Dimorphism and Phenotypic Effects of Testosterone

At 3 months of age (pre-treatment), sex differences were absent for dewlap hue, minor for dewlap brightness, and pronounced for SVL, dewlap area, and dewlap saturation (Table S2). There was no initial difference in any phenotype with respect to the treatments that were subsequently assigned (Table S2). By 8 months of age (post-treatment), control females and males had diverged substantially in all phenotypes, but sexual dimorphism was reduced (for SVL, dewlap size, and dewlap saturation) or absent (for dewlap hue and dewlap brightness) between testosterone females and males (Fig. 1, Table S3). Treatment of females with testosterone increased SVL, dewlap area, dewlap saturation, and dewlap hue while decreasing dewlap brightness (Fig. 1; Table S4). PC1 explained 49% of phenotypic variance and clearly separated control females from both male groups, with testosterone females intermediate (Fig. S2). These sex differences and treatment effects are broadly consistent with previous studies (Cox et al. 2015; 2016; 2017a,b) and confirm that subsequent comparisons of **G** and **B** involve a suite of traits that were sexually dimorphic and responsive to testosterone.

Testosterone Structures Genetic Covariance

In each experimental group, the full **G** matrix was preferred over simpler models excluding additive genetic (co)variance (Table S5). Random skewers analyses revealed

that testosterone shifted **G** of females toward an architecture similar to that of males (Fig. 2; Table S10). The mean vector correlation between evolutionary responses was low for control females when compared to control males ($r = 0.67$), testosterone males ($r = 0.64$), and testosterone females ($r = 0.63$). All three correlations fell outside the lower 5% bounds of the error distributions for each individual matrix (Fig. 2). By contrast, the mean vector correlation was high between testosterone females and either control males ($r = 0.85$) or testosterone males ($r = 0.86$), similar to the expectedly high correlation between control and testosterone males ($r = 0.83$). None of these three correlations fell outside the lower 5% bounds of the matrices being compared (Fig. 2). The same patterns of statistical separation were observed when using random skewers to compare unstandardized **G** matrices and when using several additional methods to compare genetic correlation matrices (Table S10). Therefore, elevating testosterone in females significantly altered **G**, producing a matrix that was statistically indistinguishable from that of males. Presumably, this occurred because some patterns of genetic variance and covariance that are naturally present in females were masked by the overriding "environmental" effect of elevated testosterone, whereas other patterns that are naturally "cryptic" in females were revealed via activation of underlying genes by testosterone. Consistent with this second idea, additive genetic variance for SVL increases as male anoles mature, coincident with the transcriptional activation of growth-regulatory gene networks that can also be induced experimentally by treating females with testosterone (Cox et al. 2017b).

Testosterone Structures Between-sex Genetic Covariance

The inclusion of **B** significantly improved estimation of **G** for testosterone females in combination with either control males or testosterone males, but not for the combination of control females and control males (Table S12), suggesting that the elevation of testosterone in females restores underlying between-sex genetic covariance that is naturally reduced. Only one estimate of r_{MF} for homologous traits was statistically greater than zero when estimated between control females and males (dewlap hue; Fig. 3A; Table S13), consistent with previous estimates of r_{MF} for adult anoles (Cox et al. 2017a,b). By contrast, all estimates of r_{MF} were significantly greater than zero when estimated between testosterone females and control males (Fig. 3A; Table S13), and four of five were significant between testosterone females and testosterone males (Fig. S5; Table S13). The mean strength of r_{MF} was significantly lower between control females and control males than between testosterone females and control males (paired $t = 3.13$, df = 4, one-tailed $P = 0.018$; Fig. 3A) or between testosterone females and testosterone males ($t = 3.20$, df = 4, $P = 0.016$; Fig. S5). The mean difference in r_{MF} was also significantly greater than zero when comparing matrices from the simulated REML-MVN distribution for control females and control males to matrices from either of the distributions involving testosterone females (Table S15).

High values of r_{MF} are thought to be the primary impediment to the evolution of sexual dimorphism (Lande 1980; Poissant et al. 2010). Our results support the prediction that r_{MF} can be reduced by the divergent hormonal environments in which genes are expressed in females versus males (Cox et al. 2016; Cox 2020). To our knowledge, this is the first direct experimental demonstration of an idea that traces back to Fisher (1958), but has only recently been incorporated into theory on the evolution of r_{MF} and its

implications for intralocus sexual conflict (Badyaev 2002; Poissant and Coltman 2009; Cox et al. 2017b). Corroborating lines of evidence include the tendency for r_{MF} to decrease as ontogeny progresses (Poissant and Coltman 2009; Cox et al. 2017b), and the pleiotropic effects of testosterone on organismal phenotypes and underlying patterns of gene expression (Peterson et al. 2014; Mank 2017; Cox 2020).

The mean vector correlation between male and female responses to sexually antagonistic skewers was low for the natural **G** (including **B**) matrix estimated for control females and control males ($r = 0.47$), and substantially higher when estimated between testosterone females and control males ($r = 0.79$; Fig. S4). Each of these values falls outside of the simulated distribution for the other matrix (Fig. 3B-C; Table S16), and the responses of testosterone females and testosterone males to sexually antagonistic selection were also more strongly correlated than those of control males and control females (Fig. S5; Table S16), indicating that the addition of testosterone to females produced a full **G** matrix that is significantly less permissive of sex-specific evolution under simulated sexually antagonistic selection, relative to the full **G** matrix in control animals. This agrees with a previous conclusion that the natural **B** matrix for dewlap traits is unlikely to impose a strong constraint on the short-term evolution of sexual dimorphism (Cox et al. 2017a), and extends this conclusion by implying that natural sex differences in testosterone levels directly facilitate this weakening of between-sex genetic constraint.

Synthesis and Implications

Hormonal pleiotropy is well-documented in this system and many others, but our study is the first to show that it structures the underlying patterns of genetic variance and

covariance that shape how populations evolve in response to selection. Although this phenomenon is presumably ubiquitous, it has been largely neglected by endocrinologists and evolutionary biologists alike (Poissant and Coltman 2009; Cox et al. 2016; Cox 2020). Testosterone has often been implicated as an agent of phenotypic integration (McGlothlin and Ketterson 2008; Ketterson et al. 2009; Cox et al. 2016). We extend this framework by showing that testosterone specifically alters the additive genetic components of phenotypic variance and covariance. This implies that the experimental elevation of testosterone (which has no genetic component, unlike natural variation in testosterone levels; see Cox et al. 2016) influences phenotypic expression in ways that are dependent upon underlying genetic differences among individuals. Such differences could reflect genetic variation in (1) binding proteins that mediate the availability of free testosterone, (2) cell- or tissue-specific expression of androgen receptors and cofactors necessary for initiation of transcription, (3) nucleotide motifs for androgen response elements and associated regulatory regions of androgen-responsive target genes, and (4) coding and regulatory regions of other genes and networks underlying focal phenotypes that are located downstream of genes directly responsive to testosterone (see Cox 2020 for a review). Our results imply that the extent to which these various aspects of genetic variance and covariance are available for selection will often depend upon the endocrine backgrounds in which they occur.

The internal hormonal milieu of an individual comprises the physiological environment in which its genome is translated into phenotypes. As such, our individual-level comparison of testosterone and control groups is conceptually similar to population-level comparisons of **G** between different environments. Two synthetic conclusions from

such studies are that environmentally induced differences in **G** are often as pronounced as those accumulated over thousands of generations of evolutionary divergence, and that evolutionary responses to selection will often differ dramatically across environments (Wood and Brodie 2015). Similarly, the "hormonal environment" in which a genome is translated into phenotypes should, by virtue of its effects on **G**, influence short-term evolutionary trajectories. We may often overlook this feature because the hormonal environment is both highly plastic and a property of the individual, whereas **G** is a property of the population. While this is true, there are important instances in which hormonal environments differ predictably and dramatically, either at the population level or within subsets of a population. Testosterone provides a canonical example, varying with factors such as sex, age, and season.

We have shown that distinct patterns of **G** in females and males are partly due to sex differences in circulating testosterone. Likewise, although males and females share an autosomal genome, sex differences in testosterone levels can break down between-sex genetic covariance and thereby facilitate separate evolutionary responses to sexually antagonistic selection. This evolutionary breakdown does not require upstream genetic change in testosterone production or androgen receptor expression, although such changes could contribute. It simply requires that shared autosomal genes that harbor genetic variance for phenotypes under sexually antagonistic selection become directly (e.g., *cis* regulation by androgen response elements) or indirectly (e.g., *trans* regulation by upstream genes that are responsive to testosterone) coupled to a hormone that is already sexually dimorphic. Therefore, a final key implication of our study is that a single signaling molecule, such as testosterone, provides a pleiotropic regulatory mechanism

that can potentially help to alleviate a variety of evolutionary conflicts (e.g., intersexual, ontogenetic) arising from the fundamental constraint of a shared genome that experiences conflicting selection pressures between sexes or across ontogeny.

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Author contributions

RMC and JWM conceived the study, TNW and RMC designed and implemented the experiment, TNW and CDR collected and analyzed the data, TNW and RMC drafted the initial version of the manuscript and all authors contributed to later versions of the manuscript.

Data accessibility

Code is available on GitHub: **https://github.com/ty-wittman/evo_qg_analysis_r_code**

Data are available on Dryad: **https://doi.org/10.5061/dryad.1rn8pk0tf**

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Figures

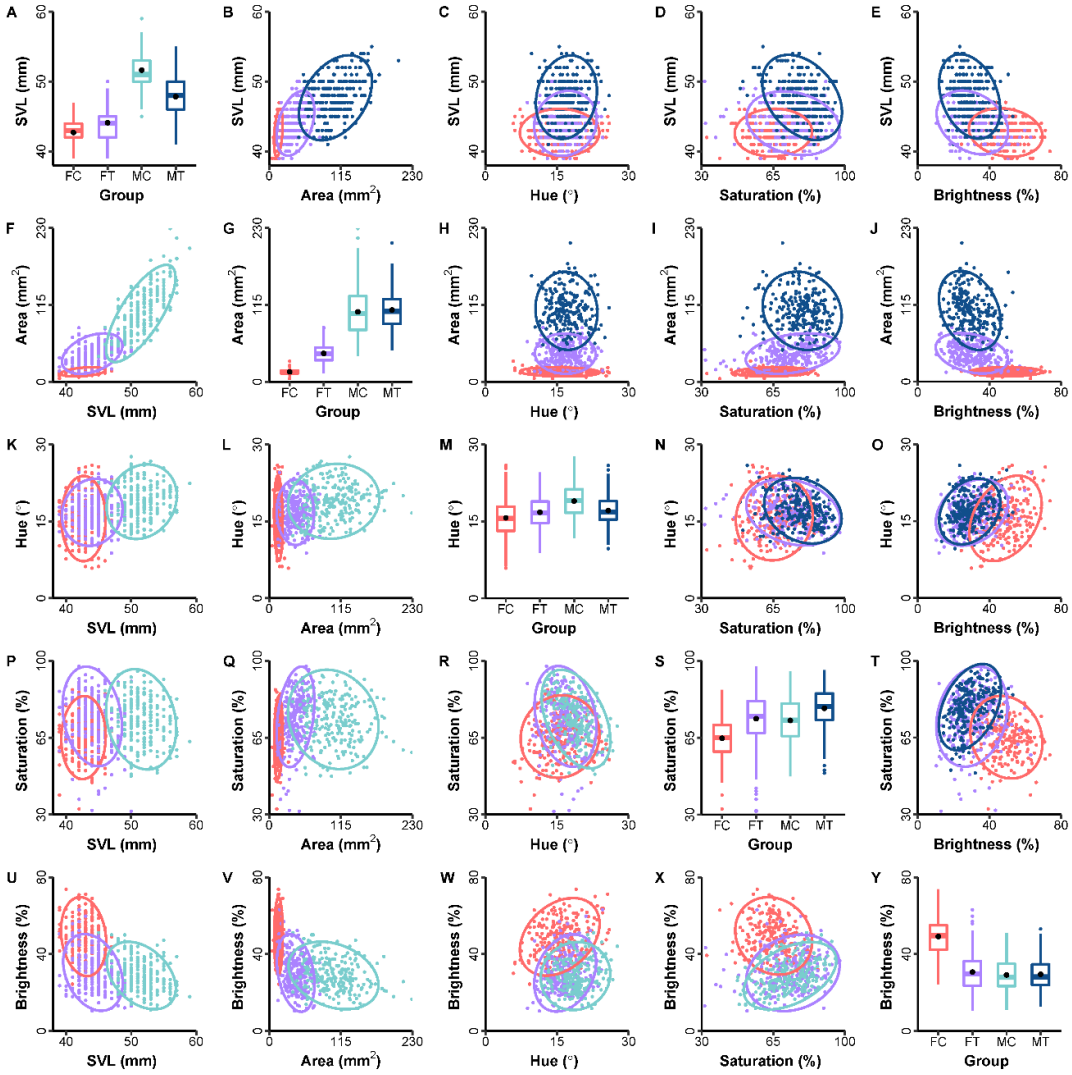

Figure 1 – Effects of sex and testosterone treatment on phenotypic means, variances, and covariances for five traits. Panels on the diagonal show the raw phenotypic distributions (dot = mean, bar = median, box = inter-quartiles, whiskers = 95% CIs) for each of four experimental groups (FC = control female, FT = testosterone female, MC = control male, MT = testosterone male). Panels above or below the diagonal show bivariate relationships between trait pairs with covariance ellipses corresponding to 95% CIs. For ease of visual comparison, control males are only plotted below the diagonal and testosterone males are only plotted above the diagonal.

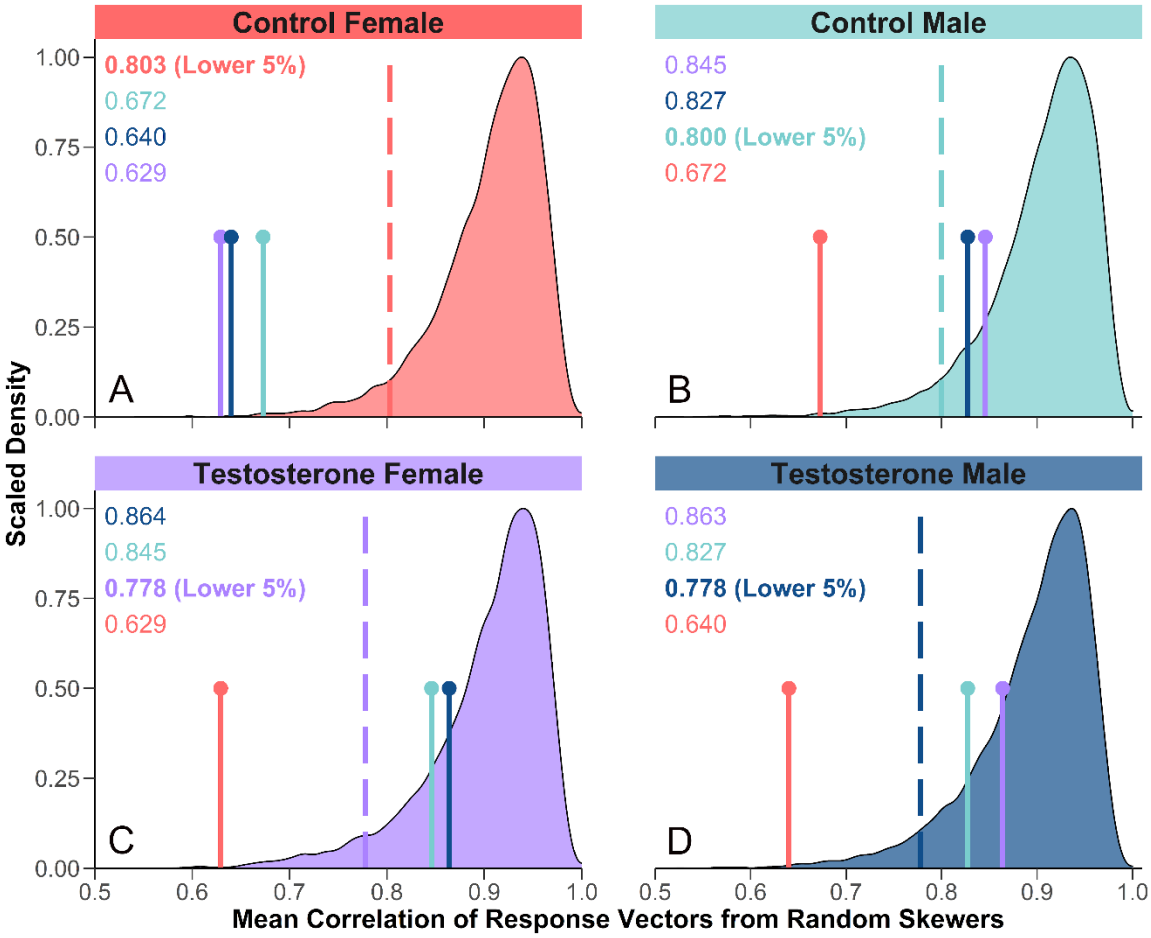

Figure 2 – Comparisons of within-sex **G** matrices across four experimental groups based on predicted evolutionary responses to random skewers. The null distribution of mean response vector correlations between the best estimate of **G** for a group and each of the 10,000 simulated matrices from its own sampling distribution is shown separately for (A) control females, (B) control males, (C) testosterone females, and (D) testosterone males. Dashed lines indicate the lower 5% bound of each distribution. Vertical pins indicate mean vector correlations between the best estimate of **G** for each of three comparison groups to that of the reference group whose null distribution is shown in that panel. Note that each vector correlation is plotted on two panels to facilitate comparison to each of the corresponding null distributions.

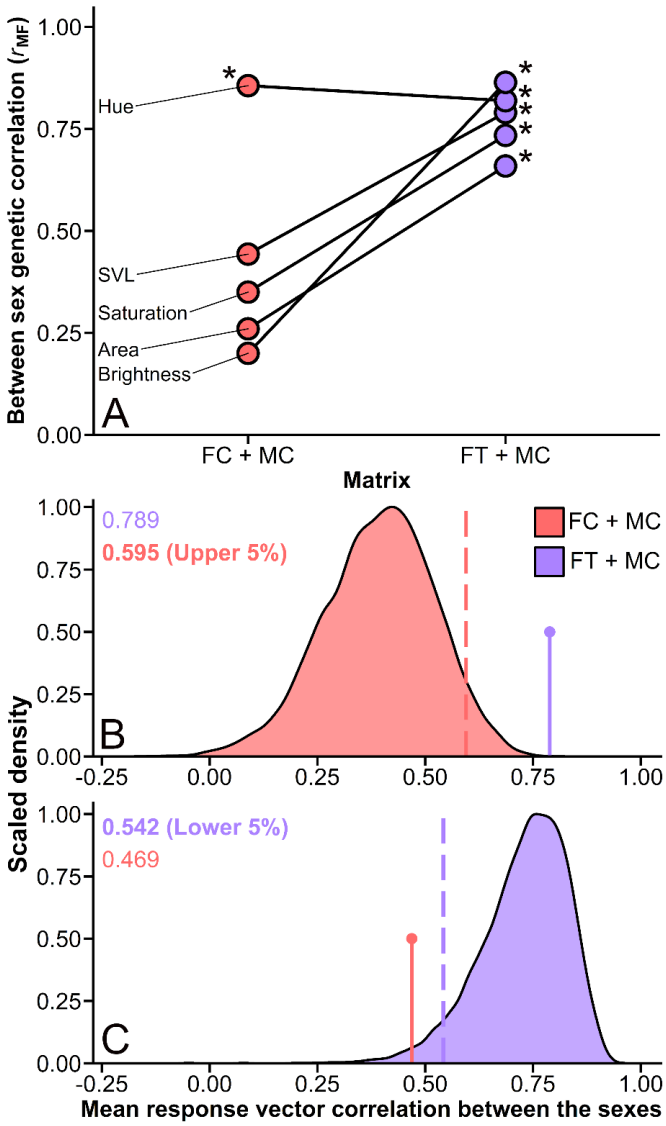

Figure 3 – (A) Point estimates of r_{MF} between five homologous traits for control females and control males (FC + MC), connected to the same r_{MF} values for testosterone females and control males (FT + MC). Asterisks indicate estimates significantly greater than zero. (B) Distribution of 10,000 mean vector correlations between female and male responses to sexually antagonistic skewers based on the simulated distribution of the full **G** matrix (including **B**) for control females and control males. The upper 5% bound of this null distribution is shown with a dashed line. The mean vector correlation between female and

male responses using the best estimate of the full **G** matrix (including **B**) for testosterone females and control males is shown with a pin and falls above the upper 5% bound. (C) The reciprocal comparison to that shown in panel B, with the mean vector correlation for control females and control males falling below the lower 5% bound of the simulated distribution for testosterone females and control males.

Supplementary Methods

The Multivariate Breeder's Equation (*Equations 1-3*)

Interest in **G** stems largely from its use in the multivariate breeder's equation (Lande 1979; Lande and Arnold 1983):

$$\boldsymbol{\Delta\bar{z}} = \mathbf{GP^{-1}S} \quad \text{or} \quad \begin{bmatrix}\Delta\bar{z}_1\\ \Delta\bar{z}_2\\ \Delta\bar{z}_3\end{bmatrix} = \begin{bmatrix}G_{11} & G_{12} & G_{13}\\ G_{12} & G_{22} & G_{23}\\ G_{13} & G_{23} & G_{33}\end{bmatrix}\begin{bmatrix}P_{11} & P_{12} & P_{13}\\ P_{12} & P_{22} & P_{23}\\ P_{13} & P_{23} & P_{33}\end{bmatrix}^{\mathbf{-1}}\begin{bmatrix}S_1\\ S_2\\ S_3\end{bmatrix} \qquad (Eqn.\ 1)$$

Here, $\boldsymbol{\Delta\bar{z}}$ is a vector describing the between-generation evolutionary change in the means of three traits (z_1, z_2, z_3) in response to a vector, **S**, of selection differentials (S_1, S_2, S_3) describing the within-generation change in trait means. S includes direct selection on a trait and indirect selection on other phenotypically correlated traits. The evolutionary response therefore depends on the **P** matrix containing phenotypic variances for each trait (P_{11}, P_{22}, P_{33}) and covariances between traits (P_{12}, P_{13}, P_{23}), and on the **G** matrix containing their underlying additive genetic variances (G_{11}, G_{22}, G_{33}) and covariances (G_{12}, G_{13}, G_{23}). When selection is expressed using multivariate selection gradients ($\beta_1, \beta_2, \beta_3$) that account for the covariance structure in **P** to reflect only the direct component of selection, the vector $\boldsymbol{\beta}$ can be substituted for $\mathbf{P^{-1}S}$:

$$\boldsymbol{\Delta\bar{z}} = \mathbf{G\beta} \quad \text{or} \quad \begin{bmatrix}\Delta\bar{z}_1\\ \Delta\bar{z}_2\\ \Delta\bar{z}_3\end{bmatrix} = \begin{bmatrix}G_{11} & G_{12} & G_{13}\\ G_{12} & G_{22} & G_{23}\\ G_{13} & G_{23} & G_{33}\end{bmatrix}\begin{bmatrix}\beta_1\\ \beta_2\\ \beta_3\end{bmatrix} \qquad (Eqn.\ 2)$$

To facilitate the study of sexual dimorphism, Lande (1980) modified the multivariate breeder's equation to separate selection, genetic architecture, and evolutionary response by sex:

$$\begin{bmatrix}\boldsymbol{\Delta\bar{z}_M}\\ \boldsymbol{\Delta\bar{z}_F}\end{bmatrix} = \frac{1}{2}\begin{bmatrix}\mathbf{G_M} & \mathbf{B}\\ \mathbf{B^T} & \mathbf{G_F}\end{bmatrix}\begin{bmatrix}\boldsymbol{\beta_M}\\ \boldsymbol{\beta_F}\end{bmatrix} \; or \; \begin{bmatrix}\Delta\bar{z}_{1M}\\ \Delta\bar{z}_{2M}\\ \Delta\bar{z}_{3M}\\ \Delta\bar{z}_{1F}\\ \Delta\bar{z}_{2F}\\ \Delta\bar{z}_{3F}\end{bmatrix} = \frac{1}{2}\begin{bmatrix}G_{11} & G_{12} & G_{13} & B_{11} & B_{12} & B_{13}\\ G_{12} & G_{22} & G_{23} & B_{21} & B_{22} & B_{23}\\ G_{13} & G_{23} & G_{33} & B_{31} & B_{32} & B_{33}\\ B_{11} & B_{21} & B_{31} & G_{11} & G_{12} & G_{13}\\ B_{12} & B_{22} & B_{32} & G_{12} & G_{22} & G_{23}\\ B_{13} & B_{23} & B_{33} & G_{13} & G_{23} & G_{33}\end{bmatrix}\begin{bmatrix}\beta_{1M}\\ \beta_{2M}\\ \beta_{3M}\\ \beta_{1F}\\ \beta_{2F}\\ \beta_{3F}\end{bmatrix} (Eqn.\ 3)$$

This example also involves three traits, but $\boldsymbol{\Delta\bar{z}}$ and **β** have been separated by sex and **G** has been expanded to include two sub-matrices describing genetic variances and covariances within each sex ($\mathbf{G_M}$, $\mathbf{G_F}$) as well as the between-sex matrix **B** and its transpose $\mathbf{B^T}$. The diagonal elements in **B** are between-sex genetic covariances for single traits (B_{11}, B_{22}, B_{33}), whereas the off-diagonal elements are between-sex genetic covariances for different traits ($B_{12}, B_{13}, B_{23}, B_{21}, B_{31}, B_{32}$). Whereas the covariances above and below the diagonal in **G** are identical, **B** is not necessarily symmetrical in the same way because the covariance between z_1 in males and z_2 in females (B_{12}) is not necessarily the same as that between z_1 in females and z_2 in males (B_{21}).

The random skewers and sexually antagonistic skewers analyses that we use to test for matrix similarity (see main text) are based on the equations above. Comparison of **G** using random skewers is achieved by randomly drawing selection gradients to produce 10,000 random vectors representing **β**, multiplying each of these 10,000 "random skewers" by an estimated or simulated **G** matrix, recording the resulting 10,000

evolutionary response vectors $\boldsymbol{\Delta\bar{z}}$, then estimating the mean vector correlation between response vectors of the two **G** matrices being compared. Sexually antagonistic skewers are created in similar fashion, with the constraint that selection on each individual trait is constrained to be opposite in sign between the sexes (i.e., $\beta_{1\mathrm{F}}$ is opposite in sign from $\beta_{1\mathrm{M}}$). For analysis, 10,000 sexually antagonistic skewers are passed through the full **G** matrix (including **B** and $\mathbf{B}^{\mathrm{T}}$) and evolutionary responses are recorded separately for $\boldsymbol{\Delta\bar{z}_{\mathbf{F}}}$ and $\boldsymbol{\Delta\bar{z}_{\mathbf{M}}}$, such that the mean vector correlation between male and female response vectors can be calculated. For any given **G** matrix, this mean vector correlation describes the extent to which males and females are constrained to exhibit a similar evolutionary response to selection that inherently favors sexual dimorphism in each trait. When two **G** matrices are compared, the matrix with the larger positive value of this mean vector correlation is inferred to impose relatively greater constraint on the evolution of sexual dimorphism.

Animal Husbandry and Breeding

We housed adults individually in plastic cages (30 cm x 20 cm x 20 cm; Lee's Kritter Keeper, San Marcos, CA, USA) with a PVC pipe for perching and hiding, a strip of fiberglass mesh for basking, and a piece of outdoor carpet as substrate. We placed each cage beneath two ReptiSun 10.0 UVB bulbs (ZooMed, San Luis Obispo, CA, USA) and maintained animals on a 13L:11D photoperiod with constant 65% relative humidity and ambient temperature of 29°C during the day and 25°C at night. We misted each cage daily with deionized water. Three times per week, we fed each adult 3-5 crickets (*Gryllodes sigillatus*, 1/2" size for adult males, 3/8" size for adult females, Ghann's Cricket Farm, Augusta, GA, USA). We dusted the crickets twice weekly with a calcium

supplement (Fluker's Repta Calcium with D_3, Fluker Farms, Port Allen, LA, USA), and once weekly with a vitamin supplement (Fluker's Reptile Vitamin).

For breeding, we placed one male and one female together in larger cages (40 cm x 23 cm x 32 cm; Lee's Kritter Keeper, San Marcos, CA, USA) that contained two screen hammocks and perches, as well as a small cup of potting soil in which females could oviposit. After two weeks, we separated sires and dams and placed the cup of soil in the female's cage. After another two weeks, we repeated this breeding protocol with the same sires and new dams to generate paternal half-sibling families. We checked each female's cup of soil for new eggs once weekly. Anoles lay one egg at a time and typically produce an egg every 7-10 days in captivity. We assigned a unique ID to each new egg, recorded its mass, then placed it into an individual plastic container filled with moist vermiculate (1g:1g ratio vermiculite to distilled water) and covered with a transparent, perforated lid to maintain moisture while permitting gas exchange. We incubated containers at constant 28°C and 80% relative humidity with a 12L:12D light cycle in a Percival Intellus 136VL. We checked each container daily for new hatchlings, which were immediately sexed and measured for mass and snout-vent length, then housed individually in small cages identical to those described for adults (above). We fed each hatchling 3-5 crickets of 1/8" size 3 times per week until they were 3 months old, after which we fed them 3-5 crickets of 3/8" size 3 times per week, with no difference in diet between sexes. New animals hatched over a 10-month period between August 2017 and June 2018. At 3 months of age, each animal was assigned to a treatment group (see main text), then received either a testosterone implant or an empty implant as a control (see main text).

Principal Component Analysis of Phenotypes

To holistically assess sex and treatment effects on phenotypes at 8 months of age (5 months post-treatment), we used PCA to visualize multivariate treatment differences in reduced phenotypic space (Fig. S2). The first principal component (PC1) explained 49% of phenotypic variance, PC2 explained 22.5%, PC3 explained 17.3%, PC4 explained 8.2%, and PC5 explained the remaining 3% of phenotypic variance. PC1 was defined primarily by positive loadings for size variables (ln SVL and ln dewlap area) and negative loadings for dewlap brightness (Fig. S2). PC2 was defined primarily by positive loadings for dewlap saturation and negative loadings for dewlap hue (Fig. S2). We used ANOVA to test the extent to which main effects of sex, treatment, and their interaction explained individual variation along PC1 and PC2. For PC1, there was a significant effect of sex ($F_{1,934} = 3499.3$, $P < 0.001$), treatment ($F_{1,934} = 295.8$, $P < 0.001$), and their interaction ($F_{1,934} = 784.4$, $P < 0.001$). Males had higher PC1 scores than females (i.e., males were larger and had larger and less bright dewlaps than females) and testosterone strongly increased PC1 scores in females (i.e., testosterone increased SVL and dewlap area while decreasing dewlap brightness, Fig. S2). For PC2, there was no effect of sex ($F_{1,934} = 1.94$, $P = 0.164$), but there was a significant treatment effect ($F_{1,934} = 114.2$, $P < 0.001$) and a weak interaction between sex and treatment ($F_{1,934} = 6.537$, $P = 0.011$). Testosterone increased PC2 scores, meaning that testosterone increased dewlap saturation and decreased dewlap hue relative to control animals (Fig. S2).

Animal Model and Estimation of **G**

We used linear mixed effect models (i.e., animal models) to estimate additive genetic variances and covariances. The animal model incorporates complex pedigree

structures, fits both fixed and additional random effects (e.g., hatch month in our analyses), and provides estimates of additive genetic variance and covariance that are unbiased for unbalanced data (Kruuk 2004). The general formula for the univariate animal model as applied in our study is:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1 a} + \mathbf{Z_2 h} + \mathbf{e} \qquad (Eqn. 4)$$

Here, **y** is a vector of individual trait values with a length equal to the number of individuals, **X** is a design matrix linking individual observations to fixed effects, **b** is a vector of fixed effects (in our case, this only includes the population mean trait value), $\mathbf{Z_1}$ is a design matrix linking individual observations to the random additive genetic effect (all individuals with observations are included in the pedigree, so $\mathbf{Z_1}$ is an identity matrix), **a** is a vector of random additive genetic effects (composed of additive genetic variance and the numerator relationship matrix), **W** is a design matrix linking individual observations to the random effect of hatch month, **h** is a vector containing the random effect of hatch month, and **e** is the vector of residual error. For multivariate estimates of **G** within each sex or treatment, we extended eqn. 4 to include five traits. For estimates of the full **G**, including **B**, we modeled homologous traits in males and females as separate traits (as in eqn. 3), creating a 10-trait matrix (Wolak et al. 2015).

Likelihood Ratio Tests for Significant Genetic (co)variance

To test for significant additive genetic variance and covariance, we used likelihood ratio tests to compare our estimates of the full **G** matrix for each experimental group with simpler models setting covariances to zero or excluding additive genetic effects entirely (Table S5). The test statistic was calculated as twice the difference in log

likelihood between models and tested against a chi-squared distribution. For each experimental group, the full **G** was preferred over simpler models, confirming the presence of significant additive genetic variance and covariance (Table S5). We used a similar approach to test for significant between-sex genetic covariance for combinations of male and female groups by comparing estimates of the full **G** (including **B**) with simpler models setting the elements in **B** to zero (Table S12). In the matrix estimated for control females and control males, the inclusion of **B** did not significantly improve the model, indicating weak between-sex genetic covariance in this "natural" condition. However, models including **B** were preferred over simpler models without **B** in matrices estimated for testosterone females and either control males or testosterone males (Table S12), indicating that between-sex genetic covariance is only significant when both sexes have elevated testosterone.

*Comparison of Phenotypic Covariance Matrices (**P**)*

The comparisons presented in the main text focus on **G** and **B**, which contain additive genetic variances and covariances. Hormonal pleiotropy is also predicted to structure **P**, the matrix of phenotypic variances and covariances (McGlothlin and Ketterson 2008; Ketterson et al. 2009; Cox et al. 2016; Cox 2020), potentially by altering non-additive or non-genetic components of phenotypic variance and covariance that are not captured by **G**. Therefore, we also used WOMBAT (Meyer 2007) to estimate **P** for each experimental group, then compared each pair of **P** matrices using random skewers as described for comparison of **G** (see main text), with the caveat that vectors derived from **P** do not correspond to "evolutionary response" per se. Because **P** is estimated with less error than **G**, null distributions for each matrix are relatively narrow, such that **P** was

statistically distinct between all groups except control and testosterone males and testosterone females and testosterone males (Fig. S3). Mean vector correlations were quantitatively higher between testosterone females and either male group ($0.97 < r < 0.98$) than between control females and either male group ($0.88 < r < 0.92$; Fig. S3; Table S10, section A).

Comparison of Unstandardized and Variance-Standardized **G** *Matrices*

The comparisons presented in the main text were conducted after variance-standardizing **G** by dividing each element in the matrix by its corresponding phenotypic variance (Table S6). This method prevents differences in the units in which traits are measured from predisposing some traits to contribute disproportionately to the size of **G** and the associated evolutionary response. The variance-standardized **G** can be used in the multivariate breeder's equation (Eqns. 2-3) when the selection gradients forming the vector $\boldsymbol{\beta}$ are estimated using traits that have been re-scaled to mean of zero and unit variance. In this formulation, the response to selection, $\boldsymbol{\Delta \bar{z}}$, is in units of phenotypic standard deviations. As a complementary approach, we repeated all of the same matrix comparisons using unstandardized estimates of **G** (Table S7). These two approaches are compared in Tables S10 and S16. Results are qualitatively similar using either approach.

Comparison of Correlation Matrices

The analyses presented above and in the main text focus on matrices whose elements are phenotypic or genetic variances (on the diagonal) or covariances (off the diagonal), as illustrated in Eqns. 1-3. We also estimated phenotypic and genetic correlation matrices, which replace covariances with correlations (covariances divided by

geometric mean variances). To compare phenotypic (Table S9) and genetic (Table S8) correlation matrices estimated within each group, we excluded diagonal elements in **P** and **G** and analyzed matrices comprised of the 10 unique correlations between 5 traits. We used two methods to compare correlation matrices.

First, we calculated the Mantel correlation between each pair of matrices (the Pearson product-moment correlation between the 10 unique correlations in each matrix) using R 3.5.3 code available on github (see Data Accessibility, main manuscript). We compared these Mantel correlations to null distributions of Mantel correlations between the best estimate of each correlation matrix and each of the 10,000 simulated matrices in the REML-MVN distribution for that experimental group, after first transforming these simulated **P** or **G** matrices into correlation matrices using the cov2cor function in R 3.5.3 (R Core Team 2019). Our method is similar to that of Goodnight and Schwartz (1997) but uses the REML-MVN method instead of their bootstrapping method to derive a null distribution. We tested whether the Mantel correlations between the best estimates of any two matrices fell below the lower 5% bound of the null distribution for either matrix. Mantel comparisons between genetic correlation matrices are reported in Table S10, those for phenotypic correlation matrices are in Table S11.

Second, we used a modified version of the T method (Roff et al. 2012) to ask whether the mean absolute value of the difference between individual elements in two matrices was greater than expected from sampling error. As with the Mantel method described above, we created a null distribution for each experimental group by calculating the mean absolute value of the differences in each element of the matrix between the best estimate of a matrix and each of the 10,000 matrices in its REML-MVN

sampling distribution. We then tested whether the mean absolute difference in elements between the best estimates of any two matrices fell below the lower 5% bound of the null distribution for either matrix. These “modified T method” comparisons between genetic correlation matrices are reported in Table S10, those for phenotypic correlation matrices are in Table S11.

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Supplemental Tables

Table S1. Summary of sample sizes for estimation of **P**, **G** and **B**. An initial sample of 60 sires and 120 dams were paired in a paternal half-sibling design. Because some pairs did not produce offspring, a total of 938 individual progeny were included in the final experiment, representing 57 sires and 104 dams. Values in the table report the mean, minimum, and maximum number of half siblings and full siblings within each experimental group.

					Half-sib family size			**Full-sib family size**		
Sex	**Treatment**	**Individuals**	**Sires**	**Dams**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**
Female	Control	224	55	92	4.00	1	10	2.43	1	8
	Testosterone	243	57	94	4.26	1	10	2.58	1	6
Male	Control	233	52	89	4.48	1	9	2.61	1	6
	Testosterone	238	56	96	4.25	1	10	2.47	1	6
Both	**Both**	938	57	104	16.46	2	34	9.01	1	22

Table S2. Phenotypic effects of sex (male, female) and treatment (control, testosterone) at two time points. Treatment is included in "Pre-treat (3 mo)" models to confirm the absence of phenotypic differences prior to manipulation. Interactions between sex and treatment were never significant in pre-treatment analyses and are therefore not shown in the table. Hatch month is included as a random effect to account for overall variation across the 10-month study. All models also included random effects of sire and dam (nested within sire), which are not shown here. Interactions between sex and ln SVL were tested in all models but only retained when significant. Ln SVL is not included in "post-treat (8 mo)" analyses of dewlap traits because males and females did not overlap in this size covariate.

Time point (age)		**Sex**		**Treatment**		**Ln SVL**		**Sex*Ln SVL**	
Phenotype	***N***	χ^2	***P***	χ^2	***P***	χ^2	***P***	χ^2	***P***
Pre-treat (3 mo)									
Ln SVL	289	23.82	< 0.001	0.75	0.386	—	—	—	—
Ln area	289	222.72	< 0.001	0.70	0.402	144.38	< 0.001	6.08	0.014
Hue	289	0.78	0.376	0.32	0.572	3.80	0.051	—	—
Saturation	289	50.93	< 0.001	0.25	0.615	1.28	0.257	—	—
Brightness	289	7.53	0.006	0.11	0.734	1.27	0.259	6.96	0.008
		Sex		**Treatment**		**Sex*Treatment**		**Sex*Ln SVL**	
		c^2	***P***	c^2	***P***	c^2	***P***	c^2	***P***
Post-treat (8 mo)									
Ln SVL	938	2128.30	< 0.001	73.11	< 0.001	348.16	< 0.001	—	—
Ln area	938	5599.27	< 0.001	810.01	< 0.001	665.82	< 0.001	—	—
Hue	938	83.05	< 0.001	4.71	0.029	58.82	< 0.001	—	—
Saturation	938	119.52	< 0.001	156.51	< 0.001	12.61	0.004	—	—
Brightness	938	361.39	< 0.001	294.69	< 0.001	306.84	< 0.001	—	—

Table S3. Phenotypic effects of sex (female, male), analysed separately for each treatment. Hatch month was included as a random effect to account for overall variation across the 10-month study. All models also include random effects of sire and dam (nested within sire), which are not shown. Sex differences were pronounced in control treatments but reduced or even eliminated when both sexes received testosterone treatments, particularly for dewlap traits (compare magnitude of c^2 statistics between Control and Testosterone groups).

Treatment		**Sex difference**	
Phenotype	***N***	χ^2	***P***
Control			
Ln SVL	457	2644.60	< 0.001
Ln area	457	6016.00	< 0.001
Hue	457	111.37	< 0.001
Saturation	457	97.27	< 0.001
Brightness	457	694.85	< 0.001
Testosterone			
Ln SVL	481	1166.60	< 0.001
Ln area	481	1221.30	< 0.001
Hue	481	2.98	0.080
Saturation	481	29.97	< 0.001
Brightness	481	2.91	0.080

Table S4. Phenotypic effects of hormone treatment (control, testosterone), analysed separately for each sex. Hatch month is included as a random effect to account for overall variation across the 10-month study. All models also included random effects of sire and dam (nested within sire), which are not shown. Ln SVL is included in analyses of dewlap traits, such that treatment differences are standardized for treatment effects on body size. Testosterone treatment influenced all phenotypes in both sexes. Treatment effects were similar when using raw phenotypic values for SVL and dewlap area without ln transformation.

Sex		**Treatment**		**Ln SVL**		**Treatment*SVL**	
Phenotype	***N***	χ^2	***P***	χ^2	***P***	χ^2	***P***
Female							
Ln SVL	467	66.04	< 0.001	—	—	—	—
Ln area	467	1390.54	< 0.001	94.25	< 0.001	0.45	0.500
Hue	467	9.68	0.002	1.75	0.185	0.22	0.636
Saturation	467	122.35	< 0.001	0.05	0.818	4.16	0.041
Brightness	467	403.92	< 0.001	14.13	0.001	0.01	0.984
Male							
Ln SVL	471	318.90	< 0.001	—	—	—	—
Ln area	471	94.83	< 0.001	161.23	< 0.001	54.12	< 0.001
Hue	471	12.18	0.004	14.17	< 0.001	1.91	0.166
Saturation	471	3.93	0.047	31.74	< 0.001	1.05	0.304
Brightness	471	11.40	0.007	29.41	< 0.001	2.52	0.110

Table S5. Summary of model comparisons testing for significant additive genetic variance (V_A) and covariance (Cov_A) in each of the four experimental groups. In each group, models that included V_A and Cov_A (i.e., the full **G** matrix) were preferred over simpler models that set $Cov_A = 0$ or excluded additive effects entirely, thus confirming significant additive genetic variance and covariance in each of the four estimates of **G**. Chi-squared values are calculated as twice the difference in log likelihood between models and are shown for each of the reduced models in comparison to the preferred full **G** model.

Experimental group	Matrix	Log Likelihood	df	χ^2	*P*
Control Female	Full **G** ($V_A + Cov_A$)	−2735.24	—	—	—
	Only V_A ($Cov_A = 0$)	−2768.28	10	66.08	< 0.001
	Null ($V_A = 0$, $Cov_A =$	−3176.31	5	816.07	< 0.001
Testosterone Female	Full **G** ($V_A + Cov_A$)	−3079.18	—	—	—
	Only V_A ($Cov_A = 0$)	−3121.10	10	83.84	< 0.001
	Null ($V_A = 0$, $Cov_A =$	−3283.30	5	324.39	< 0.001
Control Male	Full **G** ($V_A + Cov_A$)	−2849.98	—	—	—
	Only V_A ($Cov_A = 0$)	−2895.20	10	90.44	< 0.001
	Null ($V_A = 0$, $Cov_A =$	−3061.00	5	331.60	< 0.001
Testosterone Male	Full **G** ($V_A + Cov_A$)	−2933.93	—	—	—
	Only V_A ($Cov_A = 0$)	−2988.72	10	109.59	< 0.001
	Null ($V_A = 0$, $Cov_A =$	−3355.04	5	732.64	< 0.001

Table S6. Variance-standardized **G** matrices for each experimental group. Elements on the diagonal are genetic variances (from Table S7) divided by phenotypic variances (from Table S9), which corresponds to narrow-sense heritability (h^2). Elements off the diagonal are genetic covariances divided by mean phenotypic variances (from Tables S7 and S9). Numbers in parentheses are 1 SEM. **Bold** font indicates estimates >2 SEM above or below zero, approximating statistical significance. Note that the magnitudes of the genetic variances (and covariances) are independent of the units in which the phenotypes are measured (compare to Table S7). For this reason, these variance-standardized **G** matrices were used for primary analyses, though unstandardized matrices yielded equivalent results (Table S10).

Variance-standardized **G** matrix: **Control Females**

	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	0.263 (0.136)	0.103 (0.105)	0.144 (0.120)	0.021 (0.058)	0.179 (0.110)
Ln Area	0.103 (0.105)	**0.296 (0.139)**	-0.043 (0.120)	-0.038 (0.061)	0.205 (0.117)
Hue	0.144 (0.120)	-0.043 (0.120)	**0.675 (0.211)**	**0.276 (0.103)**	-0.131 (0.131)
Bright	0.021 (0.058)	-0.038 (0.061)	**0.276 (0.103)**	**0.129 (0.062)**	-0.129 (0.078)
Sat	0.179 (0.110)	0.205 (0.117)	-0.131 (0.131)	-0.129 (0.078)	**0.529 (0.177)**

Variance-standardized **G** matrix: **Control Males**

	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	0.155 (0.079)	0.070 (0.059)	-0.039 (0.085)	-0.062 (0.067)	-0.007 (0.081)
Ln Area	0.070 (0.059)	0.081 (0.062)	-0.129 (0.079)	-0.031 (0.057)	0.058 (0.073)
Hue	-0.039 (0.085)	-0.129 (0.079)	**0.591 (0.184)**	0.064 (0.097)	**-0.311 (0.142)**
Bright	-0.062 (0.068)	-0.031 (0.057)	0.064 (0.097)	**0.238 (0.105)**	**0.225 (0.095)**
Sat	-0.007 (0.081)	0.058 (0.073)	**-0.311 (0.142)**	**0.225 (0.095)**	**0.477 (0.153)**

Variance-standardized **G** matrix: **Testosterone Females**

	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	0.126 (0.077)	0.076 (0.077)	-0.049 (0.096)	-0.107 (0.071)	-0.104 (0.074)
Ln Area	0.076 (0.077)	0.247 (0.127)	-0.145 (0.105)	**-0.242 (0.105)**	-0.033 (0.067)
Hue	-0.049 (0.090)	-0.145 (0.105)	**0.601 (0.196)**	0.191 (0.109)	-0.193 (0.101)
Bright	-0.107 (0.071)	**-0.242 (0.105)**	0.191 (0.109)	**0.311 (0.120)**	0.106 (0.083)
Sat	-0.104 (0.074)	-0.033 (0.067)	-0.193 (0.101)	0.106 (0.083)	**0.256 (0.106)**

Variance-standardized **G** matrix: **Testosterone Males**

	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	0.093 (0.077)	0.017 (0.055)	-0.127 (0.089)	-0.070 (0.087)	0.067 (0.068)
Ln Area	0.017 (0.055)	0.170 (0.093)	**-0.239 (0.101)**	**-0.199 (0.097)**	-0.010 (0.075)
Hue	-0.127 (0.089)	**-0.239 (0.101)**	**0.579 (0.187)**	**0.354 (0.131)**	-0.154 (0.098)
Bright	-0.070 (0.087)	**-0.199 (0.097)**	**0.354 (0.131)**	**0.439 (0.168)**	0.109 (0.111)
Sat	0.067 (0.068)	-0.010 (0.075)	-0.154 (0.098)	0.109 (0.111)	**0.275 (0.135)**

Table S7. Unstandardized **G** matrices for each experimental group. Elements on the diagonal are genetic variances, elements off the diagonal are genetic covariances. Numbers in parentheses are 1 SEM. **Bold** font indicates estimates >2 SEM above or below zero, approximating statistical significance. Note that the magnitudes of the genetic variances (and covariances) are strongly associated with the units in which the phenotypes are measured (e.g., smaller for ln-transformed size traits, larger for color traits measured in degrees or percentages). For this reason, variance-standardized **G** matrices (Table S6) were used for primary analyses, though both methods yielded equivalent results (Table S10).

Unstandardized **G** matrix: **Control Females**						Unstandardized **G** matrix: **Control Males**				
	Ln SVL	Ln Area	Hue	Bright	Sat	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	0.0004 (0.0002)	0.0009 (0.0009)	0.0230 (0.0191)	0.0082 (0.0227)	0.0630 (0.0387)	0.0005 (0.0002)	0.0015 (0.0012)	-0.0075 (0.0162)	-0.0288 (0.0316)	-0.0042 (0.0475)
Ln Area	0.0009 (0.0009)	**0.0164 (0.0077)**	-0.0414 (0.1158)	-0.0877 (0.1415)	0.4348 (0.2491)	0.0015 (0.0012)	0.0122 (0.0093)	-0.1697 (0.1039)	-0.0984 (0.1842)	0.2352 (0.2953)
Hue	0.0230 (0.0192)	-0.0414 (0.1158)	**11.229 (3.509)**	**11.157 (4.155)**	-4.791 (4.806)	-0.0075 (0.0162)	-0.1697 (0.1039)	**6.829 (2.130)**	1.806 (2.734)	**-11.049 (5.052)**
Bright	0.0082 (0.0227)	-0.0877 (0.1415)	**11.157 (4.155)**	**12.733 (6.142)**	-11.543 (6.987)	-0.0288 (0.0316)	-0.0984 (0.1842)	1.806 (2.734)	**16.496 (7.274)**	**19.565 (8.248)**
Sat	0.0630 (0.0387)	0.4348 (0.2491)	-4.791 (4.806)	-11.543 (6.987)	**42.865 (14.373)**	-0.0042 (0.0475)	0.2352 (0.2953)	**-11.049 (5.052)**	**19.565 (8.248)**	**52.172 (19.543)**

Unstandardized **G** matrix: **Testosterone Females**						Unstandardized **G** matrix: **Testosterone Males**				
Ln SVL	0.0003 (0.0002)	0.0014 (0.0014)	-0.0080 (0.0145)	-0.0546 (0.0366)	-0.0670 (0.0476)	0.0003 (0.0003)	0.0003 (0.0009)	-0.0244 (0.0171)	-0.0358 (0.0443)	0.0400 (0.0407)
Ln Area	0.0014 (0.0014)	0.0310 (0.0160)	-0.1576 (0.1140)	**-0.8335 (0.3609)**	-0.1423 (0.2991)	0.0003 (0.0009)	0.0128 (0.0070)	**-0.2021 (0.0851)**	**-0.4485 (0.2185)**	-0.0261 (0.1970)
Hue	-0.0080 (0.0145)	-0.1576 (0.1140)	**5.652 (1.843)**	5.695 (3.237)	-7.197 (3.760)	-0.0244 (0.0171)	**-0.2021 (0.0851)**	**5.472 (1.764)**	**8.904 (3.284)**	-4.488 (2.860)
Bright	-0.0546 (0.0366)	**-0.8335 (0.3609)**	5.695 (3.237)	**29.337 (11.331)**	12.489 (9.760)	-0.0358 (0.0443)	**-0.4485 (0.2185)**	**8.904 (3.284)**	**29.296 (11.212)**	8.447 (8.633)
Sat	-0.0670 (0.0476)	-0.1423 (0.2991)	-7.197 (3.760)	12.489 (9.760)	**37.832 (15.657)**	0.0400 (0.0407)	-0.0261 (0.1970)	-4.488 (2.860)	8.447 (8.633)	**24.805 (11.255)**

Table S8. Genetic correlation matrices for each experimental group. Numbers in parentheses are 1 SEM. **Bold** font indicates estimates >2 SEM above or below zero, approximating statistical significance. Only between-trait correlations were used in corresponding matrix comparisons.

Genetic correlation matrix: **Control Females**

	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	—	0.371 (0.285)	0.342 (0.262)	0.115 (0.318)	0.479 (0.256)
Ln Area	0.371 (0.285)	—	-0.096 (0.270)	-0.192 (0.304)	**0.517 (0.229)**
Hue	0.342 (0.262)	-0.096 (0.270)	—	**0.933 (0.072)**	-0.218 (0.214)
Bright	0.115 (0.318)	-0.192 (0.304)	**0.933 (0.072)**	—	**-0.494 (0.235)**
Sat	0.479 (0.256)	**0.517 (0.229)**	-0.218 (0.214)	**-0.494 (0.235)**	—

Genetic correlation matrix: **Control Males**

	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	—	**0.629 (0.270)**	-0.130 (0.287)	-0.320 (0.303)	0.026 (0.295)
Ln Area	**0.629 (0.270)**	—	-0.588 (0.348)	-0.219 (0.388)	0.295 (0.379)
Hue	-0.130 (0.287)	-0.588 (0.348)	—	0.170 (0.249)	**-0.585 (0.159)**
Bright	-0.320 (0.303)	-0.219 (0.388)	0.170 (0.249)	—	**0.667 (0.115)**
Sat	0.026 (0.295)	0.295 (0.379)	**-0.585 (0.159)**	**0.667 (0.115)**	—

Genetic correlation matrix: **Testosterone Females**

	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	—	0.431 (0.316)	-0.180 (0.317)	**-0.539 (0.240)**	-0.582 (0.299)
Ln Area	0.431 (0.316)	—	-0.377 (0.245)	**-0.874 (0.126)**	-0.131 (0.273)
Hue	-0.180 (0.317)	-0.377 (0.245)	—	**0.442 (0.184)**	**-0.492 (0.195)**
Bright	**-0.539 (0.240)**	**-0.874 (0.126)**	**0.442 (0.184)**	—	0.375 (0.242)
Sat	-0.582 (0.299)	-0.131 (0.273)	**-0.492 (0.195)**	0.375 (0.242)	—

Genetic correlation matrix: **Testosterone Males**

	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	—	0.134 (0.430)	-0.547 (0.360)	-0.347 (0.455)	0.422 (0.469)
Ln Area	0.134 (0.430)	—	**-0.762 (0.218)**	**-0.731 (0.241)**	-0.046 (0.347)
Hue	-0.547 (0.360)	**-0.762 (0.218)**	—	**0.703 (0.139)**	**-0.385 (0.208)**
Bright	-0.347 (0.377)	**-0.731 (0.241)**	**0.703 (0.139)**	—	0.313 (0.264)
Sat	0.422 (0.469)	-0.046 (0.347)	**-0.385 (0.208)**	0.313 (0.264)	—

Table S9. Phenotypic variance-covariance (**P**) and correlation matrices for each experimental group. Elements on the diagonal are estimates of phenotypic variance, those below the diagonal are phenotypic covariances, and those above the diagonal are phenotypic correlations. Numbers in parentheses are 1 SEM. **Bold** font indicates estimates >2 SEM above or below zero, approximating statistical significance.

P and phenotypic correlation matrix: **Control Females**					
	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	**0.0015** (**0.0002**)	**0.425** (**0.078**)	-0.026 (0.092)	-0.242 (0.142)	-0.014 (0.096)
Ln Area	**0.0039** (**0.0010**)	**0.0556** (**0.0074**)	-0.084 (0.085)	-0.011 (0.118)	0.134 (0.091)
Hue	-0.0041 (0.0148)	-0.0811 (0.0829)	**16.635** (**1.958**)	**0.400** (**0.079**)	0.085 (0.085)
Bright	-0.0939 (0.0481)	-0.0250 (0.2757)	**16.189** (**4.1671**)	**98.467** (**18.045**)	-0.048 (0.107)
Sat	-0.0050 (0.0338)	0.2836 (0.1940)	3.1114 (3.1966)	-4.2905 (9.4633)	**81.041** (**9.529**)

P and phenotypic correlation matrix: **Control Males**					
	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	**0.0031** (**0.0007**)	**0.796** (**0.051**)	0.085 (0.089)	**-0.422** (**0.095**)	**-0.181** (**0.080**)
Ln Area	**0.0171** (**0.0051**)	**0.1504** (**0.0358**)	0.033 (0.088)	**-0.336** (**0.103**)	-0.114 (0.082)
Hue	0.016 (0.0169)	0.043 (0.115)	**11.549** (**1.2008**)	0.091 (0.073)	**-0.375** (**0.062**)
Bright	**-0.197** (**0.0694**)	**-1.085** (**0.456**)	2.573 (2.083)	**69.173** (**9.238**)	**0.419** (**0.056**)
Sat	-0.106 (0.0517)	-0.463 (0.348)	**-13.340** (**2.791**)	**36.421** (**6.788**)	**109.322** (**11.205**)

P and phenotypic correlation matrix: **Testosterone Females**					
	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	**0.0027** (**0.0005**)	**0.343** (**0.086**)	0.097 (0.087)	**-0.372** (**0.082**)	-0.226 (0.121)
Ln Area	**0.0064** (**0.0016**)	**0.1257** (**0.0128**)	-0.036 (0.072)	**-0.342** (**0.070**)	**0.324** (**0.074**)
Hue	0.016 (0.015)	-0.0395 (0.0794)	**9.404** (**1.058**)	**0.241** (**0.078**)	**-0.215** (**0.080**)
Bright	**-0.191** (**0.062**)	**-1.178** (**0.278**)	**7.176** (**2.385**)	**94.429** (**10.921**)	0.185 (0.094)
Sat	-0.145 (0.092)	**1.397** (**0.424**)	**-8.029** (**3.472**)	21.855 (12.468)	**147.890** (**26.740**)

P and phenotypic correlation matrix: **Testosterone Males**					
	Ln SVL	Ln Area	Hue	Bright	Sat
Ln SVL	**0.0039** (**0.0006**)	**0.450** (**0.078**)	**0.061** (**0.086**)	**-0.323** (**0.083**)	**-0.386** (**0.077**)
Ln Area	**0.0077** (**0.0022**)	**0.0757** (**0.0103**)	-0.077 (0.079)	**-0.287** (**0.080**)	-0.195 (0.086)
Hue	0.012 (0.016)	-0.064 (0.069)	**9.457** (**1.013**)	**0.311** (**0.068**)	**-0.181** (**0.075**)
Bright	**-0.165** (**0.054**)	**-0.646** (**0.215**)	**7.808** (**2.093**)	**66.768** (**8.013**)	**0.412** (**0.068**)
Sat	**-0.229** (**0.066**)	-0.509 (0.259)	**-5.293** (**2.220**)	**32.002** (**7.072**)	**90.339** (**10.479**)

Table S10. Summary of tests for differences in **G** and genetic correlation matrices across four experimental groups. The top two sections report random skewers (RS) comparisons using (A) variance standardized **G** (Table S6; Fig. 2) or (B) unstandardized **G** (Table S7). The bottom two sections report comparisons of genetic correlation matrices (Table S8) using (C) Mantel matrix correlations, or (D) a modified version of the T method (see Supplemental Methods). **Bold** values on the diagonal are modes (lower 5% bounds) of the distribution of mean vector or matrix correlations (*r*) or of mean differences in genetic correlations (D*r*) between the best estimate of a matrix and each of the 10,000 simulated matrices in its error distribution. Values off the diagonal report *r* and D*r* calculated between groups, with *P*-values corresponding to the 5% bound of the null distribution in the same column. * Indicates a significant difference between matrices.

	Control Female		**Testosterone Female**		**Control Male**		**Testosterone Male**	
A. Standardized G (RS)	***r***	***P* (5%)**	***r***	***P* (5%)**	***r***	***P* (5%)**	***r***	***P* (5%)**
Control Female	**0.938**	**(0.803)**	0.629	0.002*	0.673	0.004*	0.640	0.003*
Testosterone Female	0.629	< 0.001*	**0.940**	**(0.778)**	0.845	0.124	0.863	0.233
Control Male	0.672	0.003*	0.845	0.149	**0.935**	**(0.800)**	0.827	0.124
Testosterone Male	0.640	0.001*	0.863	0.202	0.827	0.087	**0.936**	**(0.778)**
B. Unstandardized G (RS)	***r***	***P* (5%)**	***r***	***P* (5%)**	***r***	***P* (5%)**	***r***	***P* (5%)**
Control Female	**0.983**	**(0.862)**	0.729	0.016*	0.661	0.006*	0.726	0.015*
Testosterone Female	0.729	0.006*	**0.984**	**(0.825)**	0.927	0.200	0.979	0.672
Control Male	0.661	0.002*	0.927	0.238	**0.983**	**(0.851)**	0.863	0.078
Testosterone Male	0.726	0.005*	0.979	0.689	0.863	0.059	**0.984**	**(0.834)**
C. Correlation (Mantel)	***r***	***P* (5%)**	***r***	***P* (5%)**	***r***	***P* (5%)**	***r***	***P* (5%)**
Control Female	**0.948**	**(0.564)**	0.312	0.028*	0.232	0.010*	0.518	0.049*
Testosterone Female	0.312	0.016*	**0.946**	**(0.437)**	0.770	0.313	0.657	0.118
Control Male	0.232	0.011*	0.770	0.219	**0.928**	**(0.454)**	0.685	0.140
Testosterone Male	0.518	0.039*	0.657	0.124	0.685	0.195	**0.935**	**(0.520)**
D. Correlation (Modified T)	**Δ*r***	***P* (5%)**	**Δ*r***	***P* (5%)**	**Δ*r***	***P* (5%)**	**Δ*r***	***P* (5%)**
Control Female	**0.161**	**(0.368)**	0.554	0.007*	0.466	0.009*	0.461	0.009*
Testosterone Female	0.554	< 0.001*	**0.150**	**(0.408)**	0.295	0.145	0.290	0.232
Control Male	0.466	0.009*	0.295	0.146	**0.198**	**(0.418)**	0.347	0.116
Testosterone Male	0.461	0.009*	0.290	0.156	0.347	0.070	**0.192**	**(0.408)**

Table S11. Summary of tests for differences in **P** and phenotypic correlation matrices across four experimental groups. The top section (A) reports random skewers (RS) comparisons using **P** matrices (on and below the diagonal in Table S9). The bottom two sections report comparisons of phenotypic correlation matrices (above the diagonal in Table S9) using (B) Mantel matrix correlations, or (C) a modified version of the T method (see Supplemental Methods). **Bold** values on the diagonal are modes (lower 5% bounds) of the distribution of mean vector or matrix correlations (*r*) or of mean differences in genetic correlations (D*r*) between the best estimate of a matrix and each of the 10,000 simulated matrices in its error distribution. Values off the diagonal report *r* and D*r* calculated between groups, with *P*-values corresponding to the 5% bound of the null distribution in the same column. * Indicates a significant difference between matrices.

	Control Female		Testosterone Female		Control Male		Testosterone Male	
A. P matrix (RS)	***r***	***P* (5%)**	***r***	***P* (5%)**	***r***	***P* (5%)**	***r***	***P* (5%)**
Control Female	**0.999**	**(0.974)**	0.941	0.002*	0.885	< 0.001*	0.915	< 0.001*
Testosterone Female	0.941	0.004*	**0.999**	**(0.977)**	0.974	0.004*	0.979	0.016
Control Male	0.885	< 0.001*	0.974	< 0.036*	**0.999**	**(0.987)**	0.990	0.159
Testosterone Male	0.915	< 0.001*	0.979	0.060	0.990	0.120	**0.999**	**(0.985)**
B. Correlation (Mantel)	***r***	***P* (5%)**	***r***	***P* (5%)**	***r***	***P* (5%)**	***r***	***P* (5%)**
Control Female	**0.943**	**(0.719)**	0.685	0.004*	0.560	< 0.001*	0.614	< 0.001*
Testosterone Female	0.685	0.036*	**0.957**	**(0.852)**	0.779	0.003*	0.778	0.005*
Control Male	0.560	0.011*	0.779	0.014*	**0.990**	**(0.929)**	0.891	0.038*
Testosterone Male	0.614	0.018*	0.778	0.013*	0.891	0.015*	**0.981**	**(0.904)**
C. Correlation (Modified T)	**Δ*r***	***P* (5%)**	**Δ*r***	***P* (5%)**	**Δ*r***	***P* (5%)**	**Δ*r***	***P* (5%)**
Control Female	**0.083**	**(0.141)**	0.182	0.005*	0.273	< 0.001*	0.193	0.002*
Testosterone Female	0.182	0.006*	**0.068**	**(0.131)**	0.163	0.009	0.130	0.027*
Control Male	0.273	< 0.001*	0.163	0.011*	**0.059**	**(0.124)**	0.133	0.023*
Testosterone Male	0.193	0.003*	0.130	0.053	0.133	0.035	**0.068**	**(0.117)**

Table S12. Summary of model comparisons testing for significant additive between-sex genetic covariance in three estimates of the full **G** matrix (including **B**) for different combinations of female and male treatment groups. In the two combinations that included females treated with testosterone, models that included between-sex genetic covariance (i.e., the full **G** matrix plus **B**) were preferred over simpler models that set the between-sex covariances in **B** = 0, indicating significant between-sex genetic covariance. However, in the "natural" **G** matrix estimated for control females and control males, the addition of **B** did not significantly improve model fit, indicating relatively weak between-sex genetic covariance. Chi-squared values are calculated as twice the difference in log likelihood between models and are shown for each of the reduced models in comparison to the preferred full **G** model.

Experimental groups	Matrix	Log Likelihood	df	χ^2	*P*
Control Female + Control Male	Full **G** + **B**	−5551.21	—	—	—
	Only **G**, **B** =	−5567.28	25	32.62	0.141
Testosterone Female + Control Male	Full **G** + **B**	−5887.73	—	—	—
	Only **G**, **B** =	−5913.98	25	52.49	0.001
Testosterone Female + Testosterone	Full **G** + **B**	−5979.57	—	—	—
	Only **G**, **B** =	−5999.89	25	40.65	0.025

Table S13. Full variance-standardized genetic variance-covariance matrices (**G**), including the between-sex covariance matrix (**B**), for three combinations of male and female treatments. The upper right quadrat reports corresponding between-sex genetic correlations (r_{MF}). **Bold** font indicates estimates >2 SEM above or below zero, approximating statistical significance.

A. **G** and **B** for **control males** and **control females** with between-sex genetic correlations above diagonal in box

	Ln SVL $_M$	Ln Area $_M$	Hue $_M$	Bright $_M$	Sat $_M$	Ln SVL $_F$	Ln Area $_F$	Hue $_F$	Bright $_F$	Sat $_F$
Ln SVL $_M$	**0.141**					0.443	**0.582**	-0.001	-0.190	0.255
	(0.066)					(0.263)	**(0.256)**	(0.248)	(0.281)	(0.257)
Ln Area $_M$	0.065	0.083				0.269	0.260	-0.339	**-0.630**	0.212
	(0.047)	(0.051)				(0.316)	(0.318)	(0.282)	**(0.226)**	(0.300)
Hue $_M$	-0.070	**-0.142**	**0.634**			0.067	-0.237	**0.856**	**0.856**	-0.214
	(0.075)	**(0.069)**	**(0.166)**			(0.218)	(0.224)	**(0.095)**	**(0.126)**	(0.206)
Bright $_M$	-0.060	-0.033	0.081	**0.229**		-0.125	-0.255	-0.032	0.200	-0.029
	(0.057)	(0.048)	(0.092)	**(0.093)**		(0.289)	(0.270)	(0.217)	(0.241)	(0.264)
Sat $_M$	0.023	0.065	**-0.313**	0.185	**0.437**	0.169	0.202	**-0.573**	**-0.477**	0.350
	(0.070)	(0.062)	**(0.124)**	(0.094)	**(0.156)**	(0.267)	(0.258)	**(0.166)**	**(0.225)**	(0.233)
Ln SVL $_F$	0.084	0.039	0.027	-0.030	0.057	**0.256**				
	(0.058)	(0.050)	(0.088)	(0.073)	(0.092)	**(0.105)**				
Ln Area $_F$	0.125	0.043	-0.108	-0.070	0.076	0.119	**0.327**			
	(0.067)	(0.054)	(0.104)	(0.080)	(0.101)	(0.087)	**(0.134)**			
Hue $_F$	-0.001	-0.080	**0.557**	-0.013	**-0.309**	0.173	-0.062	**0.668**		
	(0.076)	(0.069)	**(0.146)**	(0.085)	**(0.119)**	(0.095)	(0.109)	**(0.190)**		
Bright $_F$	-0.026	-0.066	**0.248**	0.035	**-0.115**	0.011	-0.043	**0.249**	**0.133**	
	(0.041)	(0.041)	**(0.072)**	(0.044)	**(0.057)**	(0.046)	(0.053)	**(0.083)**	**(0.050)**	
Sat $_F$	0.070	0.045	-0.125	-0.010	0.170	0.199	**0.239**	-0.102	-0.130	**0.538**
	(0.074)	(0.064)	(0.122)	(0.093)	(0.120)	(0.100)	**(0.115)**	(0.127)	(0.070)	**(0.181)**

B. **G** and **B** for **control males** and **testosterone females** with between-sex genetic correlations above diagonal in box

	Ln SVL $_M$	Ln Area $_M$	Hue $_M$	Bright $_M$	Sat $_M$	Ln SVL $_F$	Ln Area $_F$	Hue $_F$	Bright $_F$	Sat $_F$
Ln SVL $_M$	**0.163**					**0.790**	**0.653**	-0.389	**-0.581**	0.045
	(0.066)					**(0.130)**	**(0.168)**	(0.222)	**(0.177)**	(0.234)
Ln Area $_M$	0.073	**0.097**				0.202	**0.659**	**-0.594**	-0.282	**0.663**
	(0.040)	**(0.039)**				(0.255)	**(0.157)**	**(0.172)**	(0.215)	**(0.148)**
Hue $_M$	-0.086	**-0.141**	**0.628**			-0.303	-0.214	**0.819**	0.251	**-0.435**
	(0.072)	**(0.059)**	**(0.161)**			(0.186)	(0.227)	**(0.118)**	(0.204)	**(0.177)**
Bright $_M$	-0.063	-0.007	0.095	**0.239**		-0.422	**-0.606**	0.146	**0.864**	**0.591**
	(0.053)	(0.038)	(0.088)	**(0.078)**		(0.220)	**(0.161)**	(0.217)	**(0.108)**	**(0.173)**
Sat $_M$	0.028	0.080	**-0.332**	0.198	**0.478**	0.105	0.337	**-0.530**	**0.464**	**0.734**
	(0.062)	(0.046)	**(0.115)**	(0.070)	**(0.136)**	(0.224)	(0.193)	**(0.161)**	**(0.166)**	**(0.121)**
Ln SVL $_F$	**0.124**	0.025	-0.094	-0.080	0.028	**0.152**				
	(0.050)	(0.034)	(0.064)	(0.050)	(0.063)	**(0.056)**				
Ln Area $_F$	**0.125**	**0.097**	-0.080	**-0.140**	-0.110	0.081	**0.224**			
	(0.060)	**(0.048)**	(0.088)	**(0.053)**	(0.069)	(0.056)	**(0.090)**			
Hue $_F$	-0.120	**-0.142**	**0.499**	0.054	**-0.281**	-0.052	-0.092	**0.591**		
	(0.078)	**(0.057)**	**(0.135)**	(0.083)	**(0.114)**	(0.069)	(0.090)	**(0.189)**		
Bright $_F$	**-0.128**	-0.048	0.108	**0.230**	**0.175**	**-0.110**	-0.209	0.154	**0.296**	
	(0.058)	(0.040)	(0.091)	**(0.066)**	**(0.079)**	**(0.055)**	(0.072)	(0.101)	**(0.093)**	
Sat $_F$	0.010	**0.114**	**-0.189**	**0.159**	**0.279**	-0.072	-0.021	**-0.226**	0.123	**0.302**
	(0.053)	**(0.047)**	**(0.089)**	**(0.063)**	**(0.079)**	(0.053)	(0.054)	**(0.091)**	(0.068)	**(0.093)**

C. **G** and **B** for **testosterone males** and **testosterone females** with between-sex genetic correlations above diagonal in box

	Ln SVL $_M$	Ln Area $_M$	Hue $_M$	Bright $_M$	Sat $_M$	Ln SVL $_F$	Ln Area $_F$	Hue $_F$	Bright $_F$	Sat $_F$
Ln SVL $_M$	0.129					**0.753**	**0.684**	**-0.451**	**-0.645**	-0.363
	(0.072)					**(0.135)**	**(0.161)**	**(0.195)**	**(0.166)**	(0.236)
Ln Area $_M$	0.066	0.240				0.112	**0.611**	-0.134	-0.211	-0.214
	(0.061)	(0.141)				(0.300)	**(0.186)**	(0.236)	(0.255)	(0.244)
Hue $_M$	-0.033	-0.102	**0.566**			-0.258	**-0.663**	**0.840**	**0.491**	**-0.638**
	(0.065)	(0.095)	**(0.171)**			(0.203)	**(0.142)**	**(0.088)**	**(0.175)**	**(0.153)**
Bright $_M$	-0.084	**-0.210**	0.162	**0.267**		-0.038	-0.779	0.293	0.415	0.214
	(0.057)	**(0.101)**	(0.094)	**(0.103)**		(0.284)	(0.109)	(0.196)	(0.221)	(0.222)
Sat $_M$	-0.083	-0.026	**-0.217**	0.082	**0.262**	0.043	-0.061	-0.351	0.198	**0.844**
	(0.054)	(0.064)	**(0.089)**	(0.067)	**(0.095)**	(0.267)	(0.247)	(0.180)	(0.215)	**(0.108)**
Ln SVL $_F$	0.086	0.018	-0.062	-0.006	0.007	**0.101**				
	(0.050)	(0.050)	(0.053)	(0.047)	(0.044)	**(0.049)**				
Ln Area $_F$	0.101	0.123	**-0.205**	**-0.165**	-0.013	0.051	**0.168**			
	(0.061)	(0.069)	**(0.079)**	**(0.068)**	(0.052)	(0.041)	**(0.072)**			
Hue $_F$	-0.130	-0.053	**0.507**	0.121	-0.144	-0.166	**-0.230**	**0.642**		
	(0.074)	(0.095)	**(0.143)**	(0.091)	(0.085)	(0.076)	**(0.083)**	**(0.180)**		
Bright $_F$	**-0.155**	-0.069	**0.248**	0.144	0.068	-0.101	**-0.214**	**0.403**	**0.451**	
	(0.074)	(0.089)	**(0.106)**	(0.081)	(0.079)	(0.059)	**(0.084)**	**(0.123)**	**(0.171)**	
Sat $_F$	-0.067	-0.053	**-0.245**	0.056	**0.221**	0.029	-0.015	**-0.199**	0.062	**0.260**
	(0.056)	(0.068)	**(0.092)**	(0.059)	**(0.068)**	(0.040)	(0.051)	**(0.085)**	(0.087)	**(0.079)**

Table S14. Full unstandardized genetic variance-covariance matrices (**G**), including the between-sex covariance matrix (**B**), for three combinations of male and female treatments. The upper right quadrat reports corresponding between-sex genetic correlations (r_{MF}). **Bold** font indicates estimates >2 SEM above or below zero, approximating statistical significance.

D. G and **B** for **control males** and **control females** with between-sex genetic correlations above diagonal in box

	Ln SVL	Ln Area $_M$	Hue $_M$	Bright $_M$	Sat $_M$	Ln SVL $_F$	Ln Area $_F$	Hue $_F$	Bright $_F$	Sat $_F$
Ln SVL $_M$	**0.00048**					0.443	**0.582**	-0.001	-0.190	0.255
	(0.00022)					(0.263)	**(0.256)**	(0.248)	(0.281)	(0.257)
Ln Area $_M$	0.0015	0.0131				0.269	0.260	-0.339	**-0.630**	0.212
	(0.0011)	(0.0081)				(0.316)	(0.318)	(0.282)	**(0.226)**	(0.300)
Hue $_M$	-0.0141	**-0.1926**	**7.463**			0.067	-0.237	**0.856**	**0.856**	-0.214
	(0.0151)	**(0.0945)**	**(1.960)**			(0.218)	(0.224)	**(0.095)**	**(0.126)**	(0.206)
Bright $_M$	-0.0295	-0.0110	2.342	**16.148**		-0.125	-0.255	-0.032	0.200	-0.029
	(0.0281)	(0.1609)	(2.636)	**(6.564)**		(0.289)	(0.270)	(0.217)	(0.241)	(0.264)
Sat $_M$	0.0138	0.2699	**-11.235**	16.274	**47.930**	0.169	0.202	**-0.573**	**-0.477**	0.350
	(0.0428)	(0.2556)	**(4.442)**	(8.315)	**(17.094)**	(0.267)	(0.258)	**(0.166)**	**(0.225)**	(0.233)
Ln SVL $_F$	0.00004	0.00063	0.0037	-0.0102	0.0237	**0.00041**				
	(0.00014)	(0.00079)	(0.0121)	(0.0245)	(0.0397)	**(0.00017)**				
Ln Area $_F$	0.0017	0.0040	-0.0871	-0.1377	0.1875	0.0011	**0.0180**			
	(0.0009)	(0.0050)	(0.0835)	(0.1575)	(0.2489)	(0.0008)	**(0.0073)**			
Hue $_F$	-0.00001	-0.1301	**7.853**	-0.4330	**-13.321**	0.0284	-0.0595	**11.291**		
	(0.0182)	(0.1127)	**(2.052)**	(2.9452)	**(5.1377)**	(0.0157)	(0.1055)	**(3.212)**		
Bright $_F$	-0.0153	-0.2648	**8.598**	2.951	**-12.129**	0.0045	-0.1013	**10.334**	**13.512**	
	(0.0241)	(0.1636)	**(2.504)**	(3.745)	**(6.0277)**	(0.0184)	(0.1263)	**(3.426)**	**(5.054)**	
Sat $_F$	0.0368	0.1597	-3.845	-0.7765	15.973	0.0716	**0.5052**	-3.749	-11.824	**43.424**
	(0.0387)	(0.2287)	(3.773)	(7.0364)	(11.257)	(0.0362)	**(0.2447)**	(4.673)	(6.360)	**(14.592)**

E. **G** and **B** for **control males** and **testosterone females** with between-sex genetic correlations above diagonal in box

	Ln SVL $_M$	Ln Area $_M$	Hue $_M$	Bright $_M$	Sat $_M$	Ln SVL $_F$	Ln Area $_F$	Hue $_F$	Bright $_F$	Sat $_F$
Ln SVL $_M$	0.00053					0.790	0.653	-0.389	-0.581	0.045
	(0.00022)					(0.130)	(0.168)	(0.222)	(0.177)	(0.234)
Ln Area $_M$	0.00162	0.0144				0.202	0.659	-0.594	-0.282	0.663
	(0.00089)	(0.0057)				(0.255)	(0.157)	(0.172)	(0.215)	(0.148)
Hue $_M$	-0.0166	-0.1853	7.2480			-0.303	-0.214	0.819	0.251	-0.435
	(0.0139)	(0.0781)	(1.8576)			(0.186)	(0.227)	(0.118)	(0.204)	(0.177)
Bright $_M$	-0.0295	-0.0227	2.6424	16.127		-0.422	-0.606	0.146	0.864	0.591
	(0.0284)	(0.1194)	(2.4678)	(5.2814)		(0.220)	(0.161)	(0.217)	(0.108)	(0.173)
Sat $_M$	0.0164	0.3209	-11.723	16.906	51.725	0.105	0.337	-0.530	0.464	0.734
	(0.0370)	(0.1854)	(4.0539)	(6.0173)	(14.760)	(0.224)	(0.193)	(0.161)	(0.166)	(0.121)
Ln SVL $_F$	0.00038	0.00005	-0.0168	-0.0348	0.0155	0.00042				
	(0.00015)	(0.00067)	(0.0115)	(0.0215)	(0.0345)	(0.00016)				
Ln Area $_F$	0.0025	0.0131	-0.0952	-0.4026	-0.4015	0.0015	0.0273			
	(0.0012)	(0.0065)	(0.1045)	(0.1533)	(0.2518)	(0.0010)	(0.0110)			
Hue $_F$	-0.0213	-0.1699	5.2586	1.3978	-9.0813	-0.0084	-0.0999	5.6832		
	(0.0137)	(0.0685)	(1.4253)	(2.1230)	(3.6655)	(0.0112)	(0.0974)	(1.8139)		
Bright $_F$	-0.0707	-0.1785	3.5625	18.309	17.618	-0.0565	-0.7069	4.6312	27.874	
	(0.0320)	(0.1506)	(2.9822)	(5.2665)	(8.0070)	(0.0280)	(0.2451)	(3.0356)	(8.7690)	
Sat $_F$	0.0068	0.5214	-7.6611	15.531	34.585	-0.0452	-0.0880	-8.3342	14.248	42.884
	(0.0357)	(0.2179)	(3.5908)	(6.1465)	(9.8424)	(0.0334)	(0.2241)	(3.3640)	(7.8309)	(13.208)

F. **G** and **B** for **testosterone males** and **testosterone females** with between-sex genetic correlations above diagonal in box

	Ln SVL $_M$	Ln Area $_M$	Hue $_M$	Bright $_M$	Sat $_M$	Ln SVL $_F$	Ln Area $_F$	Hue $_F$	Bright $_F$	Sat $_F$
Ln SVL $_M$	0.00036					0.753	0.684	-0.451	-0.645	-0.363
	(0.00020)					(0.135)	(0.161)	(0.195)	(0.166)	(0.236)
Ln Area $_M$	0.0012	0.0296				0.112	0.611	-0.134	-0.211	-0.214
	(0.0011)	(0.0173)				(0.300)	(0.186)	(0.236)	(0.255)	(0.244)
Hue $_M$	-0.0054	-0.1100	5.3855			-0.258	-0.663	0.840	0.491	-0.638
	(0.0105)	(0.1034)	(1.6299)			(0.203)	(0.142)	(0.088)	(0.175)	(0.153)
Bright $_M$	-0.0429	-0.7121	4.8405	24.946		-0.038	-0.779	0.293	0.415	0.214
	(0.0291)	(0.3446)	(2.8088)	(9.5911)		(0.284)	(0.109)	(0.196)	(0.221)	(0.222)
Sat $_M$	-0.0538	-0.1125	-8.2109	9.7947	39.530	0.043	-0.061	-0.351	0.198	0.844
	(0.0354)	(0.2776)	(3.3662)	(7.9941)	(14.340)	(0.267)	(0.247)	(0.180)	(0.215)	(0.108)
Ln SVL $_F$	0.00029	0.00039	-0.0120	-0.0038	0.0054	0.00004				
	(0.00017)	(0.00111)	(0.0102)	(0.0285)	(0.0337)	(0.00002)				
Ln Area $_F$	0.0014	0.0118	-0.1736	-0.4395	-0.0431	0.00089	0.0127			
	(0.0008)	(0.0066)	(0.0668)	(0.1818)	(0.1741)	(0.00072)	(0.0054)			
Hue $_F$	-0.0218	-0.0587	4.9536	3.7178	-5.6073	-0.0331	-0.2002	6.4498		
	(0.0124)	(0.1055)	(1.4010)	(2.7891)	(3.3094)	(0.0152)	(0.0726)	(1.8107)		
Bright $_F$	-0.0674	-0.1991	6.2552	11.390	6.8337	-0.0522	-0.4813	10.465	30.158	
	(0.0323)	(0.2566)	(2.6715)	(6.4699)	(7.9258)	(0.0304)	(0.1882)	(3.1858)	(11.449)	
Sat $_F$	-0.0336	-0.1787	-7.1945	5.1857	25.801	0.0176	-0.0383	-6.0075	4.8275	23.617
	(0.0285)	(0.2259)	(2.7024)	(5.4687)	(7.9655)	(0.0241)	(0.1327)	(2.5738)	(6.7801)	(7.1778)

Table S15. Summary of tests for effects of testosterone on the magnitude of between-sex genetic correlations (r_{MF}) for five homologous traits (diagonals in the between-sex genetic correlation matrices in Tables S13-S14). To compare any two matrices, the value of r_{MF} in one matrix was subtracted from the value of r_{MF} for the same trait in the other matrix, with the direction of subtraction indicated in the first column. The mean difference in r_{MF} across all five traits was then calculated. The second column reports this point estimate of the mean difference across five r_{MF} values when using the best estimate of each correlation matrix (Tables S13-S14). To test for significant differences in the magnitude of r_{MF} of across matrices, the same process was repeated for each of the 10,000 matrices in the simulated error distribution of each matrix, yielding 10,000 estimates of the mean difference in r_{MF} averaged across the five traits in the matrix. If the lower 5% of this simulated distribution includes zero, the two matrices do not differ significantly in the average magnitude of r_{MF}, as seen when comparing the two matrices in which females received testosterone (FT + MT and FT + MC, bottom row of table). However, both of these matrices exhibited significantly higher average values of r_{MF} when compared to the "natural" correlation matrix estimated between control females and control males (FC + MC).

Between-sex correlation matrices being compared, direction of comparison	**Mean difference in r_{MF} between the best estimates of each correlation matrix**	**Simulated distributions of mean differences in r_{MF} from error distributions of each matrix**			
		Mean	**Mode**	**Lower 5%**	***P***
(FT + MC) – (FC + MC)	0.351	0.355	0.360	0.138	0.006*
(FT + MT) – (FC + MC)	0.283	0.292	0.266	0.065	0.021*
(FT + MC) – (FT + MT)	0.068	0.062	0.063	– 0.110	0.275

Table S16. Summary of sexually antagonistic skewers comparison of the full **G** matrix (including **B**) for control females and control males versus those estimated for testosterone females and control males or for testosterone females and testosterone males. Comparisons are made using variance-standardized **G** matrices (as in Fig. 3 and Table S13) and unstandardized **G** matrices (Table S14). The bold values on the diagonal report the mean and upper or lower 5% bound of the distribution of mean vector correlations between male and female evolutionary responses, derived from passing 10,000 sexually antagonistic skewers through each of the 10,000 matrices simulated from error associated with the best estimate of **G**. The values above and below the diagonal replot the same mean between-sex vector correlations alongside *P*-values corresponding to the comparison of that estimate with the 5% upper or lower bound of the distribution in the same column. These are one-tailed tests because the a priori hypothesis is that the mean vector correlation for control females and control males should be lower than the mean vector correlation for the other two matrices that include testosterone females.

	Control Female + Control Male		**Testosterone Female + Control Male**		**Testosterone Female + Testosterone Male**	
Standardized G (+B)	***r***	***P* (upper 5%)**	***r***	***P* (lower 5%)**	***r***	***P* (lower 5%)**
C Female + C Male	**0.469**	**(0.595)**	0.469	0.017*	0.469	0.084
T Female + C Male	0.789	< 0.001*	**0.789**	**(0.542)**	0.789	0.875
T Female + T Male	0.705	0.002*	0.705	0.364	**0.705**	**(0.425)**
Unstandardized G (+B)	***r***	***P* (upper 5%)**	***r***	***P* (lower 5%)**	***r***	***P* (lower 5%)**
C Female + C Male	**0.125**	**(0.531)**	0.125	0.002*	0.125	0.059
T Female + C Male	0.739	0.003*	**0.739**	**(0.452)**	0.739	0.870
T Female + T Male	0.544	0.045*	0.544	0.127	**0.544**	**(0.101)**

Supplemental Figures

Figure S1. Representative images of individuals from each sex and treatment at 8 months of age (5 months post-treatment), illustrating effects of testosterone on dewlap phenotypes.

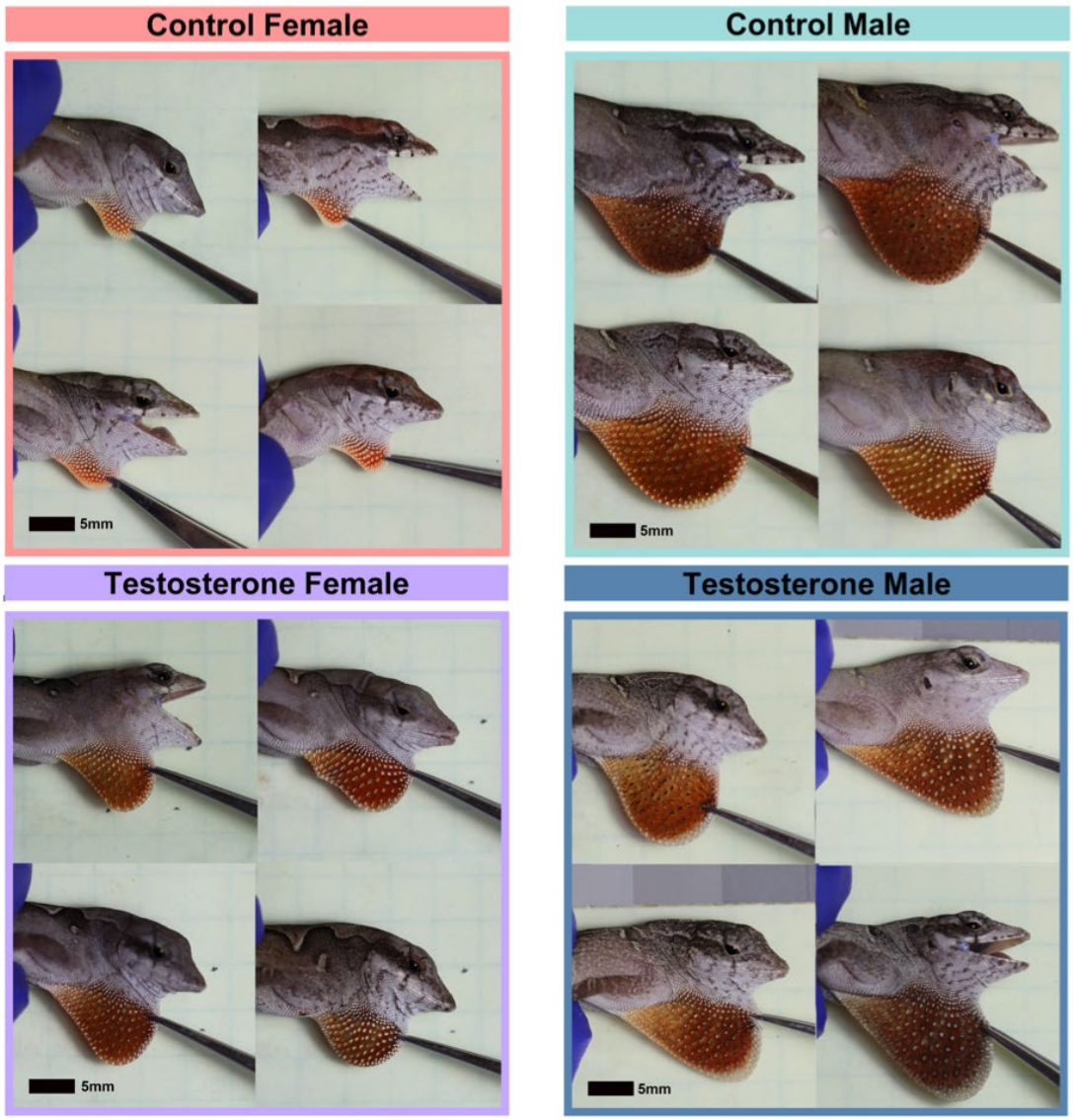


Figure S2. (A) Separation of experimental groups based on the first two principal components, which explain 71.5% of the variance in 5 phenotypes. Dots represent individuals and ellipses are 95% confidence intervals. For PC1 (positive loading for SVL and dewlap area, negative loading for dewlap brightness), control females are distinct from control and testosterone males, whereas testosterone females are intermediate. For PC2 (positive loading for dewlap saturation, negative loading for dewlap hue), individuals in control groups are slightly left-shifted, whereas individuals in testosterone treatments are slightly right-shifted. (B) Statistical separation of treatment groups on PC1. Solid line = median, box = interquartile range, whiskers = 95% CI, dots = individual outliers. SVL and dewlap area were ln-transformed prior to analysis.

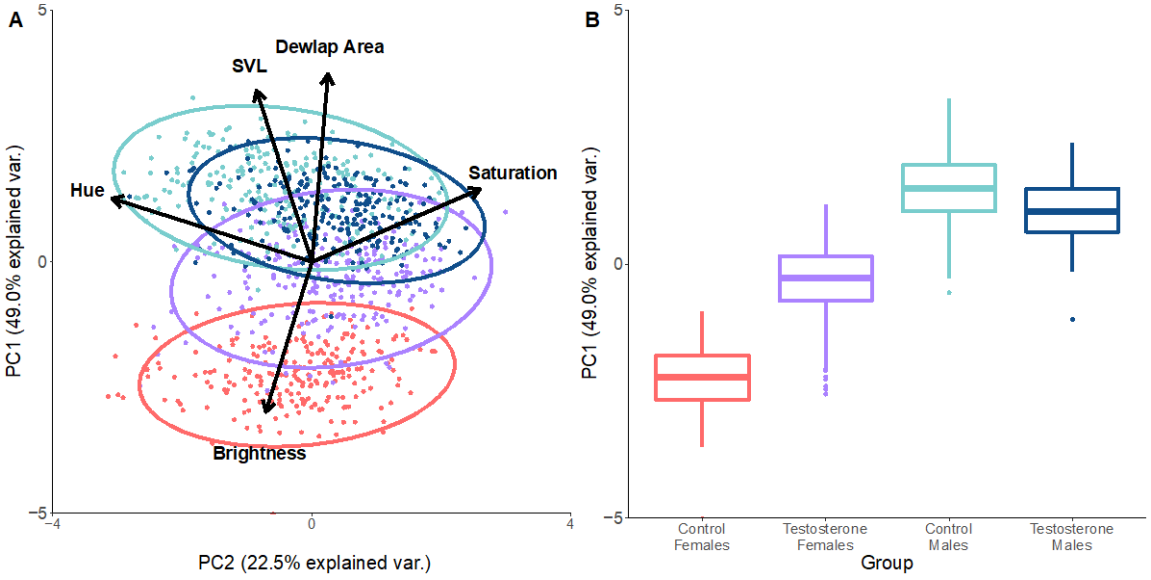


Figure S3. Comparisons of within-sex **P** matrices across four experimental groups based on response vectors from random skewers. The null distribution of mean vector correlations between the best estimate of **P** for a group and each of the 10,000 simulated matrices from its own sampling distribution is shown separately for (A) control females, (B) control males, (C) testosterone females, and (D) testosterone males. Dashed lines indicate the lower 5% bound of each distribution. Vertical pins indicate mean vector correlations between the best estimate of **P** for each of three comparison groups to that of the group whose null distribution is shown in that panel. Each vector correlation is plotted on two panels to facilitate comparison to each of the corresponding null distributions.

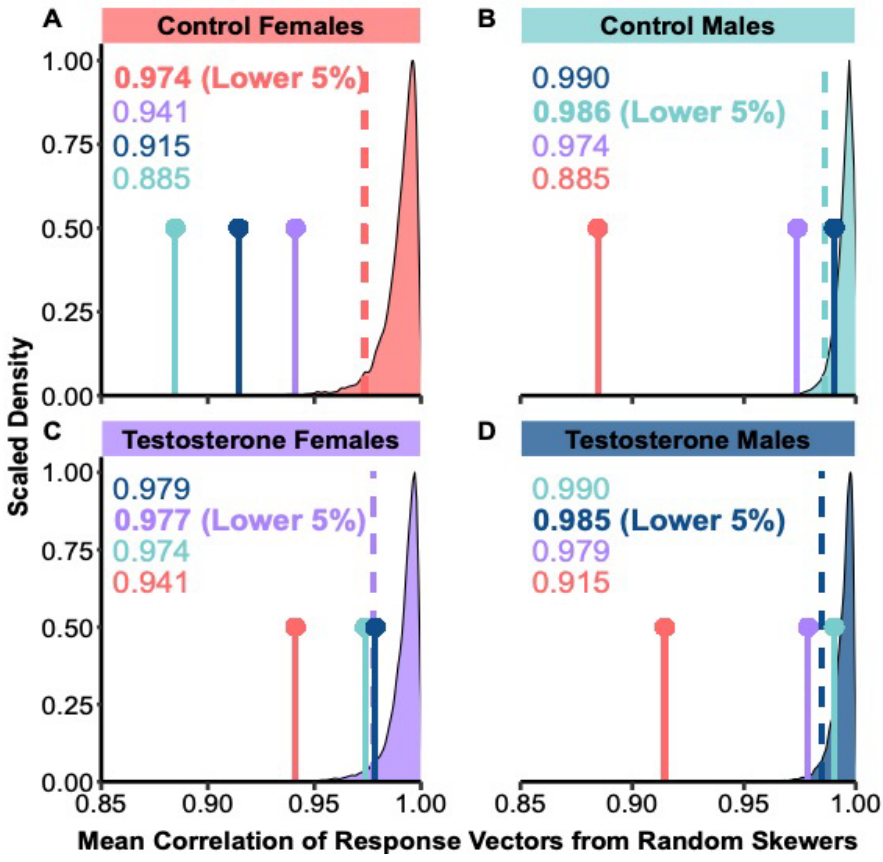


Figure S4. Distribution of between-sex selection vector correlations for 10,000 randomly drawn sexually antagonistic skewers (gray distribution), shown alongside corresponding distributions of 10,000 between-sex response vector correlations derived from passing these sexually antagonist skewers through the best estimate of the **G** matrix (including **B**) derived from control females and control males (coral distribution) or from testosterone females and control males (purple distribution). Although both **G** matrices bias the predicted evolutionary response such that it is positively correlated between males and females across the majority of sexually antagonistic selection vectors, this constraint is much stronger when **G** is estimated for testosterone females and males.

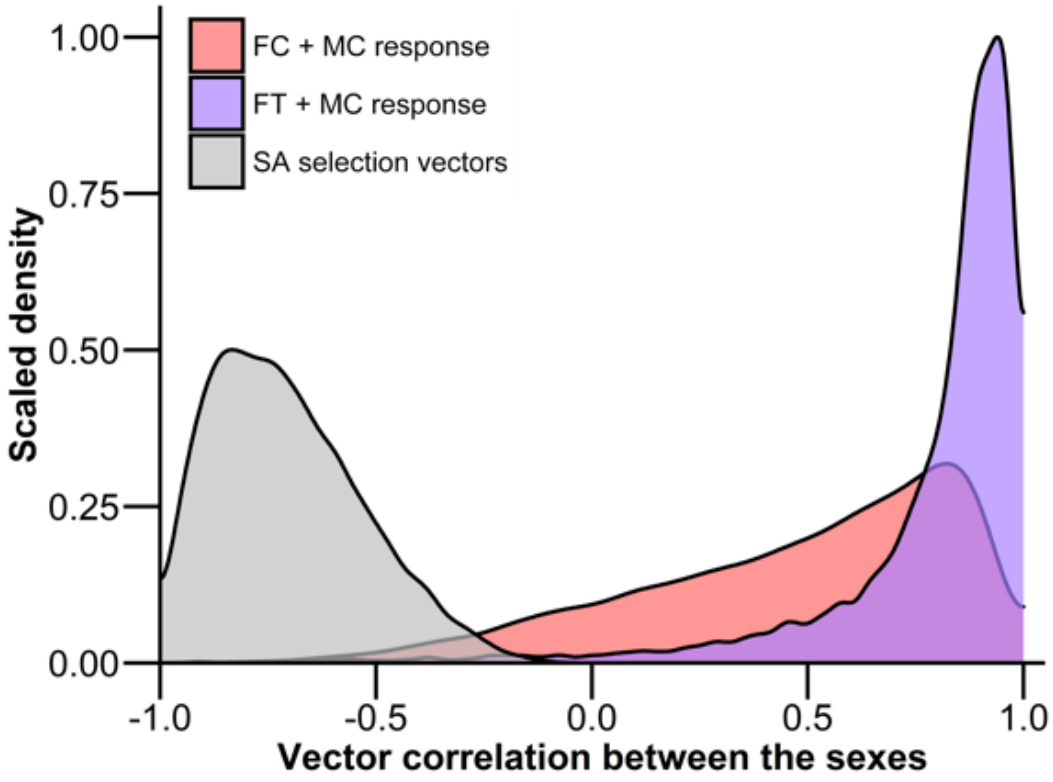
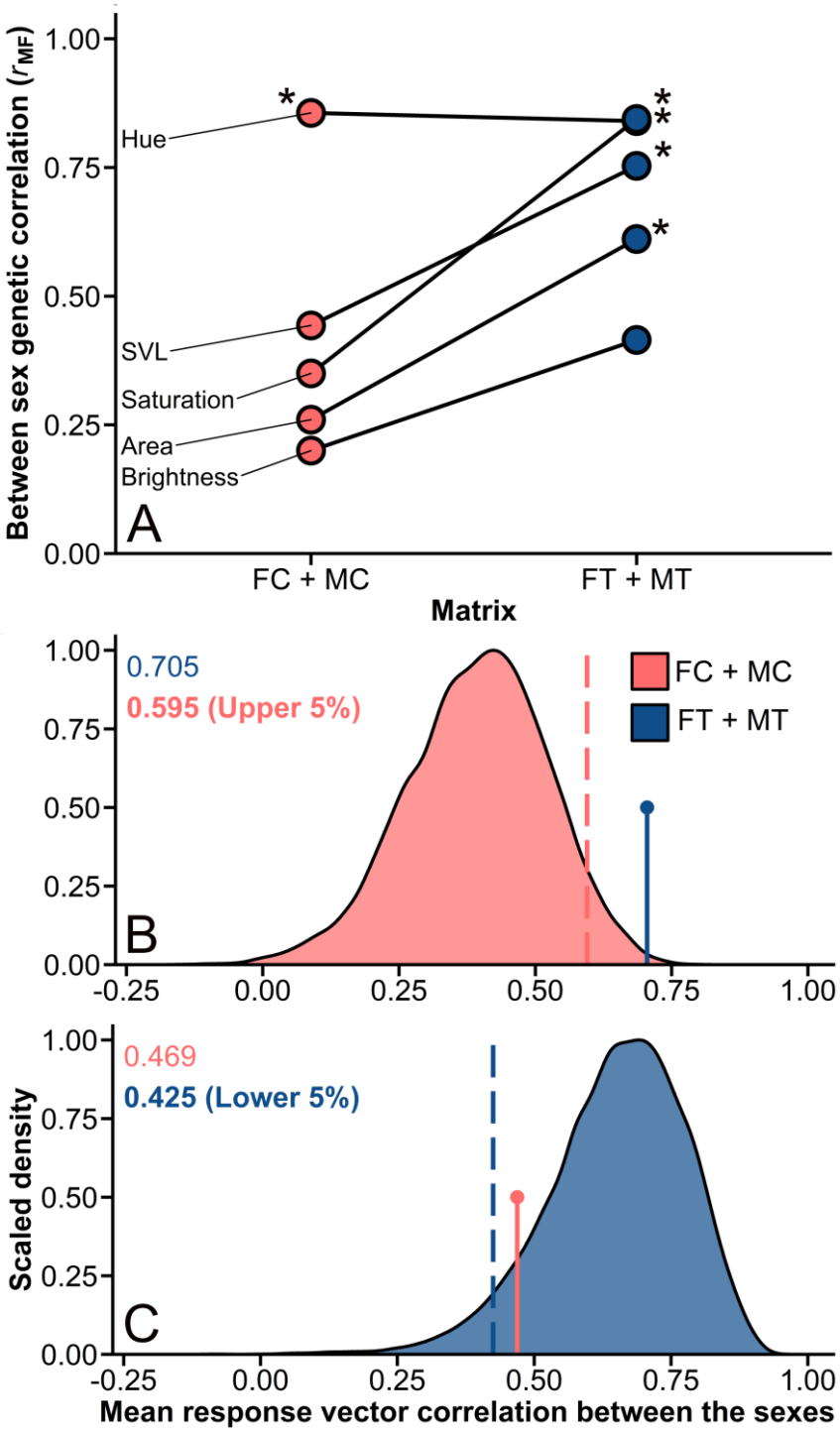


Figure S5. (A) Point estimates of r_{MF} between five homologous traits for control females and control males (FC + MC), connected to the same r_{MF} values for testosterone females and testosterone males (FT + MT). Asterisks indicate estimates significantly greater than zero. (B) Distribution of 10,000 mean vector correlations between female and male responses to sexually antagonistic skewers based on the simulated distribution of the full **G** matrix (including **B**) for control females and control males. The upper 5% bound of this null distribution is shown with a dashed line. The mean vector correlation between female and male responses using the best estimate of the full **G** matrix (including **B**) for testosterone females and testosterone males is shown with a pin and falls above the upper 5% bound. (C) The reciprocal comparison to that shown in panel B, with the mean vector correlation for control females and control males falling just within the 95% bound of the simulated distribution for testosterone females and males.


Between sex genetic correlation (r_{MF})
1.00
0.75
0.50
0.25
0.00
Hue
SVL
Saturation
Area
Brightness
A
FC + MC
FT + MT
Matrix
0.705
0.595 (Upper 5%)
FC + MC
FT + MT
B
-0.25
0.00
0.25
0.50
0.75
1.00
0.469
0.425 (Lower 5%)
Scaled density
C
Mean response vector correlation between the sexes

Chapter Two:

The evolution of monogamy is associated with reversals from male to female bias in the survival cost of parasitism[1]

[1] As published in: Wittman, T.N., and Cox, R.M. 2021. The evolution of monogamy is associated with reversals from male to female bias in the survival cost of parasitism. Proceedings of the Royal Society B, 288: 20210421. https://doi.org/10.1098/rspb.2021.0421

Abstract

The extent to which parasites reduce host survival should depend upon how hosts balance trade-offs between reproduction and survival. For example, parasites are predicted to impose greater survival costs under polygynous or promiscuous mating systems in which competition for mates favors increased reproductive investment, particularly in males. We provide, to our knowledge, the first comparative test of the hypothesis that the mating system of the host is an important determinant of (i) the extent to which parasites reduce survival, and (ii) the extent to which males and females differ in the survival cost of parasitism. Using meta-analysis of 85 published estimates of the survival cost of parasitism from 72 studies of 64 species representing diverse animal lineages, we show that parasites impose a mean 3.5-fold increase in the odds of mortality on their hosts. Although this survival cost does not differ significantly across monogamous, polygynous and promiscuous mating systems, females incur a greater survival cost than males in monogamous species, whereas males incur a greater survival cost than females in polygynous and promiscuous species. Our results support the idea that mating systems shape the relative extent to which males and females invest in reproduction at the expense of defense against parasites.

Introduction

By definition, parasites reduce host fitness, and they can do so through a variety of mechanisms, such as lowering feeding rate, decreasing fat stores, triggering costly immune responses and inducing behavioural changes that may lead to increased predation (Lafferty & Morris 1997, Scantlebury et al. 2007, Careau et al. 2010, Cox et al. 2015). Collectively, the deleterious effects of parasites can substantially impact the survival of their hosts (Robar et al. 2010). The magnitude of the survival cost of parasitism, as frequently inferred from the relative survival rates of infected versus non-infected hosts, is highly variable across host taxa (Robar et al. 2010). This variation is partially explained by differences in the parasites themselves (e.g. parasites with complex life cycles have greater effects on host survival) as well as extrinsic environmental factors (e.g. the risk of host mortality associated with parasitism decreases as latitude increases) (Robar et al. 2010). However, concepts from sexual selection and life-history theory suggest that the magnitude of the survival cost of parasitism may often depend upon how hosts balance the trade-off between survival (including resistance, the ability to limit or reduce parasite load, and tolerance, the ability to reduce the costs of a given parasite load) and reproduction (mate acquisition and offspring production) (Roff 2002, Lee et al. 2008, Råberg et al. 2009, Cox et al. 2010, Rauw 2012, Cox 2014). Moreover, the trade-off between reproduction and survival is often expected to differ as a function of sex (Trivers 1972, Delph 1999, Zuk & Stoehr 2002, Kotiaho & Simmons 2006, Hoffman et al. 2008, Santos & Nakagawa 2012) and mating system (Trivers 1972, Promislow 1992, Rever 1994, Weatherhead & Teather 1994, Karlsson 1996, Kolm et al. 2007, Simmons & García-González 2008, Innocenti et al. 2014). Therefore, host sex and mating system may

be important determinants of the extent to which hosts differ in the survival cost of parasitism. Here, we provide, to our knowledge, the first comparative test of the hypothesis that host mating system influences the extent to which parasites reduce host survival, as well as the extent to which males and females differ in this fitness cost of parasitism.

Survival and reproduction are the two primary components of Darwinian fitness. Owing to various constraints limiting maximal investment in both processes, organisms are often forced to trade current repro- duction against survival and future reproduction (Clutton-Brock et al. 1989, Stearns 1989, Cox et al. 2010, Hoffman et al. 2013, Reedy et al. 2016, Reedy et al. 2019). Because mating systems can structure the opportunity for sexual selection and fecundity selection, they can also influence the optimal levels of investment in current repro- duction versus survival for one or both sexes (Holland & Rice 1999, Martin et al. 2004, Kolm et al. 2007, Innocenti et al. 2014, Tidière et al. 2015, García-Navas et al. 2016). Given that the immune system is intimately related to survival (Pennington & Ehrie 1978, Eleftherianos et al. 2006) and energetically expensive to develop, maintain and use (Lockmiller & Deerenberg 2000, Lee 2006, Demas 2012) and that variation in immune function explains variation in survival (Møller & Saino 2004, Cichon & Dubiec 2005, Bowers et al. 2014, Seppälä 2015), the immune system is likely to be one of a number of factors mediating the trade-off between reproduction and survival. For example, in a variety of insect species, selection lines evolved under experimentally enforced monogamy have improved survival (Holland & Rice 1999, Martin & Hosken 2003, Maklakov et al. 2007, Maklakov et al. 2009) and increased immune function (Hosken 2001, McKean & Nunney 2008,

McNamara et al. 2013) relative to lines evolved under promiscuity. More broadly, mating system and reproductive investment are correlated with and known to causally affect longevity, survival and immune function (Holland & Rice 1999, Martin & Hosken 2003, Sorci et al. 1996, Höglund & Sheldon 1998, Møller et al. 1999, Fedorka et al. 2004, Previtali et al. 2012, Grazer et al. 2014, Hollis et al. 2017). Through this mechanism of immune function, variation in sexual selection and reproductive investment is hypothesized to explain variation in the survival cost of parasitism (Roff 2002). Given that polygynous and promiscuous mating systems lead to increased sexual selection relative to monogamy, we expect the survival cost of parasitism to be greater in those systems. However, in some systems and circumstances, sexual selection may reinforce natural selection (Long et al. 2012, Yun et al. 2018), and thus has the potential to drive evolutionary increase in host resistance and tolerance to parasites. Moreover, if mating carries an increased risk of infection, polygynous and promiscuous systems may favour increased immune function over monogamous systems (Hangartner et al. 2015). Males and females often use different life-history strategies, with females favoured to balance current repro- ductive success against survival and future reproduction, whereas males are often favoured to maximize current mating success at the expense of survival (Bateman 1948, Trivers 1972, Schärer 2012, Fritzsche & Arnqvist 2013, Collet et al. 2014, Cox 2014, Janicke et al. 2016, Hämäläinen 2018). Sex differences in allocation to reproduction versus survival may be mediated in part through differential investment in immune function, leading to sex differences in the fitness costs associated with parasitism (Joop et al. 2006, Stoehr & Kokko 2006). For example, in broiler chickens (*Gallus gallus domesticus*) descended from promiscuous

jungle fowl, males have weaker antibody responses and greater hatchling mortality than females, and also suffer greater mortality than females when challenged with pathogenic bacteria (Leitner et al. 1989). By contrast, the reproductive interests of males should largely overlap those of females under monogamy, and selection on patterns of allocation between mating success and survival should generally be more similar between the sexes under monogamy than under polygyny or promiscuity. Therefore, any sex differences in the survival cost of parasitism in monogamous species should be shaped primarily by sex differences in aspects of reproductive investment other than mating, such as egg and offspring provisioning, which should reduce male bias in the survival cost of parasitism and could even lead to greater survival costs in females (Bolund et al. 2016).

While there is no general pattern of male- or female-biased parasitism across all animals, it is common for one sex or the other to exhibit a higher parasite burden in any given lineage or species (McCurdy et al. 1998, Sheridan et al. 2000, Moore & Wilson 2002, Cox & John-Alder 2007). Often, such evidence of sex-biased parasitism has been implicitly assumed to translate into a sex bias in the cost of parasitism. However, males and females may also differ in their tolerance of parasites (Bordes et al. 2012, Vincent & Sharp 2014), such that a sex bias in parasite burden does not necessarily correspond to a sex difference in the fitness costs of parasitism. Although previous meta-analyses have shown that sex biases in parasitism are common in many taxa (McCurdy et al. 1998,Sheridan et al. 2000, Moore & Wilson 2002) and that parasites generally impose a substantial fitness cost in terms of host survival (Robar et al. 2010), it is presently unknown whether there is an overall trend towards male or female bias in the survival

cost of parasitism, or whether host characteristics such as mating system influence the magnitude and direction of sex bias in this cost. Here, we address these questions using a meta-analysis of experimental and descriptive studies in which survival is reported separately for individuals that are either parasitized or not. In addition to estimating the overall extent to which parasites reduce host survival, we also test (i) whether the magnitude of the survival cost of parasitism differs as a function of host mating system, (ii) whether male and female hosts differ in the survival cost of parasitism, and (iii) whether the magnitude and direction of sex bias in the survival cost of parasitism differs as a function of host mating system. Relative to monogamous species, we predict that polygynous and promiscuous species will (i) suffer a greater survival cost of parasitism and (ii) exhibit a relatively larger male bias in the survival cost of parasitism.

Methods

Data acquisition

We conducted a systematic literature search for studies investigating the effects of parasitism on survival using Web of Science and including any studies published before July 2020. We searched using logical combinations of the following keywords: parasit*, pathogen, virus, viral, protist, gregarine, fluke, trematode, cestode, mite, tick, nematode, acanthocephal*, botfly, ectoparasite, flea, louse, surviv*, mortality, virulen*, longevity, removal, treatment, infect*, experiment, medicine, medication, anthelmintic, anti, fumigation, exposure, inject*, male bias, female bias and sex bias. We included studies that quantified the survival of parasitized versus unparasitized individuals using experimental infection with parasites, experimental removal of parasites or natural variation in the presence or absence of parasites. We excluded studies that did not

provide information on costs of parasitism separately for each sex. We included all animal taxa with distinct male and female sexes except for domesticated animals. We included only those studies that reported a measure of variance associated with survival, and induced a significant change in the parasite load with experimental infection or removal. A PRISMA flow diagram depicting the filtering of the studies is provided in the supplementary material, figure S1. When infection studies used more than one dose of parasites, we took the weighted average across parasite doses, with each dose class weighted by its respective variance (Borenstein et al. 2009).

We quantified the effect size for each study as the natural log of the odds ratio for mortality (LOR), which is calculated by classifying each individual as either parasitized (*P*) or unparasitized (*U*) at the beginning of an interval, recording each individual as either dead (*D*) or alive (*A*) at the end of that interval, using these count data to calculate the odds of mortality for parasitized and unparasitized groups, and then calculating their ratio as

$$ln(\text{odds ratio}) = ln\left(\frac{P_D / P_A}{U_D / U_A}\right) = ln\left(\frac{P_D\, U_A}{U_D\, P_A}\right) = LOR$$

The sampling variance in the LOR is calculated as the sum of the inverse of each cell (P_D, P_A, U_D, U_A) (Korichev et al. 2013). If any of these four cells contained a value of five or less, we used Jewell's small sample size correction to calculate the LOR and its associated sampling variance (Jewell 1986). If count data were not presented in a study, we calculated them using data on the proportion of alive or dead individuals in each

category and their respective sample sizes. When data were unavailable to calculate the odds ratio directly, we used the compute.es package in R v. 4.0 to calculate another effect size and convert it to LOR (Del Re 2013).

We classified host mating system as monogamous, polygynous or promiscuous. We defined monogamous systems as those in which both males and females tend to have a single mate throughout a reproductive season. Because many 'socially monogamous' species may actually produce a high percentage of extrapair offspring (Griffith et al. 2002), we also collected genetic data on extrapair paternity whenever possible for all putatively monogamous species. With one exception, all putatively monogamous species for which we obtained genetic parentage data produce fewer than 11% of their clutches with multiple sires (mean = 7.4%, range 4–10% extrapair paternity), so we considered them effectively monogamous (Griffith et al. 2002). We defined polygynous systems as those in which males tend to have multiple mates whereas females tend to have a single mate. This classification includes species with diverse mating tactics, ranging from the gelada baboon (*Theropithecus gelada*) in which males hold a harem of females to the housefly (*Musca domestica*) in which females are only receptive to a single mating. We defined promiscuous systems as those in which males and females both tend to have multiple mates. We initially included an additional category for polyandrous species, defined as those in which females have multiple mates whereas males tend to have a single mate, but our final dataset did not include any species that fit this criterion. Information on host mating system was gathered from the references listed in the supplementary material, table S1. For each study, we also recorded the following measures as potential confounding sources of variation in the survival cost of parasitism:

parasite type (ectoparasite, bacteria, fungus, helminth, protist or virus), study method (parasite removal, parasite infection or natural variation in parasitism), host taxon (arthropod, bird, fish or mammal) and host age (adult, juvenile). The studies and species are given in table S1, and the full dataset is available online as a spreadsheet (see the Data accessibility statement).

Data Analysis

For analysis, we weighted effect sizes (LORs) from each study by the inverse of their respective sampling variances. For the overall cost of parasitism, we used the weighted average of the LOR of males and females from each study with the sampling variance for the average calculated as the inverse of the sum of the male and female weights (inverse of their respective sampling variances) (Borenstein 2009). To assess sex-specific effects of parasitism, we calculated the sex difference for each study (male LOR – female LOR), and calculated the associated sampling variance of this difference as the sum of male and female sampling variances minus the covariance between male and female LOR (Borenstein 2009). The correlation between male LOR and female LOR across the dataset was $r = 0.76$. Study and species were included as random factors in all models. We used profile plots of random-effect estimates to test for over-parametrization. We give estimates of heterogeneity as their raw values (τ^2 or σ^2) as well as their per cent contribution to the total variance (I^2), and for statistical tests of heterogeneity, we used Cochran's Q statistic. We tested for publication bias using a regression of the residuals from our meta-analytic models on the inverse of their respective standard errors (a measure of precision) and performed a trim-and-fill analysis to estimate the number of

missing studies and model their potential impact (Egger et al. 1997, Duval & Tweedie 2000). We used the R_0 estimator for the trim-and-fill analysis (Duval & Tweedie 2000). We detected significant publication bias for the average cost of parasitism (intercept = 0.55, s.e. = 0.18, $p = 0.003$), and trim-and-fill analysis indicated funnel plot asymmetry and missing studies (7, s.e. = 4, $p = 0.004$, supplementary material, figure S2). We did not find any indications of publication bias for the sex difference in the LOR (intercept = 0.20 ± 0.15 s.e., $p = 0.18$), nor did trim-and-fill analysis indicate funnel plot asymmetry or missing studies (1, s.e. = 2, $p = 0.25$; supplementary material, figure S3).

First, to test the hypothesis that parasites impose a survival cost on their hosts, we used the metafor package for R v. 4.0 (Viechtbauer 2010, R Core Team 2017) to perform a random-effects meta-analysis and test whether the grand mean effect size (LOR) describing the survival cost of parasitism differs significantly from zero across our entire dataset ($k = 85$ from 64 species and 72 studies; figure 1*b*). Second, to test the hypothesis that the survival cost of parasitism is influenced by host mating system, and to account for any potential influence of the additional moderator variables (host taxon, parasite type, study method and host age), we built mixed-effect meta-analytic models containing all additive combinations of all moderator variables using the multcomp package for R v. 4.0 (Calcagno & de Mazancourt 2010) ($k = 84$ from 63 species; figure 1*b*). We compared these models using the small sample size-corrected Akike information criterion (AICc) and retained the top supported model. The top 5 models, with their associated AICc values, are provided in the supplementary material, table S2. Additionally, for each moderator, we calculated an importance value as the sum of the weights of all models containing that moderator (supplementary material, table S3). Third, we tested whether

the overall sex difference in the survival cost of parasitism differed from zero using a random-effects meta-analysis (k= 85 from 64 species; figure 1*c*). Fourth, we used the same model building and comparison approach described above to test whether the sex difference in the survival cost of parasitism is influenced by host mating system while accounting for the suite of other potential moderator variables (k = 84 from 63 species; figure 1*c*). The top 5 supported models, with their associated AICc values, are provided in the supplementary material, table S4, while the moderators and their importance values are provided in the supplementary material, table S5.

For models investigating the average cost of parasitism, visual analysis of qqplots indicated minor departures of residuals from normality, while qqplots for the sex bias in the cost of parasitism indicated normality (supplementary material, figures S4 and S5). Although non-normality is not a major concern in mixed-effects models and does not bias estimates of fixed effects, it may impact estimates of their standard errors (Kontopantelis & Reeves 2012). We, therefore, used smoothed-cases bootstrapping to generate standard errors and confidence intervals (CIs) for the model coefficients from models investigating the average cost of parasitism (DiCiccio & Efron 1996, Davison & Hinkley 1997, Polansky & Schucany 1997, Canty & Ripley 2021). Smoothing was done to improve the coverage of confidence intervals, which may be negatively biased for bootstraps with small sample sizes (Davidson & Hinkley 1997). We used the boot package for R 4.0 to generate 10,000 bootstrap samples of the associated dataset (Cantly & Ripley 2021). For each data point in each bootstrap sample, we added a simulated random deviate from the standard gaussian kernel (mean = 0, variance = 1) multiplied by the smoothing bandwidth h (Polansky & Schucany 1997, Silverman 1986). We estimated h using Silverman's rule

of thumb, with the inter quartile range as our measure of scale (Silverman 1986). Each bootstrap sample was run through its associated meta-analytic model to create the bootstrap distributions of model coefficients. We generated bias-corrected and accelerated bootstrap confidence intervals for each coefficient using the boot package in R 4.0 (Cantly & Ripley 2021).

To assess the need for phylogenetic control when estimating the overall survival cost of parasitism and when testing our *a priori* hypotheses about host mating system, we first tested for phylogenetic signal in the survival cost of parasitism (LOR) and in the residuals of the regression of the survival cost of parasitism on the potential moderator variables (Revell 2010). We used this same procedure to investigate phylogenetic signal in the sex difference in the survival cost of parasitism. We created a phylogeny (figure 1*a*) using the TimeTree database (Kumar et al. 2017), which uses time since divergence to calculate branch lengths. Twenty-three of the species in our dataset were not represented in the TimeTree database, so we placed them on the phylogeny using the closest related lineage available (supplementary material, table S6). We tested for phylogenetic signal using Pagel's λ with the phylosig function in the R package phytools (Pagel 1999, Revell 2012). To account for phylogenetic signal when it was present, we included the λ-transformed phylogenetic correlation matrix as the correlation structure for the random effect of *species* in our meta-analytic models (Revell 2012, Nakagawa & Santos 2012). We used the package APE in R v. 4.0 to calculate a distance matrix from the phylogenetic tree using branch lengths and used phytools to λ-transform the phylogenetic variance–covariance matrix, after which we converted it to a correlation

matrix (Paradis et al. 2004). We only found significant phylogenetic signal for the mean survival cost of parasitism ($\lambda = 0.68$, $p = 0.04$) (upplementary material, table S7).

Results

Survival cost of parasitism

Across all species, while accounting for phylogeny, we found that parasitism imposes a significant survival cost (mean LOR = 1.25, CI = 1.03–1.55, χ^2_1 =9.57, $p = 0.002$), with parasitized individuals facing an average of 3.5 times greater odds of mortality than unparasitized individuals. Accounting for phylogeny leaves a significant amount of residual between-study heterogeneity in the survival cost of parasitism across the dataset ($Q_{84} = 944.38$ $p < 0.0001$; supplementary material, table S8). The best supported model as judged by AICc contains the predictors host mating system, study method and host age (supplementary material, table S2), which together explain a significant amount of variation in the survival cost of parasitism (χ^2_5 =18.65 ,$p = 0.002$) while leaving a significant amount of residual between-study heterogeneity ($Q_{78} = 641.97$, $p < 0.0001$; supplementary material, table S8). Of these three factors, study method is the only individually significant factor in the model (method: χ^2_2 =9.03, $p = 0.01$; host mating system: χ^2_2 =4.52, $p = 0.10$, figure 2; host age: χ^2_1 =2.61, $p = 0.11$). Comparison of levels within study method shows that studies using experimental removal or natural variation have significantly lower estimated costs of parasitism than those using experimental infections (supplementary material, table S9). In model comparisons, study method has the highest importance value of all moderators (0.81), while host age (0.53) and host

mating system (0.47) are less important and host taxon (0.19) and parasite type (0.01) appear in few well-supported models (supplementary material, table S3 and figure S6).

Sex differences in the survival cost of parasitism

We found a significant overall male bias in the survival cost of parasitism (mean sex difference in LOR = 0.24, CI = 0.07–0.40, χ_1^2 =7.77, p = 0.005) and significant between-study heterogeneity in this estimate of sex bias (Q_{84} = 195.30, p < 0.0001; supplementary material, table S10). The best supported model as judged by AICc contains the single moderator of mating system (supplementary material, table S4). Mating system explains a significant amount of variation in the sex difference in the survival cost of parasitism (χ_2^2 =27.79, p < 0.0001) while leaving significant residual between-study heterogeneity (Q_{81} = 125.22, p = 0.001; supplementary material, table S10). Both polygynous and promiscuous species have significantly more male-biased costs of parasitism than monogamous species (polygynous–monogamous = 0.90, CI = 0.53–1.26, p < 0.0001; promiscuous–monogamous = 0.79, CI = 0.46–1.13, p < 0.0001), whereas sex bias does not differ between promiscuous and polygynous species (promiscuous–polygynous = −0.10, CI = −0.41 to 0.21). Further, promiscuous and polygynous species have significantly male-biased costs while monogamous species have significantly female-biased costs (figure 3). The next best supported model contained the terms mating system and age (supplementary material, table S4), and investigation of this model revealed significant differences between the levels of mating system (as in the case of the best model), but no differences among the levels of age at any level of mating system (mating system: χ_2^2 =29.19, p < 0.0001; age: χ_1^2 =0.48, p = 0.48). Further, mating system had the maximum importance value of 1, while age had a substantially smaller

importance value of 0.28 (supplementary material, figure S7).

Discussion

On average across diverse animal lineages, we found that parasitized individuals have 3.5 times greater odds of mortality compared to unparasitized individuals. This result strengthens the findings of a previous meta-analysis on a smaller dataset (Robar et al. 2010) by confirming that parasites typically impose severe survival costs on host populations. Importantly, we extend this work by providing, to our knowledge, the first evidence that mating system is also a significant predictor of sex bias in the survival cost of parasitism. Specifically, we show that females experience greater survival costs of parasitism than males in monogamous species, whereas males tend to experience greater survival costs of parasitism in non-monogamous species. This is in line with previous work showing that male bias in parasite burden is associated with shifts from monogamy to polygyny in mammals (Moore & Wilson 2002). The importance of host mating system and the underlying dynamics of sexual selection and life history may also help explain previous work suggesting that there is no general pattern of male- or female-biased parasitism across animals, despite pronounced sex bias in many lineages and species (McCurdy et al. 1998, Sheridan et al. 2000, Cox & John-Alder 2007, Moore & Wilson 2002). Collectively, our results are consistent with the idea that different mating systems may predispose males and females to different immune strategies for parasite resistance and/or tolerance while also shaping the relative extent to which males and females invest in reproduction at the expense of defense against parasites.

Increased survival costs of parasitism in males of polygynous and promiscuous species may potentially be explained by the negative relationship between mating rate

and survival (Nady et al. 2013), which may be mediated through immune function. For example, male Indian meal moths (*Plodia interpunctella*) in populations with higher mating rates evolved lower levels of phenoloxidase, an immune defense that correlates with parasite resistance in insects, relative to males in populations with lower male mating rates (McNamara et al. 2013). This interpretation is supported by our finding that polygynous and promiscuous species exhibit a stronger male bias in the survival cost of parasitism than do monogamous species. However, we also note that many non-monogamous species exhibit a female bias in the survival cost of parasitism, and that the overall survival cost of parasitism is not significantly elevated in promiscuous species, which presumably have the highest mating rates. In monogamous systems where the mating rate is presumably reduced, costs of reproduction other than mating may become more important in structuring the survival cost of parasitism, potentially also driving the evolution of female-biased survival costs (Promislow 1992).

Male bias in the survival cost of parasitism has been proposed as a consequence of divergence in mating and life-history strategies between males and females, with females generally benefiting more than males from investment in survival and future reproduction (Rolff 2002). In monogamous species, selection on males for investment in current mating success may be reduced in favour of investment in survival and future reproduction, producing a life history more similar to that of females (Promislow 1992, Liker & Székely 2005, Tidière et al. 2015). In these situations, offspring production, deposition and maternal care, which can be costly in terms of energy, nutrients and immune defense (Shine 1980, Clutton-Brock et al. 1989, Hanssen et al. 2005, Cox & Calsbeek 2009), may lead to relatively higher parasite-mediated mortality in females.

Thus, in many monogamous species (and potentially some polygynous and polyandrous species), female reproductive investment may impose a cost of parasite-associated mortality that is equal to or greater than that arising from male investment in courtship and competition for mates. Across mammals, parasitism and overall mortality tend to be sexually equivalent or female-biased in monogamous mating systems, but male-biased in polygynous systems (Promislow 1992, Moore & Wilson 2002). Our findings provide support for the idea that parasitism contributes to overall patterns of sex-biased mortality (Promislow 1992, Promislow et al. 1992), thereby shaping sex-specific patterns of life history and longevity.

The measurement of the survival cost of parasitism used here is directly interpretable as the log of the ratio of the opportunity for survival selection in parasitized populations relative to that in unparsitized populations. Thus, parasites on average cause a 3.5-fold increase in the opportunity for survival selection. By shaping the opportunity for selection, parasites may be key determinants of the strength of selection on a variety of phenotypes. Further, males tend to experience a greater opportunity for parasite-mediated survival selection than females in promiscuous and polygynous species, whereas the opposite occurs in monogamous species. Thus, parasites may also be important in shaping sex-specific patterns of selection via survival and potentially driving sexual conflict. Parasites may also increase the variance in reproductive fitness in a sex-specific manner. For example, in *Drosophila nigrospiracula*, mite parasitism increases the variance in mating success, with males experiencing a greater increase than females (Polak & Markow 1995). Likewise, in *Drosophila melanogaster*, parasite infection increases the fitness costs of mutations to a greater extent in males than in females,

driving sex-specific selection (Sharp & Vincent 2015). Therefore, sex differences in the fitness costs of parasitism have the potential to influence a variety of important evolutionary dynamics.

One caveat to our correlative study is that we cannot tease apart whether host mating system causally influences the survival cost of parasitism, or vice versa. Processes related to reproductive success in both males (increased growth rate, rapid development) and females (fecundity, offspring provisioning) are known to trade off with immune function and survival upon immune challenge (Cox et al. 2015). However, parasites may also select for these traits in their hosts, and can potentially cue plastic adjustments of phenotypes (Agnew et al. 2000). For example, both sexes of the freshwater snail, *Potamopyrgus antipodarum*, had an increased mating rate and a greater number of mating partners when exposed to a parasitic trematode (Soper et al. 2014).

Our dataset comprises a relatively small number of species (64 total), with the majority of studies (97%) conducted on arthropods, mammals or birds, which may temper the generality of our findings across other lineages. Moreover, our dataset contains relatively few monogamous species (14 total), with only three major lineages (arthropods, mammals and birds) representing clearly independent evolutionary transitions to monogamy. Nonetheless, nearly all of these monogamous species (13 of 14) exhibit a female bias in the survival cost of parasitism, which differs from the strong general trend towards male bias in non-monogamous species. Therefore, we consider our results suggestive, though not conclusive, of an evolutionary association between host mating system and sex differences in the extent to which parasites reduce survival. How

and why mating system influences the survival cost of parasitism are open questions, and could prove fruitful directions for future research. To allow for a more informative synthesis, future studies should aim to quantify the sex-specific costs of parasitism using both survival and reproduction as measures of fitness.

Data accessibility

All data used in the analysis are available as a spreadsheet from the Open Science Framework at the following link https://osf.io/z62br, doi:10.17605/OSF.IO/Z62BR.

Authors' contributions

T.N.W.: conceptualization, data curation, formal analysis, methodology, visualization, writing—original draft, writing—review and editing; R.M.C.: conceptualization, resources, supervision, visualization, writing—review and editing. Both authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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Figures

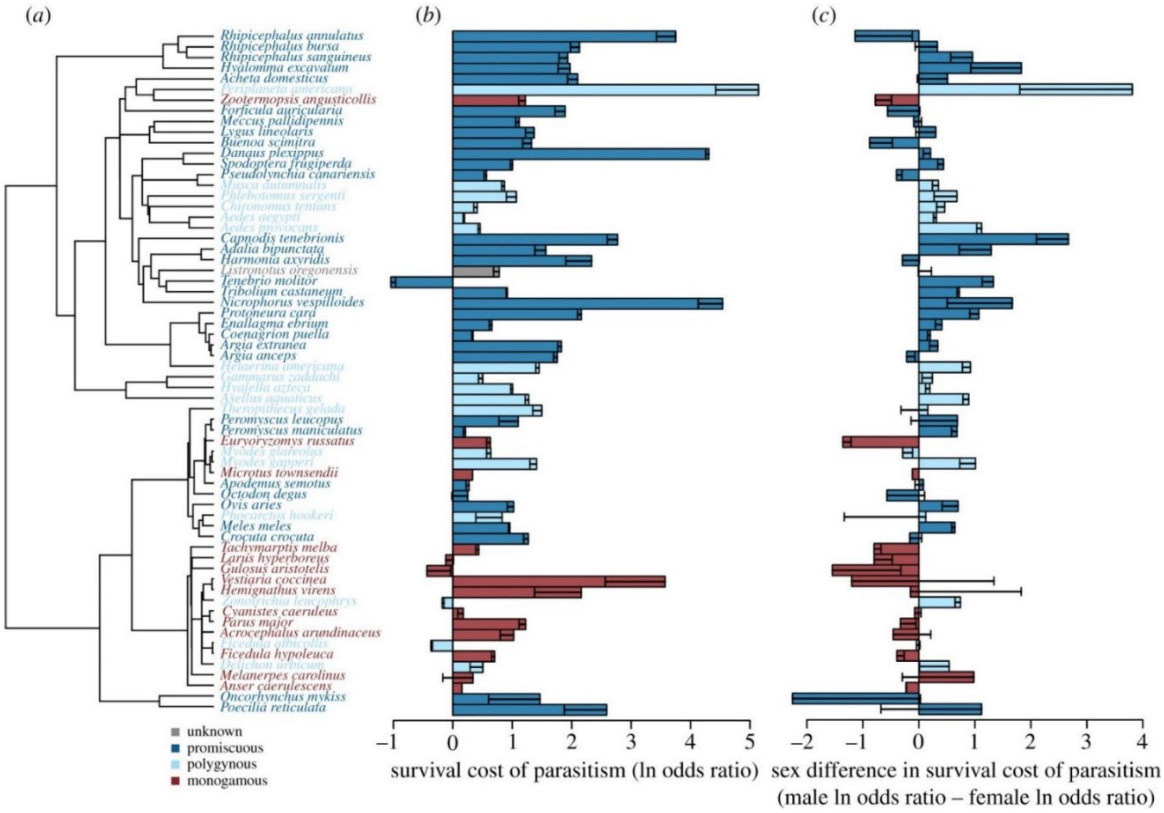

Figure 1. (A) The phylogenetic relationships of the species used; the species are color coded by mating system. (B) The mean (+ or – the sampling variance) survival cost of parasitism (mean of the ln odds ratio across studies and/or sexes) for each species. (C) The mean (+ or – the sampling variance) difference in the survival cost of parasitism between males and females (sex difference in ln odds ratio across studies).

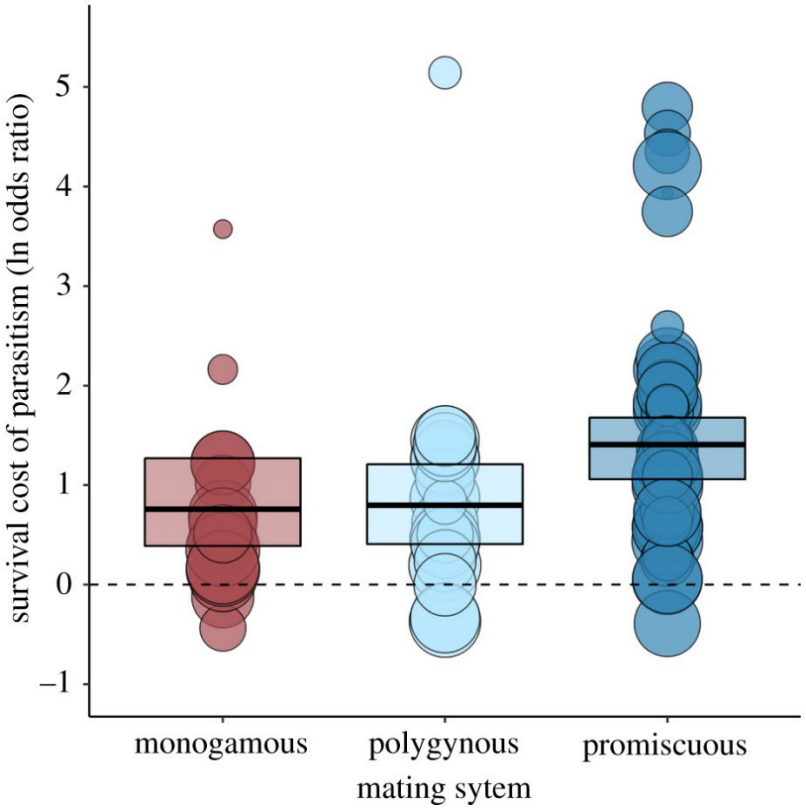

Figure 2. Estimates of the survival cost of parasitism grouped by host mating system. Means from the meta-analytic model are given by the center line in each box, and the upper and lower edges are the 95% confidence intervals. Points represent means of each individual data set, with size proportional to the inverse of the variance in the ln odds ratio. The mean survival cost of parasitism does not differ as a function of host mating system.

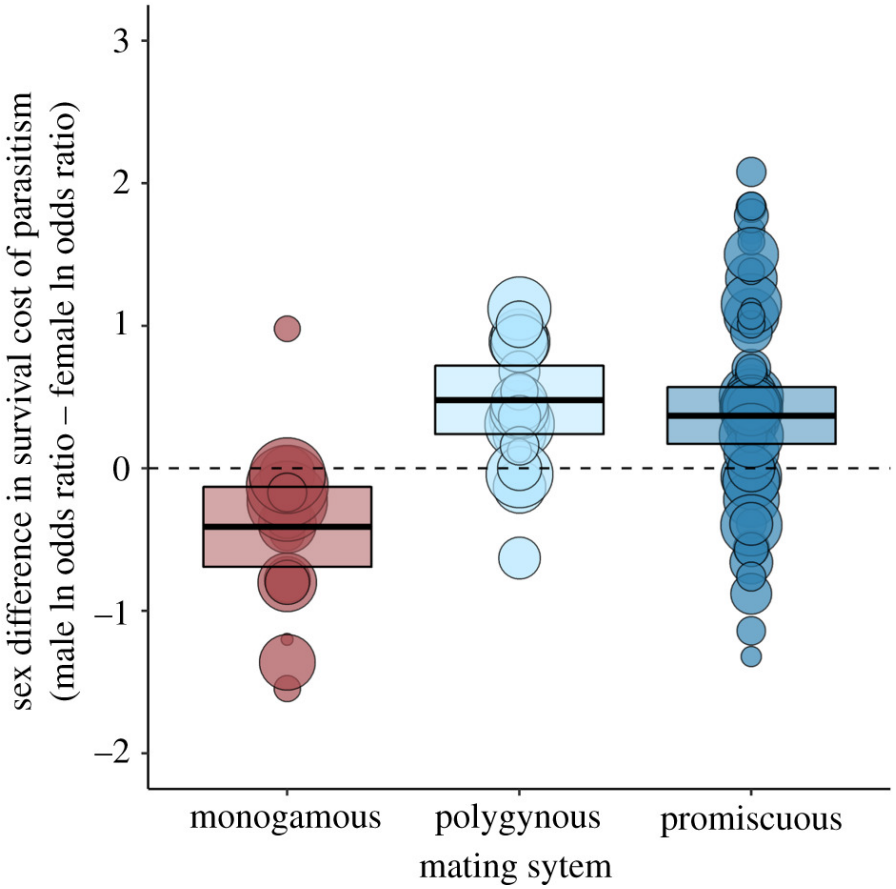

Figure 3. Sex differences in the survival cost of parasitism for species in which males and females were paired within a study, grouped by host mating system. Mean differences are given by the center line in each box, and the upper and lower edges are the 95% confidence intervals. Points are the sex difference in male and female means for each data set, with their size proportional to the inverse of the variance in the difference. The mean sex difference in the survival cost of parasitism differs significantly across host mating systems.

Supplementary Tables

Table S1. Full dataset (k = 85 observations from 64 species) used to quantify the overall survival cost of parasitism, its sex difference, and test the effect of mating system on both. *Study* is the publication from which the log odds ratio was extracted. Full references are given in the reference list below (pages 109-115). *Species* gives the species that the information in the respective row is for. *Mating System* describes the mating system of the associated species (Promiscuous, Polygynous, and Monogamous). *Reference for Mating System* lists the publication from which the mating system for a species was characterized, the full references are given below (pages 115-119). *Taxon* describes the broad taxonomic group of the species (Arthropod, Bird, Fish, or Mammal).

Study	Species	Mating System	Reference for mating system	Taxon
Gray 1998	*Acheta domesticus*	Promiscuous	Gray 1998	Arthropod
Asghar 2015	*Acrocephalus arundinaceus*	Monogamous	Griffith 2002	Aves
Webberley 2002	*Adalia bipunctata*	Promiscuous	Haddrill 2008	Arthropod
Webberley 2002	*Adalia bipunctata*	Promiscuous	Haddrill 2008	Arthropod
Bedhomme 2004	*Aedes aegypti*	Polygynous	Richardson 2015	Arthropod
Rau 1991	*Aedes provocans*	Polygynous	Yuval 2006	Arthropod
Souchay 2013	*Anser caerulescens*	Monogamous	Griffith 2002	Aves
Jhan-Wei 2014	*Apodemus semotus*	Promiscuous	Shaner 2018	Mammal
Lo 2015	*Apodemus semotus*	Promiscuous	Shaner 2018	Mammal
Córdoba-Aguilar 2013	*Argia anceps*	Promiscuous	Caesar 2012	Arthropod
Córdoba-Aguilar 2013	*Argia extranea*	Promiscuous	Caesar 2012	Arthropod
Benesh 2007	*Asellus aquaticus*	Polygynous	Jormalainen 1998	Arthropod
Lanciani 1982	*Buenoa scimitra*	Promiscuous	Rowe 1994	Arthropod
Morton 2009	*Capnodis tenebrionis*	Promiscuous	Bonsignore 2014	Arthropod
Morton 2013	*Capnodis tenebrionis*	Promiscuous	Bonsignore 2014	Arthropod

Weiberg 1997	*Chironomus tentans*	Polygynous	Eberhard 2004	Arthropod
Braune 2001	*Coenagrion puella*	Promiscuous	Thompson 2011	Arthropod
Joop 2006	*Coenagrion puella*	Promiscuous	Thompson 2011	Arthropod
Marescot 2018	*Crocuta crocuta*	Promiscuous	East 2003	Mammal
Marescot 2018	*Crocuta crocuta*	Promiscuous	East 2003	Mammal
Podmokla 2016	*Cyanistes caeruleus*	Monogamous	Griffith 2002	Aves
de Roode 2006	*Danaus plexippus*	Promiscuous	Svard 1988	Arthropod
Lindsey 2009	*Danaus plexippus*	Promiscuous	Svard 1988	Arthropod
Magallanes 2017	*Delichon urbicum*	Polygynous	Griffith 2002	Aves
Leonard 1999	*Enallagma ebrium*	Promiscuous	Fincke 1982	Arthropod
Leonard 1999	*Enallagma ebrium*	Promiscuous	Forbes 1991	Arthropod
Bergallo 2000	*Euryoryzomys russatus*	Monogamous	Bergallo 2004	Mammal
Kulma 2013	*Ficedula albicollis*	Polygynous	Griffith 2002	Aves
Kulma 2013	*Ficedula hypoleuca*	Monogamous	Griffith 2002	Aves
Arcila 2020	*Forficula auricularia*	Promiscuous	Sandrin 2015	Arthropod
Arundell 2019	*Gammarus zaddachi*	Polygynous	Jormalainen 1998	Arthropod
Granroth-Wilding 2015	*Gulosus aristotelis*	Monogamous	Griffith 2002	Aves
Gegner 2018	*Harmonia axyridis*	Promiscuous	Awad 2015	Arthropod
Riddick 2010	*Harmonia axyridis*	Promiscuous	Awad 2015	Arthropod
Atkinson 2000	*Hemignathus virens*	Monogamous	Ripper 1987	Aves
Córdoba-Aguilar 2013	*Hetaerina americana*	Polygynous	Córdoba-Aguilar 2011	Arthropod
Duclos 2006	*Hyalella azteca*	Polygynous	Wen 1993	Arthropod
Kokkotis 2005	*Hyalella azteca*	Polygynous	Wen 1993	Arthropod
Samish 2000	*Hyalomma excavatum*	Promiscuous	Cutulle 2010	Arthropod
Bustnes 2006	*Larus hyperboreus*	Monogamous	Brouwer 2019	Aves
Gagnon 2018	*Listronotus oregonensis*	Unknown		Arthropod
Musser 2012	*Lygus lineolaris*	Promiscuous	Brent 2010	Arthropod
Martinez-Sanchez 2007	*Meccus pallidipennis*	Promiscuous	Vitta 2009	Arthropod
Schrader 2003	*Melanerpes carolinus*	Monogamous	Griffith 2002	Aves
McDonald 2014	*Meles meles*	Promiscuous	Dugdale 2007	Mammal
Wilkinson 1999	*Meles meles*	Promiscuous	Dugdale 2007	Mammal
Boonstra 1980	*Microtus townsendii*	Monogamous	Lambin 1991	Mammal

Boonstra 1980	*Microtus townsendii*	Monogamous	Lambin 1991	Mammal
Steen 2002	*Microtus townsendii*	Monogamous	Wolff 2007	Mammal
Arimoto 2012	*Musca autumnalis*	Polygynous	Mansour 1987	Arthropod
Lemaitre 2009	*Myodes gapperi*	Polygynous	Tisell 2019	Mammal
Cayol 2018	*Myodes glareolus*	Polygynous	Garcia-Navas 2015	Mammal
Kallio 2007	*Myodes glareolus*	Polygynous	Garcia-Navas 2015	Mammal
Miller 2018	*Nicrophorus vespilloides*	Promiscuous	Scott 1998	Arthropod
Botto-Mahan 2012	*Octodon degus*	Promiscuous	Ebensperger 2019	Mammal
Currie 2007	*Oncorhynchus mykiss*	Promiscuous	Seamons 2004	Fish
Craig 2009	*Ovis aries*	Promiscuous	Soulsbur 2010	Mammal
Gulland 1992	*Ovis aries*	Promiscuous	Soulsbur 2010	Mammal
Gulland 1992	*Ovis aries*	Promiscuous	Soulsbur 2010	Mammal
Gulland 1993	*Ovis aries*	Promiscuous	Soulsbur 2010	Mammal
Lachish 2012	*Parus major*	Monogamous	Griffith 2002	Aves
Morton 2013	*Periplaneta americana*	Polygynous	Bell 2007	Arthropod
Vandergrift 2008	*Peromyscus leucopus*	Promiscuous	Xia 1991	Mammal
Fuller 1996	*Peromyscus maniculatus*	Promiscuous	Xia 1991	Mammal
Luis 2012	*Peromyscus maniculatus*	Promiscuous	Xia 1991	Mammal
Wilde 2019	*Peromyscus maniculatus*	Promiscuous	Xia 1991	Mammal
Lantova 2011	*Phlebotomus sergenti*	Polygynous	Yuval 2006	Arthropod
Chilvers 2009	*Phocarctos hookeri*	Polygynous	Foote 2018	Mammal
Dargent 2015	*Poecilia reticulata*	Promiscuous	Neff 2008	Fish
Cordoba-aguilar 2013	*Protoneura cara*	Promiscuous	Nava Bolanos 2011	Arthropod
Waite 2012	*Pseudolynchia canariensis*	Promiscuous	Bonomi 2011	Arthropod
Samish 2000	*Rhipicephalus annulatus*	Promiscuous	Cutulle 2010	Arthropod
Samish 2000	*Rhipicephalus bursa*	Promiscuous	Cutulle 2010	Arthropod
Samish 2000	*Rhipicephalus sanguineus*	Promiscuous	Cutulle 2010	Arthropod
Simmons 1994	*Spodoptera frugiperda*	Promiscuous	Murua 2008	Arthropod
Bize 2005	*Tachymarptis melba*	Monogamous	Martins 2002	Aves
Hurd 2001	*Tenebrio molitor*	Promiscuous	Drnevich 2003	Arthropod

Nguyen 2015	*Theropithecus gelada*	Polygynous	Mitani 1996	Mammal
Hangartner 2015	*Tribolium castaneum*	Promiscuous	Pai & Bernasconi 2008	Arthropod
Kramarz 2014	*Tribolium castaneum*	Promiscuous	Pai & Bernasconi 2008	Arthropod
Kramarz 2016	*Tribolium castaneum*	Promiscuous	Pai & Bernasconi 2008	Arthropod
Shostak 2015	*Tribolium castaneum*	Promiscuous	Pai & Bernasconi 2008	Arthropod
Atkinson 1995	*Vestiaria coccinea*	Monogamous	Kuntz 2008	Aves
Zylberberg 2015	*Zonotrichia leucophrys*	Polygynous	Poesel 2011	Aves
Rosengaus 2000	*Zootermopsis angusticollis*	Monogamous	Nalepa & Jones 1991	Arthropod

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Table S2. The top five models by AICc value for the survival cost of parasitism. *Model structure* gives the set of factors included in the meta-analytic mode, all models also include study and species as random effects. Descriptions of the factors and their levels can be found in the supplementary data file whose link can be found in the data availability statement of the main manuscript. *AICc* gives the small sample corrected Akaike information criterion value for the respective model.

Model structure	**AICc**
~1 + Method + Age + Mating System	241.03
~1 +Method + Age	241.14
~1 + Method + Mating System	241.31
~1 + Method	241.37
~1 + Age + Taxon	244.05

Table S3. Moderators for the survival cost of parasitism and their model averaged importance, calculated as the sum of the weights of the models they appear in.

Moderator	**Importance**
Method	0.81
Age	0.53
Mating System	0.47
Taxon	0.19
Parasite type	0.01

Table S4. The top five models by AICc value for the sex-difference in the survival cost of parasitism. *Model structure* gives the set of factors included in the meta-analytic mode, all models also include study and species as random effects. Descriptions of the factors and their levels can be found in the supplementary data file whose link can be found in the data availability statement of the main manuscript. *AICc* gives the small sample corrected Akaike information criterion value for the respective model.

Model structure	**AICc**
~1 + Mating System	187.34
~1 + Mating System + Age	189.23
~1 + Mating System + Method	191.54
~1 + Mating System + Method + Age	193.53
~1 + Mating System + Taxon	193.71

Table S5. Moderators for the sex difference in the survival cost of parasitism and their model averaged importance, calculated as the sum of the weights of the models they appear in.

Moderator	**Importance**
Mating System	1.00
Age	0.28
Method	0.11
Taxon	0.04
Parasite	0.03

Table S6. List of the twenty three species used in our meta-analysis that were not represented on TimeTree, alongside the closest related species on TimeTree that was used to place the focal species on the phylogenies depicted in Figs. 1-2.

Focal species in original study	**Closest related species on TimeTree**
Ageneotettix deorum	*Ceracris kiangsu*
Aedes sierrensis	*Ochlerotatus triseriatus*
Paracalliope novizealandiae	*Paralicella caperesca*
Paracalliope novizealandiae	*Paralicella caperesca*
Pseudolynchia canariensis	*Glossina morsitans*
Capnodis tenebrionis	*Anthaxia hungarica*
Hyalella Azteca	*Caprella mutica*
Gammarus zadachi	*Gammarus fossarum*
Listronotus oregonensis	*Listronotus cryptops*
Musca autumnalis	*Musca domestica*
Lygus lineolaris	*Lygus rugulipennis*
Meccus pallidipennis	*Rhodnius prolixus*
Phlebotomus sergenti	*Phlebotomus papatasi*
Aedes provocans	*Ochlerotatus triseriatus*
Buenoa scimitra	*Enithares tibialis*
Protoneura cara	*Neoneura maria*
Hyalomma excavatum	*Amblyomma triguttatum*
Rhipicephalus annulatus	*Rhipicephalus sanguineus*
Rhipicephalus bursa	*Amblyomma triguttatum*
Argia anceps	*Coenagrion scitulum*
Argia extranea	*Coenagrion caerulescens*
Capnodis tenebrionis	*Anthaxia hungarica*
Adalia bipunctata	*Psyllobora vigintiduopunctata*

Table S7. Test statistics and *P*-values of tests for phylogenetic signal. Residuals are from the regression of species average log odds ratios (LOR, Table S1) on the moderators, or from the regression of sex differences in LOR (male LOR – female LOR, Table S1) on the moderators.

Trait	Pagel's lambda	*P*
Average LOR	0.68	0.04
Residuals of LOR on Mating System	0.03	0.67
Residuals of LOR on Method	0.000	1
Residuals of LOR on Age	0.22	0.12
Residuals of LOR on Taxon	0.000	1
Residuals of LOR on Parasite type	0.28	0.053
Average Sex Difference in LOR	0.11	0.10
Residuals of Sex Difference on Mating System	0.001	1
Residuals of Sex Difference on Method	0.04	0.47
Residuals of Sex Difference on Age	0.000	1
Residuals of Sex Difference on Taxon	0.000	1
Residuals of Sex Difference on Parasite type	0.099	0.14

Table S8. Between-study heterogeneity statistics for models of the survival cost of parasitism.

Model	σ^2 Species (95%CI)	σ^2 Study (95%CI)	I^2
Intercept only	0.79 (0.22 to 1.68)	0.45 (0.18 to 0.95)	96.04
Mating System + Method + Age	0.32 (0.01 to 0.79)	0.50 (0.20 to 1.06)	91.31

Table S9.

Survival cost of parasitism (LOR) as a function of *Method*, which is a significant modifier of the survival cost of parasitism ($QM_2 = 9.03$, $P = 0.01$). The abbreviations for the methods given within comparison are, Exp. Inf is experimental infection, Exp. Rem is experimental removal, and Nat. Var is natural variation. LOR estimates are derived from a meta-analytic mixed-effects model with *Species* and *Study* as random effects and *Method*, *Mating System*, and *Age* as main effects, while confidence intervals are derived from smoothed-cases bootstrapping.

Comparison	**Estimate**	**95%CI**	***P***
Exp. Inf. - 0	1.48	(0.80, 2.10)	< 0.0001
Exp. Rem. - 0	0.51	(-0.39, 1.36)	0.27
Nat. Var. - 0	0.97	(0.16, 1.71)	0.02
Exp.Rem - Exp.Inf	-0.97	(-1.65, -0.34)	0.002
Nat.Var - Exp.Inf	-0.51	(-0.97, -0.07)	0.04
Exp.Rem - Nat.Var	-0.46	(-1.14, 0.14)	0.15

Table S10. Between-study heterogeneity statistics for models of the sex difference in the survival cost of parasitism.

Model	$\sigma^2_{\text{Species (95\%CI)}}$	$\sigma^2_{\text{Study (95\%CI)}}$	I^2
Intercept only	0.12 (0.00 to 0.35)	0.09 (0.00 to 0.35)	63.70
Mating System	0.06 (0.00 to 0.20)	0.03 (0.00 to 0.18)	40.03

Supplementary Figures

Figure S1. A PRISMA flow diagram describing the selection of the pool of potential studies and the filtering of these studies down to the final set used in the meta-analysis.

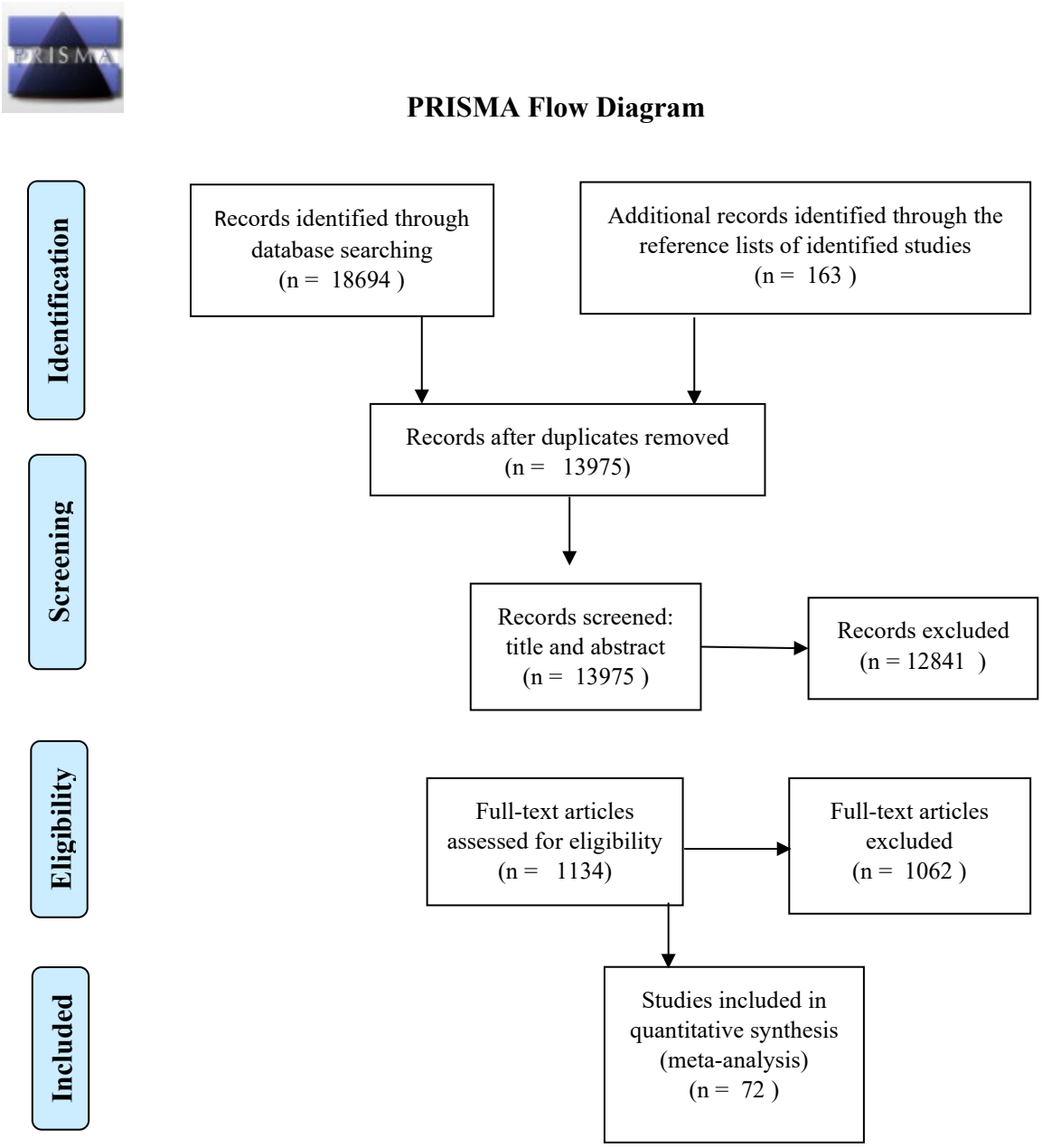


Figure S2. Funnel plot for the survival cost of parasitism. Black points are observed studies while white points are estimated missing studies from a trim-and-fill analysis.

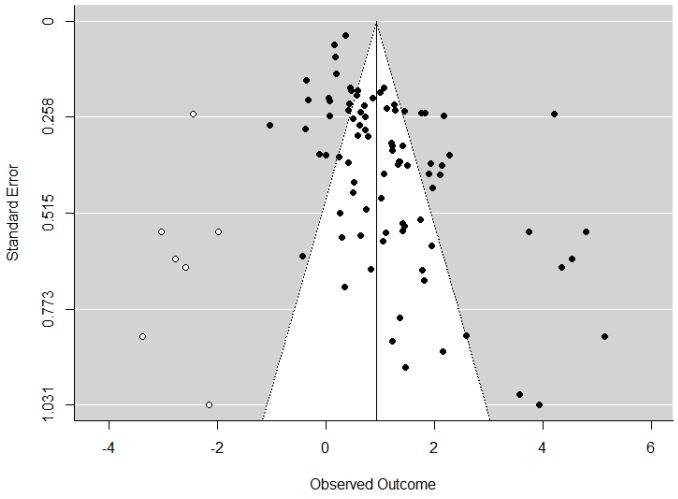


Figure S3. Funnel plot for the sex difference in the survival cost of parasitism. Black points are observed studies while white points are estimated missing studies from a trim-and-fill analysis.

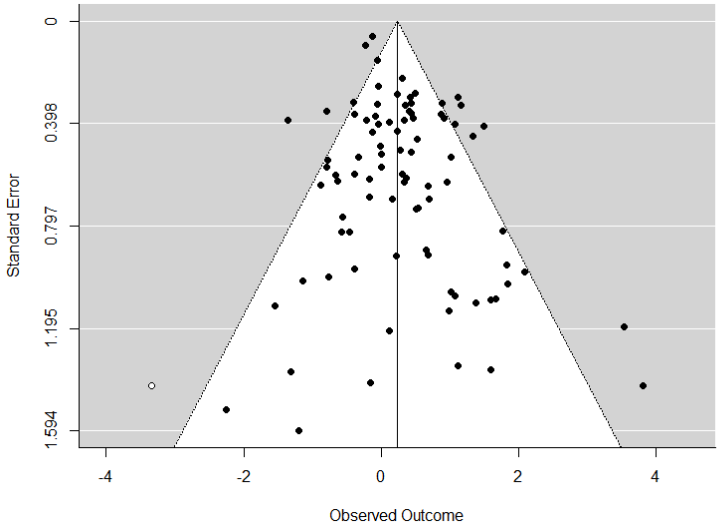


Figure S4. QQplot from the base meta-analytic model for the survival cost of parasitism. Due to non-normality indicated by this plot we used smoothed-cases bootstrapping to estimate standard errors and confidence intervals for models estimating the survival cost of parasitism.

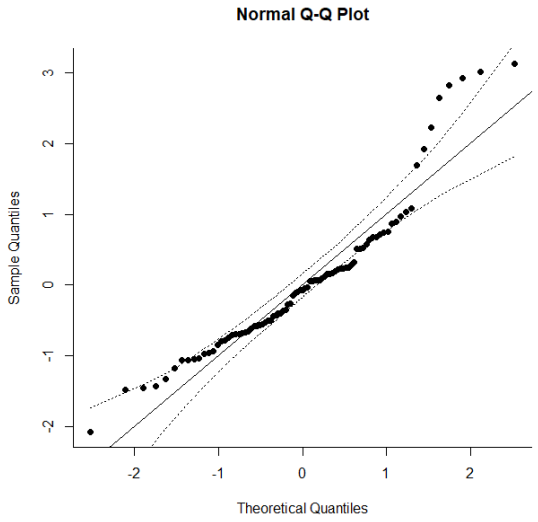


Figure S5. QQplot from the base meta-analytic model for the sex difference in the survival cost of parasitism. This plot did not indicate any departure from normality.

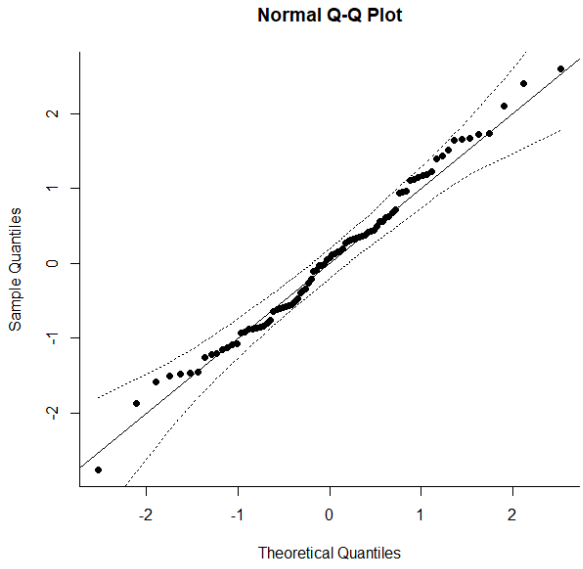


Figure S6. Moderators for the survival cost of parasitism and their model-averaged importance, which is calculated as the sum of the weights of the models they appear in.

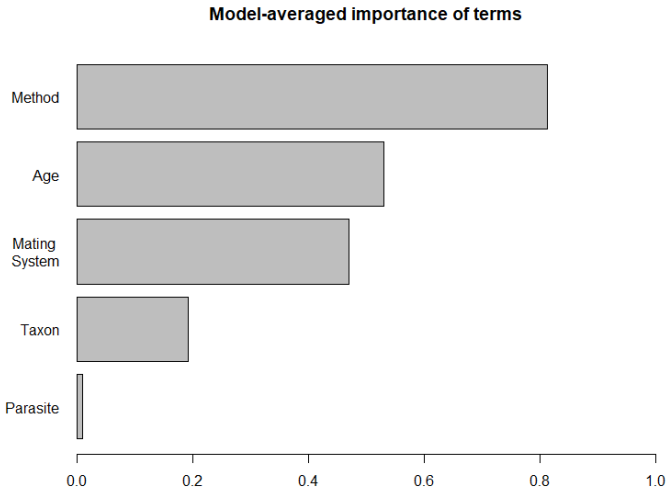
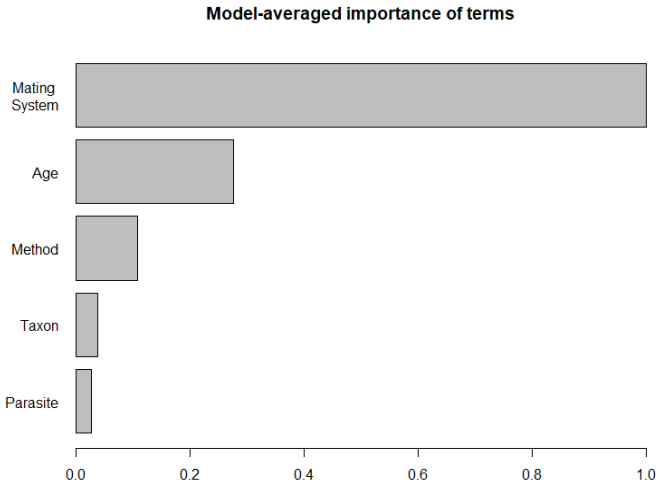


Figure S7. Moderators for the sex difference in the survival cost of parasitism and their model-averaged importance, which is calculated as the sum of the weights of the models they appear in.



Chapter Three:

Experimental removal of nematode parasites increases growth, sprint speed, and mating success in brown anole lizards[1,2]

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[1] Published in part in: Wittman, T.N., Carlson, T.A., Robinson, C.R, Bhave, R.S., & Cox, R.M. 2022. Experimental removal of nematode parasites increases growth, sprint speed, and mating success in brown anole lizards. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 1-15. https://doi.org/10.1002/jez.2644

[2] This chapter includes additional data from a replicate experiment that I performed after the preparation of the published manuscript. The data on juveniles from 2021 is not in the published manuscript.

Abstract

Parasites interact with nearly all free-living organisms and can impose substantial fitness costs by reducing host survival, mating success, and fecundity. Parasites may also indirectly affect host fitness by reducing growth and performance. However, experimentally characterizing these costs of parasitism is challenging in the wild because common anti-parasite drug formulations require repeated dosing that is difficult to implement in free-living populations, and because the extended-release formulations that are commercially available for livestock and pets are not suitable for smaller animals. To address these challenges, we developed a method for the long-term removal of nematode parasites from brown anole lizards (*Anolis sagrei*) using an extended-release formulation of the anti-parasite drug ivermectin. This treatment eliminated two common nematode parasites in captive adult males and dramatically reduced the prevalence and intensity of infection by these parasites in wild adult males and females. Experimental parasite removal significantly increased the sprint speed of captive adult males, the mating success of wild adult males, and the growth of wild juveniles of both sexes. Although parasite removal did not have any effect on survival in wild anoles, parasites may influence fitness directly through reduced mating success and indirectly through reduced growth and performance. Our method of long-term parasite manipulation via an extended-release formulation of ivermectin should be readily adaptable to many other small vertebrates, facilitating experimental tests of the extent to which parasites affect host phenotypes, fitness, and eco-evolutionary dynamics in the wild.

Introduction

Parasites interact with nearly all free-living organisms and are known to impose a variety of costs on their hosts, including decreases in host growth, fat storage, and performance, increases in host metabolism and changes in host behavior (Lafferty & Morris 1996, Tierney et al. 1996, Wedekind & Milinski 1996, Forbes et al. 2002, Eraud et al. 2005, Careau et al. 2010, Hawley et al. 2012, Moore 2013, Cox et al. 2015, Binning et al. 2017, Finnerty et al. 2017, Kelehear et al. 2019). The costs are in part mediated through activation of energetically expensive immune responses and collateral damage from inflammation and the production of reactive oxygen species (Sadd & Siva-jothy 2006, Dowling & Simmons 2009, Demas et al. 2012, Ashley et al. 2012, Hasselquist & Nilsson 2012). Ultimately, these costs are expected to accrue in terms of fitness, and parasites have frequently been found to reduce the survival and reproductive success of their hosts (Schall 1983, Pai & Yan 2003, Newey & Thirgood 2004, Robar et al. 2010, Patterson et al. 2013, Albery et al. 2021, Wittman & Cox 2021, however, Raveh et al. 2011 and Raveh et al. 2015). Through their detrimental effects on host fitness, parasites may regulate host populations and influence their evolutionary dynamics (Anderson & May 1978, Pedersen & Fenton 2015).

Early studies on the effects of parasitism in natural populations were predominantly observational, and their inferences often relied on age structure of parasite burden, associations between individual fitness and parasite burden, or associations between parasitism and proxies for host fitness (Minchella & Scott 1991, Rousset et al. 1996, Pedersen & Fenton 2015, Coulson et al. 2018). However, many processes can create positive or negative correlations between parasite burden and host characteristics

in the absence of a direct causal relationship (e.g., individual hosts that feed more may grow more and also ingest more parasites; hosts in poor condition may have both weakened immune responses and lower clearance of parasites). Therefore, observational studies cannot establish the causal influence of parasitism on individual hosts or, by extension, on host populations. Experimental infection studies have increased our understanding of the costs of parasitism in terms of host survival and reproductive success, but they are generally performed in a laboratory or controlled environment in which organisms are removed from natural conditions that they experience in the wild (Atkinson et al. 1995, Ebert et al. 2000, Blaser & Schmid-Hempel 2005, Ilmonen et al. 2008, Vincent & Sharp 2014). The experimental removal of parasites in a framework that tracks individual hosts through time and under natural conditions provides a powerful approach to study the causal effects of parasitism on host populations. As this experimental approach has become more common, it has led to new insights on the effects of parasitism on host phenotypes (growth, body condition, performance, behavior) and fitness (survival and reproduction) while also increasing our understanding of co-infection dynamics and epidemiological processes (Pedersen & Antonovics 2013, Pedersen & Fenton 2015, Budischak et al. 2016, Binning et al. 2017, Sanchez et al. 2018, Sweeny et al. 2020). While the popularity of parasite removal experiments has grown, the taxonomic scope of such experiments in vertebrates has been focused on mammals and birds. Non-avian reptiles are poorly represented in experimental parasite removal and infection studies (Main & Bull 2000, Oppliger et al. 1999), particularly for tests of the fitness costs of parasitism (Pedersen & Fenton 2015). Expanding the taxonomic breadth of studies that experimentally test for costs of parasitism will allow for better

comparative tests of theories relating pace-of-life and metabolic rate with immune strategy and parasite defense, and theories implicating parasitism in the evolution of endothermy (Lee 2006, Sandmeier & Tracey 2014, Sanchez et al. 2018, Logan 2019, Casadevall & Damman 2020).

A common challenge associated with experimental parasite removal in the wild is the recommended timing for repeated dosing with anti-parasite drugs (Barragry 1987, Soll 1989). Conducting frequent recaptures for retreatment is logistically difficult and potentially stressful for the host, and experimental subjects may be missed during a recapture census and not receive a supplemental dose. Yet, frequent dosing may be required if wild hosts are continually exposed to parasites, such that treated individuals face reinfection as the anti-parasite drug is excreted and falls below therapeutic levels. Over time, these factors can reduce any differences in parasite prevalence and intensity between treatment groups while increasing variance within treatment groups, thereby complicating inferences about treatment effects, especially when the study is conducted over a time span that exceeds the half-life of the drug (Wahid 1989, Easterly et al. 1992, Ranjan et al. 1997, Irvine et al. 2000, Ezenwa et al. 2010, Knowles et al. 2013, Thomas & Morgan 2013, Friant et al. 2016). Therefore, extended-release drug formulations have many advantages over traditional drug formulations that require frequent dosing to maintain their effectiveness against parasites (Ezenwa et al. 2010, Carlsson et al. 2012).

Although extended-release formulations of anti-parasite drugs have been developed for the livestock production industry and are commercially available, they are generally unsuitable for use in smaller animals that are often studied in ecological and evolutionary research (Soll et al. 1990, González Canga et al. 2009, Geng et al. 2016,

Boehringer Ingelheim 2019). This is because the mechanism of release for some commercially available products is only appropriate for ruminants, or because formulations for large animals are too concentrated to be accurately administered by simply reducing the volume given to small animals. Of the available extended-releases formulations, *in situ* gelling injections are particularly promising for long-term parasite removal in small vertebrates. Gelling compounds can be easily and affordably produced in the laboratory, allowing researchers to tailor the formulation to their specific host and parasite system and can sustain an effective level of drug release for many months (Geng et al. 2016).

In this study, we developed an extended-release formulation of the antihelminth drug ivermectin and then tested its efficacy for the removal of nematode parasites in the brown anole lizard*, Anolis sagrei*. Ivermectin is tolerated well by most mammals, amphibians, fish, birds, and squamate reptiles, has broad-spectrum action against nematodes and arthropods, has a wide range between therapeutic and toxic doses, and can be used for extended periods with minimal side effects (Boyce et al. 1992, Letcher & Glade 1992, Wilson & Carpenter 1996, Davies & Rodger 2000, Jacobson 2007, Camargo et al. 2013, Langford et al. 2013). Ivermectin causes paralysis in nematodes and arthropods by selectively binding to and opening glutamate-gated chloride ion channels in nerve and muscle tissue (Turner & Schaeffer 1989). We specifically tested the efficacy of an extended-release formulation of ivermectin for the removal of *Physaloptera* sp. (Physalopteridae) and *Cyrtosomum penneri* (Atractidae), two nematodes that are common parasites of *A. sagrei* adults (see Methods, below). In addition to testing the effects of extended-release ivermectin formulations on these two parasites in captivity (where post-

treatment exposure to parasites is unlikely) and in the wild (where repeated exposure to parasites is likely), we also tested for any detrimental effects of ivermectin on performance by measuring sprint speed shortly after treatment. We then used this technique to test for predicted costs of nematode parasites with respect to performance, growth, survival, and mating success in adult *A. sagrei* as well as growth and survival in juvenile *A. sagrei*. Relative to individuals in control groups that received only the gelling vehicle for drug delivery, we predicted that individuals receiving an extended-release formulation of ivermectin would exhibit (1) reduced prevalence and intensity of infection by *Physalopetera* and *Cyrtosomum,* (2) increased sprint speed when measured two months post-treatment, (3) increased growth and mass gain as both juveniles and adults, (4) increased survival as both juveniles and adults, and (5) increased mating success, as measured by inferred copulation rates of males in each treatment.

Materials and Methods

Lizard hosts and nematode parasites

The brown anole (*Anolis sagrei*) is a small lizard that is native to Cuba and The Bahamas and invasive across much of the southeastern United States, including our study populations in northeast Florida. Lizards used in our laboratory study were collected from Palm Coast, Florida (29°35’59” N, 81°11’49” W). Our field experiments were conducted on two nearby spoil islands located in the Guana Tolomato Matanzas National Estuarine Research Reserve (GTM NERR, 29°37’43” N, 81°12’42” W and 29°37’58” N, 81°12’46” W). Field work was conducted under permits from the GTM NERR and all procedures involving lizards were approved by the University of Virginia Animal Care and Use Committee (protocol 3896).

Anolis sagrei harbors a variety of internal macroparasites (Acanthocephala, Nematoda, Pentastomida, Trematoda), of which nematodes are usually the most prevalent (Goldberg & Bursey 2000; Langford et al. 2013; Reedy et al. 2016; Thawley et al. 2019). In our study populations about half (56%) of all wild adult anoles are infected with *Physaloptera* sp. (Physalopteridae) and infection intensities range from 1-15 worms per infected anole (T.N. Wittman, unpublished data). *Physaloptera* is a large (5-15 mm total length) nematode that attaches to the mucosa of the stomach and uses the feces of anoles and other vertebrates to transmit its eggs, which are then ingested by intermediate arthropod hosts (e.g., Blattodea, Coleoptera, Hymenoptera, Orthoptera) that are common prey of anoles (Petri 1950, Lee 1957, Fincher et al. 1969, Lincoln & Anderson 1975, King et al 2013). A second nematode parasite, *Cyrtosomum penneri* (Atractidae), is much smaller (~1 mm length), resides in the rectum near the cloacal opening, reproduces viviparously within the host, and is only transmitted sexually (Langford et al. 2013). Infections of *C. penneri* can range upwards of 500 worms per anole, and nearly all adults harbor this parasite, whereas juveniles do not (Goldberg et al. 2002, Langford et al. 2013, Reedy et al. 2016). Experiments show that *C. penneri* does not infect anoles via oral transmission, nor does it infect snails or crickets that consume infected anole feces (Langford et al. 2013). However, 70% of male and 100% of female anoles acquire *C. penneri* after mating with an infected partner (Langford et al. 2013). Manipulations of reproduction via gonadectomy reduce the prevalence (percentage of individuals infected) of *C. penneri* infection in adult anoles of both sexes but have no effect on the prevalence or intensity (average number of parasites infecting individuals) of *Physaloptera* infection (Reedy et al. 2016). While some populations of *A. sagrei* harbor ectoparasites, we did

not detect any ticks, mites, or other ectoparasites on the lizards used in this study (Reedy et al. 2016).

Formulation of ivermectin gelling solution

We made an *in situ* gelling solution from the solvent N-Methyl-2-pyrrolidone (NMP; Sigma-Aldrich, 328634; 15% v/v), the polymers polylactic acid (PLA; Polysciences, PA, USA, 22505; 5% m/v) and sucrose acetate isobutyrate (SAIB; Sigma-Aldrich, MO, USA, W518107; 85% v/v), and the active anti-parasite drug ivermectin (IVM; Sigma-Aldrich, MO, USA, I18898; 1.5 μg/μl) (Camargo et al. 2013; Geng et al. 2016). Briefly, we melted PLA on a hot plate at 150°C, then added NMP and SAIB while stirring at 100°C until the PLA was fully dissolved and the solution was of uniform consistency, then reduced the heat to 50°C added ivermectin and continued stirring the solution until the ivermectin was fully dissolved. We stored this solution at 4°C and heated the solution at 50°C while stirring to reduce its viscosity prior to injection. Upon subcutaneous injection of this solution, the solvent diffuses into the aqueous environment of the organism while the hydrophobic polymers (SAIB and PLA) form a porous, semi-solid gel containing the active drug ivermectin (Lin et al. 2012). This gel matrix then slowly degrades over several months, releasing ivermectin and the by-products lactic acid and sucrose (Phillips et al. 1976, Göpferich 1996).

Experiment 1: Captive adult males

To test the safety and efficacy of our gelling compound, we captured 38 adult male *A. sagrei* lizards in Palm Coast, Florida (29°35’59” N, 81°11’49” W) in July 2018 and transported them to our animal facility at the University of Virginia. We housed each

lizard individually in a plastic cage (30 cm x 20 cm x 20 cm; Lee's Kritter Keeper, San Marcos, CA, USA) with a strip of outdoor carpet as a substrate, a section of PVC pipe for perching and hiding, and a strip of fiberglass mesh for basking under two ReptiSun 10.0 UVB bulbs (ZooMed, San Luis Obispo, CA, USA) suspended above the cage. We maintained animals on a 13L:11D photoperiod with a temperature of 29°C during the day, 25°C at night, and constant 65% relative humidity. We misted each cage daily with deionized water. Three times per week, we fed 3-5 crickets of 1/2" length (*Gryllodes sigillatus*, Ghann's Cricket Farm, Augusta, GA, USA) to each lizard. We dusted the crickets twice weekly with a calcium supplement (Fluker's Repta Calcium with D_3, Fluker Farms, Port Allen, LA, USA), and once weekly with a vitamin supplement (Fluker's Reptile Vitamin). We allowed animals to acclimate to laboratory conditions for 30 days prior to treatment.

Seven days prior to and 60 days after treatment, we measured snout-vent length (SVL) to the nearest 1 mm with a ruler and body mass to the nearest 0.01 g with a digital balance (Ohaus Scout Pro: SP202). We assigned each lizard to one of two treatments: (1) ivermectin (n = 19), in which animals received 1 µl per g body mass of the 1.5 µg/µl ivermectin gelling solution described above (1.5 µg ivermectin per g body mass), and (2) control (n = 19), in which animals received 1 µl per g body mass of the gelling vehicle without ivermectin. We haphazardly assigned treatment groups to individuals and confirmed that SVL and mass did not differ between treatment and control groups prior to treatment (SVL: t = 1.73, P = 0.09; Mass: t = 0.88, P = 0.38). We injected the drug or vehicle compounds subcutaneously, approximately 5 mm posterior and medial to the right shoulder, using a 25-µl Hamilton syringe (702 LT SYR) and a 26-gauge needle. We

used 70% isopropyl alcohol swabs to sanitize the site of injection and the needle before and after each injection, and we used a new needle every five injections.

Upon injection of the gelling solution, dissipation of the solvent causes an initial burst release of the drug before the polymer matrix forms (Geng et al. 2016). Because of the initial burst of ivermectin released into the blood, any potential toxic effects of ivermectin are likely to occur in the days immediately following injection. Impaired locomotor performance is a common side effect of ivermectin toxicity (Lovell 1990, Kim & Crichlow 1995, Clayton et al. 2013, Verdú et al. 2018). To assess the effect of the gelling vehicle and the burst release of ivermectin on lizard performance during this immediate post-treatment period, we compared the maximum sprint speed of lizards two days pre-treatment with the same measure at two days post-treatment. To test whether experimental removal of parasites improves performance, we also measured maximum sprint speed at two months post-treatment. We measured sprint speed at the same temperature and humidity in which lizards were housed (29°C, 65% relative humidity) by racing each lizard up a flat wooden track at an incline of 45°. The track was 1.2 m in length and 6 cm in width with sides 8 cm in height. We shrouded the upper end of the track to offer lizards a refugium to sprint towards. Four infrared sensors were spaced 10 cm apart starting 40 cm up the track (TrackMate Racing IRSENSORS, British Columbia, Canada). We recorded the time a lizard passed each sensor using TrackMate Racing Sc Timer software (Version 9.42), then calculated velocity (cm/s) over each 10-cm interval between sensors, which resulted in three measures of velocity per trial. We raced each lizard in three successive trials and selected the highest velocity across the nine measures (3 intervals x 3 trials) as the maximum sprint speed for each individual. Two lizards had

maximum sprint speeds that were over 100 cm/s faster than the next fastest lizard in the dataset. These values were significant outliers as assessed by Rosner's test, so these individuals were removed prior to analysis.

To test for a potential detrimental effect of either the gelling vehicle or the burst release of ivermectin on sprint speed, we used a linear mixed-effects model with time (2 d pre-treatment, 2 d post-treatment) and treatment (ivermectin, control) as fixed effects with interaction, plus individual ID as a random effect. A significant decrease in sprint speed at 2 d post-treatment (main effect of time) would indicate a detrimental effect of the gelling vehicle or injection procedure, whereas a significant decrease in only the ivermectin group (time*treatment interaction) would indicate a detrimental effect of ivermectin. To test for the effect of parasites on sprint speed, we used a similar mixed-effects model with a different post-treatment time point (2-m post treatment), with the expectation (subsequently confirmed) that ivermectin would eliminate parasites by that point. We predicted that any beneficial effects of parasite removal on performance would be evident as a significant time*treatment interaction. We also tested for effects of body length and body mass on sprint speed, but neither were significant, so they were not included as covariates in the model. We additionally tested if ivermectin males experienced a significant increase in sprint speed 2 m post-treatment compared to their pre-treatment values. To do this, we used a mixed-effect model that included only ivermectin males, with time as a fixed effect and individual ID as a random effect. For visualization, we expressed change in performance by subtracting each male's pre-treatment sprint speed from its post-treatment speed.

We tested for effects of parasite removal on growth in SVL (mm/d) and mass (g/d) using linear models with a fixed effect of treatment and initial body size (SVL or mass) as a covariate. Initial size was included as a covariate because growth rates decrease with size. At 70 days post-treatment, we euthanized each lizard, dissected out its gastrointestinal tract, and stored it in 100% ethanol. We sectioned the gastrointestinal tract into the stomach, small intestine, large intestine, and rectum, then counted all nematode parasites in each section under a 10x stereoscope. The two nematode genera are easily distinguished by their relative size and location; *Physaloptera* sp. is 5-15 mm in length and found embedded in the gastric mucosa, whereas *Cyrtosomum penneri* is only 1 mm in length and mainly found in the rectum and the posterior end of the large intestine. To test for treatment effects on prevalence of parasite infection (proportion of hosts infected), we used generalized linear models with a binomial error distribution and each individual scored as infected (1) or not (0) by each parasite type. To test for treatment effects on intensity of parasite infection (number of parasites per host), we used count data for each parasite type and generalized linear models with a negative binomial error distribution and a log link function (Alexander 2012). We performed all analysis in R v4.0.2 (R Core Team 2020).

Experiment 2: Wild adults

Using a population of *A. sagrei* on a small spoil island located in the GTM NERR (29°37’43”N, 81°12’42”W), we captured adult males and females at the beginning of the breeding season (March 2019) and treated individuals with an injection of either (1) our gelling formulation of ivermectin (n = 91 males, 90 females), or (2) the gelling vehicle as a control (n = 87 males, 87 females). Prior to treatment, we gave each individual a unique

toe clip for identification, measured its SVL and body mass (see above), and sorted individuals by these measures of size to create size-matched treatment groups within each sex. Treatment groups did not differ in initial size for males (SVL: $t = 0.06$, $P = 0.95$; mass: $t = 0.36$, $P = 0.71$) or females (SVL: $t = 0.85$, $P = 0.39$; mass: $t = 0.57$, $P = 0.56$). Within 24 h of capture and treatment, we released each animal at its site of initial capture.

We resampled the island in May, July, and October of 2019. During each recapture census, we measured SVL and body mass to calculate growth in length (mm/d) and mass (g/d). We re-treated the animals with ivermectin or control injections calibrated to their new body mass during the July census. Because our final October sampling effort was not exhaustive, we did not include this census in capture-mark-recapture models of survival and recapture rate. Individuals captured in October were euthanized and dissected to assess treatment effects on parasite load, as described above, with the addition of sex as a fixed effect. We did not detect any significant treatment*sex interactions for the prevalence or intensity of either parasite, so we present results from models without an interaction term. We tested for effects of parasite removal on growth in SVL (mm/d) and mass (g/d) using linear models with a fixed effect of treatment and initial body size (SVL or mass) as a covariate. Initial size was included as a covariate because growth rates decrease with size. Because the distributions of the covariates (initial mass and initial SVL) have little overlap between the sexes, we tested for treatment effects on growth separately for adult males and females. To assess homogeneity of slopes, we tested for an interaction between initial size and treatment. For growth in body mass of adult males, we found a significant interaction between treatment and initial body mass, which we included when testing for main effects of

treatment. To make the main effect in this interaction model interpretable as an effect at mean body mass, we transformed initial body mass to have a mean of zero. There were no significant interactions between initial body size and treatment for any other models of adult growth (SVL growth in males: initial SVL*treatment: $F_{1,96} = 1.50$, $P = 0.22$; SVL growth in females: initial SVL*treatment: $F_{1,94} = 0.29$, $P = 0.59$; mass growth in females: initial mass*treatment: $F_{1,94} = 0.08$, $P = 0.78$), so we present the results from ANCOVA without the interaction term. Numbers of recaptured adults were low in July and October, so we restricted our analysis of growth to the interval between March and May.

We used generalized linear models with a binomial error distribution and a logit link function to test for effects of sex, treatment, and their interaction on apparent survival (observed survival, uncorrected for our estimated probability of recapture) between March and May and between March and July. One male was injured upon capture in May and had to be euthanized; this animal was censored when measuring survival between March and July. To estimate survival while also estimating and accounting for recapture probability between censuses in March, May, and July, we built Cormack-Jolly-Seber capture-mark-recapture models using the Rmark interface for the program MARK (Laake 2013). We used a model comparison approach to test for the effects of treatment, sex, their interaction, and time on monthly survival rate and recapture rate. We tested the significance of factors using log likelihood ratio tests between full and reduced models (Table S1).

In May, during the peak of the breeding season, we performed an additional experiment to test whether parasite removal increased the mating success of males. On our first recapture day, we exclusively captured males, then grouped them according to

their treatment (ivermectin, control, or previously unmanipulated). Immediately prior to release, we dusted the venter of each male with non-toxic fluorescent powder (A/AX Series, Day-Glo Color Corp., OH, USA) of a color unique to its treatment group (ivermectin $n = 40$; control, $n = 37$; unmanipulated, $n = 73$). We allowed males to mate undisturbed for two days, then returned to the island to capture both experimental and unmanipulated females and checked their venters under UV light to detect any fluorescent powder transferred during copulation. Females with two or more different colors of powder ($n = 14$) were counted as two or more copulation events. To estimate the population mean copulation rate, we divided the total number of inferred copulation events by the total number of recaptured females. To test for an effect of parasite removal on male copulation success, we assessed whether the observed number of copulations in each group differed from null expectation using a chi-square test with 2 degrees of freedom. We calculated the expected number of copulations for each of the three groups of males by multiplying the relative frequencies of powdered males in each group by the total number of inferred copulation events.

Experiment 3: Wild juveniles

3a: July to October 2019 and 2021

Using a separate population of *A. sagrei* on a different small spoil island in the GTM NERR (29°37’58” N, 81°12’46” W), we captured juvenile males and females during July 2019 (hatched in 2019) and during July 2021 (hatched in 2021) at which point juveniles ranged from about 1-70 days of age, depending on their hatch date (hatching typically begins in the last week of May). We gave each animal a unique toe clip, measured its SVL and mass, then treated all individuals that weighed at least 0.5 g with an injection of

either (1) our *in situ* gelling formulation of ivermectin (2019: $n = 40$ males, $n = 37$ females; 2021: $n = 58$ males, $n = 62$ females), or (2) the gelling vehicle as a control (2019: $n = 45$ males, $n = 38$ females; 2021: $n = 55$ males, $n = 60$ females). Prior to assigning treatment, we sorted individuals by SVL and mass to create size-matched treatment groups within males (2019: SVL: $t = 0.02$, $P = 0.99$, mass: $t = 0.35$, $P = 0.72$; 2021: SVL: $t = 0.29$, $P = 0.77$, mass: $t = 0.08$, $P = 0.93$) and females (2019: SVL: $t = 0.05$, $P = 0.96$,$n =$ mass: $t = 0.18$, $P = 0.85$; 2021: SVL: $t = 0.29$, $P = 0.77$, mass: $t = 0.82$, $P = 0.42$). Within 24 h, we released each treated individual at its site of capture. We resampled the island extensively in October 2019 to recapture the animals marked in July 2019, and in October 2021 to recapture the animals marked in July 2021. We measured growth in SVL and mass and survival across this period, which is prior to lizards entering their first winter. We tested for treatment effects on growth in SVL (mm/d) and in body mass (g/d) using linear models with fixed effects of sex, treatment and year plus initial size as a covariate. To assess homogeneity of slopes we tested for interactions of the fixed effects with the covariate of initial body size. For SVL growth we did not find any significant interactions between the fixed effects and initial SVL (initial SVL * Treatment: $F_{1,172} = 1.06$, $P = 0.30$; initial SVL * Sex: $F_{1,172} = 0.03$, $P = 0.86$; initial SVL * year: $F_{1,172} = 1.73$, $P = 0.19$). However, we did find a significant interaction between year and sex ($F_{1,174} = 11.63$, $P = 0.0008$), which we included in our final model testing for treatment effects. For mass growth there was no significant interaction between initial mass and treatment ($F_{1,172} = 1.03$, $P = 0.31$), however we found significant interactions between initial body mass and sex ($F_{1,172} = 5.97$, $P = 0.015$), and initial body mass and year ($F_{1,172} = 10.45$, $P = 0.001$). When analyzed separately within each sex we did not

find an interaction between initial body mass and year in males ($F_{1,82} = 3.26$, $P = 0.07$) or females ($F_{1,88} = 0.01$, $P = 0.92$). We thus analyze treatment effects on growth in body mass within the two years separately for each sex. We analyzed apparent survival using a generalized linear model with a binomial error distribution and a logit link function, we included the fixed effects of treatment, sex, and year. We did not detect any significant two- or three-way interactions (Sex * Treatment: $\chi^2_1 = 0.52$, $P = 0.47$; Sex * Year: $\chi^2_1 = 0.47$, $P = 0.49$; Treatment * Year: $\chi^2_1 = 1.83$, $P = 0.17$; Sex * Treatment * Year: $\chi^2_1 = 2.87$, $P = 0.09$), so we present results from the additive model. Because we only have one resampling period, we cannot estimate recapture rate and survival independently.

Results

Efficacy of parasite removal treatment

In the laboratory (Experiment 1), one injection of the gelling ivermectin formulation achieved complete removal of both gastric *Physaloptera* (Prevalence: $\chi^2_1 = 17.51$, $P < 0.0001$) and cloacal *Cyrtosomum* (Prevalence: $\chi^2_1 = 25.12$, $P < 0.0001$) in adult males (Fig. 1A-B). In control males, the prevalence of *Physaloptera* infection was 53% (10 of 19), and the prevalence of *Cyrtosomum* infection was 68% (13 of 19). In the field (Experiment 2), adult males and females did not differ in the prevalence or intensity of infection by either parasite, and two injections of the gelling ivermectin formulation reduced the prevalence of *Physaloptera* infection (sex: $\chi^2_1 = 0.14$, $P = 0.70$; treatment: $\chi^2_1 = 6.70$, $P = 0.009$), the intensity of *Physaloptera* infection (sex: $\chi^2_1 = 0.12$, $P = 0.73$; treatment: $\chi^2_1 = 8.12$, $P = 0.004$), the prevalence of *Cyrtosomum* infection (sex: $\chi^2_1 =$

1.79, $P = 0.18$; treatment: $\chi^2_1 = 13.40$, $P = 0.0002$), and the intensity of *Cyrtosomum* infection (sex: $\chi^2_1 = 0.87$, $P = 0.34$; treatment: $\chi^2_1 = 3.95$, $P = 0.046$) (Fig. 1.C-D).

Effects of parasite removal on performance

In the laboratory study of adult males (Experiment 1), treatment groups did not differ in sprint speed prior to treatment, and neither the injection itself nor the drug ivermectin had any short-term effect on sprint speed (time: $\chi^2_1 = 0.17$, $P = 0.67$; treatment: $\chi^2_1 = 2.13$, $P = 0.14$; time*treatment: $\chi^2_1 = 0.20$, $P = 0.65$) (Fig. 2A). However, ivermectin treatment significantly increased sprint speed above that of control animals at two months post-treatment (time: $\chi^2_1 = 1.31$, $P = 0.25$; treatment: $\chi^2_1 = 0.56$, $P = 0.45$; time*treatment: $\chi^2_1 = 6.14$, $P = 0.01$) (Fig. 2B). Further, ivermectin treatment significantly increased sprint speed at two months post-treatment above the pre-treatment sprint speeds for the same individuals ($\chi^2_1 = 3.97$, $P = 0.046$).

Effects of parasite removal on growth

In the laboratory study of adult males (Experiment 1), we did not detect any effects of ivermectin treatment on growth in SVL (treatment: $F_{1,35} = 0.51$, $P = 0.48$; initial SVL: $F_{1,35} = 11.12$, $P = 0.002$) or growth in mass (treatment: $F_{1,35} = 0.60$, $P = 0.44$; initial mass: $F_{1,35} = 13.16$, $P = 0.009$). In the field study of adult males (Experiment 2), we did not detect any effects of ivermectin treatment on growth in SVL (treatment: $F_{1,97} = 0.06$, $P = 0.79$; initial SVL: $F_{1,97} = 112.41$, $P < 0.0001$) or growth in mass (treatment: $F_{1,96} = 1.57$, $P = 0.21$; initial mass: $F_{1,96} = 0.029$, $P = 0.86$; treatment*initial mass: $F_{1,96} = 8.01$, $P = 0.006$) (Fig. S1). The significant interaction was included because males treated with ivermectin exhibited the expected negative relationship between initial body mass and

growth whereas control males did not (Fig. S1). In the field study of adult females (Experiment 2), we did not detect any effects of ivermectin treatment on growth in SVL (treatment: $F_{1,95} = 0.05$, $P = 0.82$; initial SVL: $F_{1,95} = 118.27$, $P < 0.0001$) or growth in mass (treatment: $F_{1,95} = 0.02$, $P = 0.88$; initial SVL: $F_{1,95} = 75.59$, $P < 0.0001$) (Fig. S1).

For juveniles in the field (Experiment 3), ivermectin treatment significantly increased SVL growth, males grew faster than females, and males grew faster in 2021 than 2019 (treatment: $F_{1,174} = 7.54$, $P = 0.006$; initial SVL: $F_{1,174} = 110.43$, $P < 0.0001$; sex: $F_{1,174} = 269.11$, $P < 0.0001$; year: $F_{1,174} = 0.43$, $P = 0.51$; sex * year: $F_{1,174} = 11.63$, $P = 0.0008$) (Fig.3A). For growth in mass, ivermectin treatment significantly increased growth in females, and females grew faster in 2021 than 2019 (treatment: $F_{1,89} = 5.00$, $P = 0.03$; initial Mass: $F_{1,89} = 35.09$, $P < 0.0001$; year: $F_{1,89} = 13.61$, $P = 0.0003$) (Fig.3B). There was no effect of ivermectin treatment in males and growth was faster in 2021 than 2019 (treatment: $F_{1,83} = 1.60$, $P = 0.20$; initial Mass: $F_{1,83} = 0.29$, $P = 0.58$; year: $F_{1,83} = 60.01$, $P < 0.0001$) (Fig.3C).

Effects of parasite removal on survival

For adults in the field (Experiment 2), apparent survival was similar between sexes and treatment groups from March to May (sex: $\chi^2_1 = 0.24$, $P = 0.61$; treatment: $\chi^2_1 = 0.40$, $P = 0.52$; sex*treatment: $\chi^2_1 = 0.64$, $P = 0.42$) and from March to July (sex: $\chi^2_1 = 2.01$, $P = 0.16$; treatment: $\chi^2_1 = 0.22$, $P = 0.63$; sex*treatment: $\chi^2_1 = 0.04$, $P = 0.83$) (Table 1). Our best-supported CJS capture-mark-recapture model had a single recapture rate (0.84) that did not vary across treatment groups or sexes, and a time-varying survival rate (survival probability per month) (March to May: 0.77, May to July: 0.69; $\chi^2_1 = 4.15$, $P = 0.04$) (Table S1). We found no evidence for effects of treatment or sex on either

survival (sex: $\chi^2_1 = 1.65$, $P = 0.20$, treatment: $\chi^2_1 = 0.39$, $P = 0.53$, sex*treatment: $\chi^2_1 = 0.20$, $P = 0.65$) or recapture probability (sex: $\chi^2_1 = 0.46$, $P = 0.49$, treatment: $\chi^2_1 = 0.28$, $P = 0.60$, sex*treatment: $\chi^2_1 = 0.51$, $P = 0.46$).

For juveniles in the field (Experiment 3) there was no effect of treatment on apparent survival between July and October and survival was lower in 2021 than 2019 (sex: $\chi^2_1 = 0.78$, $P = 0.37$; treatment: $\chi^2_1 = 1.16$, $P = 0.28$; year: $\chi^2_1 = 17.28$, $P < 0.001$) (Table 1).

Effects of parasite removal on mating success

We detected 77 copulations across 272 recaptured females (0.28 mating frequency, $n = 63$ females copulated at least once). The number of copulations attributed to each of the three male groups differed slightly from the null expectation based on their frequencies in the population ($\chi^2_1 = 5.94$, $P = 0.051$), such that more copulations were attributed to males treated with ivermectin than expected, fewer copulations were attributed to unmanipulated males than expected, and the number of copulations attributed to control males was similar to the null expectation (Fig. 4).

Discussion

We found that removal of nematode parasites from anole hosts with a novel, *in situ* gelling formulation of ivermectin increased the sprint speed and mating success of adult males and also increased growth in length for juveniles of both sexes and growth in body mass for females. We interpret these results as evidence for costs of parasitism with respect to performance, juvenile growth, and one aspect of reproductive fitness, although we found no evidence for any effects of parasite removal on the survival of juveniles or

adults of either sex. We did not measure reproductive success directly, but it is possible that the positive effects of parasite removal on juvenile growth and body condition that we observed could indirectly enhance both male mating success and female fecundity by increasing adult body size, which correlates with reproductive success (Duryea et al. 2016, Kamath & Losos 2018). Non-avian reptiles are poorly represented in experimental parasite removal and infection studies (Main & Bull 2000, Oppliger et al. 1999), particularly for tests of the fitness costs of parasitism (Pedersen & Fenton 2015). Expanding the taxonomic breadth of studies that experimentally test for costs of parasitism will allow for better comparative tests of theories relating pace-of-life and metabolic rate with immune strategy and parasite defense (Lee 2006, Sandmeier & Tracey 2014, Sanchez et al. 2018).

We found that the small, sexually transmitted nematode *Cyrtosomum penneri* occurred at a higher prevalence and intensity of infection than the large, trophically transmitted nematode *Physaloptera* sp., and we found no evidence for a sex difference in the prevalence or intensity of infection by either parasite. These patterns are largely consistent with other studies of parasitism in the brown anole (Goldberg et al. 1994, Goldberg & Bursey 2000, Norval et al. 2011, Reedy et al. 2016). Although ivermectin eliminated both parasites in the laboratory, it was somewhat more effective at lowering the prevalence and intensity of infection by *Physaloptera* compared to *Cyrtosomum* in the field (Fig. 1), perhaps because of the high prevalence of *Cyrtosomum* in the untreated portion of the adult population (100%) and the high likelihood of reinfection during mating (Langford et al. 2013), which appears to happen frequently based on our fluorescent powdering data (23% of females had mated at least once within several days

of their capture). Although our experimental design does not allow us to disentangle the independent effects of *Physaloptera* and *Cyrtosomum* on anoles*,* the detrimental effects on growth, performance, and mating success that we observed in control animals may be largely due to *Physaloptera.* Nematodes in the genus *Physaloptera* embed in the gastric epithelium and feed on gastric mucosa and blood, which causes tissue damage in the mucosal lining of the stomach, inflammation, and invasion of the gastric tissue with immune cells (Naem et al. 2006, Hoseini et al. 2014). By contrast, *Cyrtosomum penneri* is a sexually transmitted nematode infecting the rectum of its hosts, where it may feed primarily on feces rather than host tissue. In *Sceloporus* lizards, infection by *C. penneri* was not associated with inflammation of the rectum or tissue damage, but its potential pathological effects have not been well studied (Pearce 1972). Therefore, any costs stemming from *C. penneri* may reflect collateral damage from immune activation and associated tissue inflammation, rather than direct damage to host tissues by the parasite.

Neither the gelling injection vehicle nor the ivermectin treatment had any detrimental short-term (two days) effect on sprint speed in adult males. However, two months post-treatment, males given ivermectin were free of both species of nematode parasite and had significantly increased sprint speed, whereas males given the control vehicle showed no change in sprint speed. This increase in performance of the ivermectin group is likely due to the concomitant reduction in parasitism and not due to ivermectin itself, because ivermectin tends to depress the central nervous system and reduce motor coordination (Moriera et al. 2017). A meta-analysis across host and parasite taxa showed that, on average, parasitism reduces host performance, and that endurance measures tend to be reduced more than speed (McElroy & de Buron 2014). However, of the previous

studies investigating the relationship between parasitism and running performance in lizards, none involved nematodes and all were observational. Reductions in performance were associated with parasitism by apicomplexans (haemogregarines, *Plasmodium*), but not by mites or ticks (Schall et la. 1982, Oppliger et al. 1996, Main & Bull 2000, Ekner-Grzyb et al. 2013, Garrido & Pérez-Mellado 2014). One mechanism through which parasitism may affect performance is by compromising the ability of blood to effectively transport nutrients and oxygen to muscles under stress. In the lizard *Lacerta vivipara* hemogregarine parasitism is associated with reduced hemoglobin, increased numbers of immature red blood cells, and reduced mean sprint speed (Oppliger et al. 1996). Although this mechanism is intuitive for hemogregarines and other blood parasites, there is also some evidence that *Physaloptera* infections can cause anemia (Al-Obaidi 2012, Lértora et al. 2016). Protein loss from healing damaged gastric epithelium due to parasitic nematodes also leads to a decrease in enzymes and myoglobin used for oxygen transport during muscle activity (Fuge et al. 1968). Additionally, the energetic cost of mounting an immune response may trade off with performance. Lizards treated with the bacterial cell wall component lipopolysaccharide (LPS) to induce an immune response have reduced sprint speed, possibly due to altered energy balance favoring immune response (Zamora-Camacho et al. 2015, Hudson et al. 2021). However, in mammals the antimicrobial immune response induced by LPS is distinct from the response against macroparasites and involves pro-inflammatory type 1 responses (Spellberg & Edwards 2001, Ashley et al. 2012, Annunziato et al. 2014).

We found no effects of parasites on growth in either length or mass of adult anoles in either captivity or the wild (Fig. S1.). Although growth is indeterminate in *A.*

sagrei, it decreases asymptotically with body size. Therefore, adults that are not rapidly growing may not face growth costs from parasitism, or the similar costs may be more difficult to detect than in juveniles, where we found small but significant effects of parasite removal on growth (Fig. 3). For the early juvenile period, when expressed relative to the average initial SVL for each sex, individuals treated with ivermectin grew 0.019 mm more per day than control individuals, corresponding to a 20% increase in growth rate of females and a 10% increase in growth rate of males. Further, the effect of parasite removal on growth was consistent in direction across years which differed greatly in average growth rate. These growth costs of parasitism may have important consequences for fitness as adults, given that body size at the beginning of the breeding season is positively associated with offspring production that year (Duryea et al. 2016).

Growth costs of parasitism have been found in a variety of taxa, including birds (O'Brien & Dawson 2007, Fassbinder-Orth et al. 2018), amphibians (Finnerty et al. 2017), mammals (Sacks & Blejwas 2000, Stien et al. 2002), fish (Hansen et al. 2006, Hoffnagle et al. 2006), arthropods (Polak 1998, Botto-Mahan et al. 2017), annelids (Field & Michiels 2005), and molluscs (O'Connel-Milne et al. 2016). These studies also encompass a broad range of parasite types, suggesting that growth reduction is a general cost of parasitism. However, many other studies have found no effect of parasitism on growth or body size (Tompkins et al. 1999, Hillegass et al. 2010, Reed et al. 2012, Roznik et al. 2020), and in some systems, parasites are known to increase the body size of their hosts, though usually at the expense of reproduction (Sorensen & Minchella 2001, Ebert et al. 2004). Variability in the reported growth costs of parasitism is likely due in part to variation in study methodology (e.g., observational study, experimental

infection, experimental removal, effectiveness of removal, reinfection rate, length of study, field versus laboratory, measure of body size) as well as the specifics of the host-parasite system (variation in parasite virulence and host tolerance), and the time period of the study relative to host lifespan.

Parasites are likely more costly when resources are limited or during energetically demanding periods of the host life cycle, such as during rapid growth periods preceding maturity or during reproduction (Francis 1961, Bruns et al. 2017, Albery et al. 2021). Because parasites directly utilize host resources, they potentially decrease the total amount of resources that hosts can allocate towards growth, somatic maintenance, and reproduction (van Noordwijk & de Jong 1986, de Jong 1993, Sheldon & Verhulst 1996, Zera & Harshman 2001). This reduction may be especially detrimental for juveniles that are in a period of rapid, energetically demanding growth. In the chipmunk (*Tamias striatus*), botfly parasitism depresses growth and increases resting metabolic rate in juveniles, but not in adults (Careau et al. 2010). Juvenile chickens that are experimentally infected with the nematode *Ascaridia galli* have decreased mass gain and increased mortality, and this effect is greatest in younger chicks (Ackert & Herrick 1928). In terms of growth and survival, juveniles may be less likely to compensate for the increased energetic demands imposed by parasitism because their foraging opportunities are potentially more limited than those of adults, and because they cannot divert energy from reproduction. In addition to shrinking the pool of available resources through direct consumption, parasites may reduce the size of the resource pool that can be allocated to growth, given that many hosts show a depressed feeding rate upon infection (Arneberg et al. 1996, Adamo 2010, Sargent et al. 2014, Finnerty et al. 2017). Further, parasites may

render foraging more energetically costly for their hosts. In European shags (*Phalacrocorax aristotelis*), there is a positive association between parasite burden and energy expended on foraging flights (Hicks et al. 2018). The energetic cost of mounting an immune response to an active nematode infection may also trade off against growth, and growth costs of immune activation have been seen in many organisms (Uller et al. 2006, Devevey et al. 2009, Bascuñán-García et al. 2010, Demas et al. 2011 Bonneaud et al. 2016).

Male anoles with their parasites removed had greater mating success than expected, while the mating success of control and unmanipulated males was at or below expectation, as inferred through transfer of florescent powder to the female venter, which presumably occurred during copulation. Parasite removal may have increased the competitive ability of males in intra-sexual interactions, thereby increasing their access to females (Gómez-Llano et al. 2020). Additionally, parasite removal could have increased the number of encounters a male has with females. Tick removal increases range size in the common pheasant (*Phasianus colchicus*), and thereby increases the likelihood of males acquiring mates (Hoodless et al. 2002). In *A. sagrei,* male home range size is positively associated with female encounter rate, and the proportion of a female's offspring sired (Kamath & Losos 2018). Parasites have been shown to reduce reproductive success and offspring production in other species (Newey & Thirgood 2004, Worden et al. 2000, Patterson et al. 2013, however, see Raveh et al. 2011), but further work involving genetic parentage assignment will be necessary to determine whether the effects of parasite removal on juvenile growth and male mating success that we observed translate into increased reproductive success.

Across a variety of host-parasite systems, parasites usually reduce host survival (Robar et al. 2010, Wittman & Cox 2021). Therefore, it is somewhat surprising that we found no effects of nematode parasitism on survival in juvenile or adult brown anoles. Although experimental data for non-avian reptiles are scarce, observational studies of mite and hemogregarine parasitism have shown little evidence for survival costs in this group (Sorci et al. 1996, Brown et al. 2006, Bonneaud et al. 2017, Paterson & Blouin-Demers 2020, but see Shaner et al. 2013), suggesting some level of mortality tolerance to parasites. The immune strategy of short-lived ectotherms may rely more heavily on constitutive rather than induced aspects of the immune system, or on tolerance (reducing the fitness costs of a given parasite burden) than on resistance (reducing the parasite burden) than long-lived ectotherms or endotherms with a comparable pace-of-life (Råberg et al. 2009, Palacios et al. 2010, Previtali et al. 2012, Sandmeier & Tracy 2014). Alternatively, mortality may be largely stochastic in this system, such that any effects of parasites on survival are obscured by other sources of mortality, such as predation, which may occur randomly with respect to parasite load.

Conclusion

Our extended-release formulation of ivermectin safely and effectively decreased nematode parasitism of brown anole hosts in the laboratory and field, suggesting that this new technique has promise for use in field studies of parasitism in a variety of vertebrate host species. Our experimental results indicate that nematode parasites can impose costs in terms of growth, performance, and mating success, but we see no evidence for a survival cost of parasitism in *A. sagrei*. The lack of a survival cost is somewhat surprising given substantial evidence for this cost in a variety of other vertebrate and invertebrate

hosts, though lizards are poorly represented in experimental tests for survival costs of parasitism in the wild. Given that our results suggest a variety of modest costs of parasitism that may collectively influence lifetime reproductive success, future studies should seek to measure survival, mating success, and total lifetime reproductive success to understand how parasites directly and indirectly affect host fitness.

Data Availability Statement: The data used in all analysis is publicly available through the Open Science Foundation, and can be accessed through the following link, https://doi.org/10.17605/OSF.IO/JQ8UM.

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Tables

Table 1. Summary of survival estimates across ages, time intervals, sexes, and treatment groups for the two field studies involving adults (Experiment 2) and juveniles (Experiment 3). The standard error was calculated as $\sqrt{\frac{probability\ of\ survival * probability\ of\ mortality}{n}}$.

Age	Interval	Sex	Treatment	Alive	Dead	Survival	1 SE
Adult	March-May	Male	Control	47	40	0.54	0.053
			Ivermectin	56	35	0.62	0.051
		Female	Control	53	34	0.61	0.052
			Ivermectin	54	36	0.60	0.052
Adult	March-July	Male	Control	15	72	0.17	0.041
			Ivermectin	18	72	0.20	0.042
		Female	Control	21	66	0.24	0.045
			Ivermectin	23	67	0.26	0.046
Juvenile	July-October 2019	Male	Control	25	20	0.55	0.074
			Ivermectin	21	19	0.54	0.077
		Female	Control	22	16	0.58	0.080
			Ivermectin	25	12	0.68	0.077
Juvenile	July-October 2021	Male	Control	20	35	0.36	0.065
			Ivermectin	21	37	0.36	0.064
		Female	Control	29	31	0.48	0.065
			Ivermectin	17	45	0.27	0.058

Figures

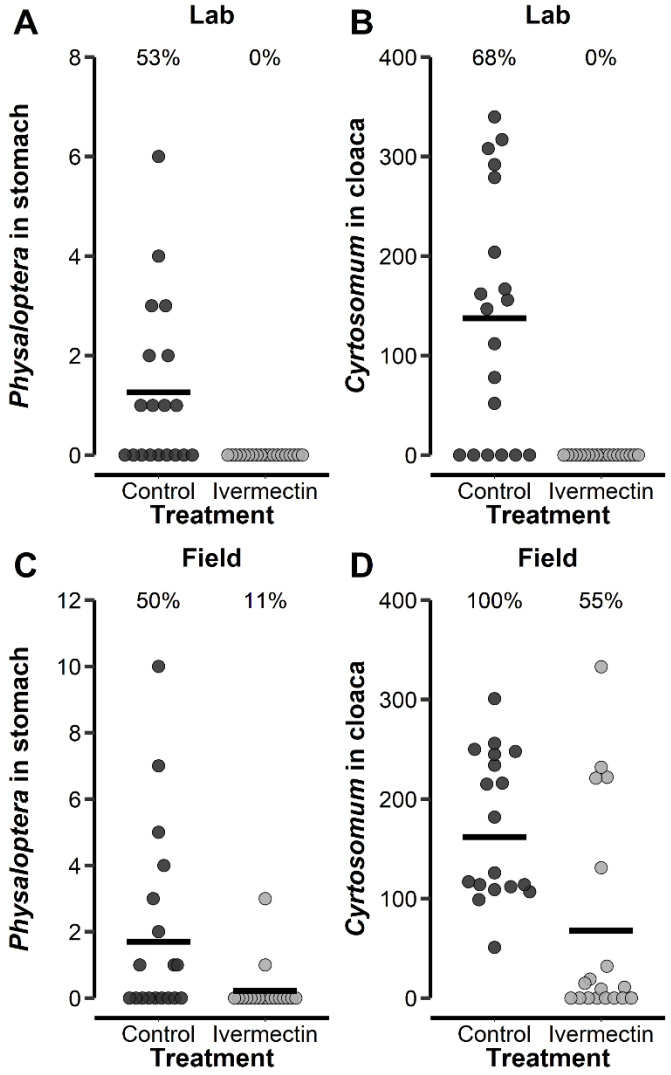

Figure 1. Nematode counts in adult brown anoles in the control (black) and ivermectin (grey) treatment groups based on dissections from the lab experiment on adult males (A and B) and the field experiment on adult males and females (C and D, sexes pooled). Points are jittered horizontally to avoid overplotting. Bars give the mean for each treatment group. Percentages above each column of data indicate the prevalence of infection (percentage of lizards infected). Ivermectin reduced the prevalence and intensity of both nematode parasites in both the lab and the field.

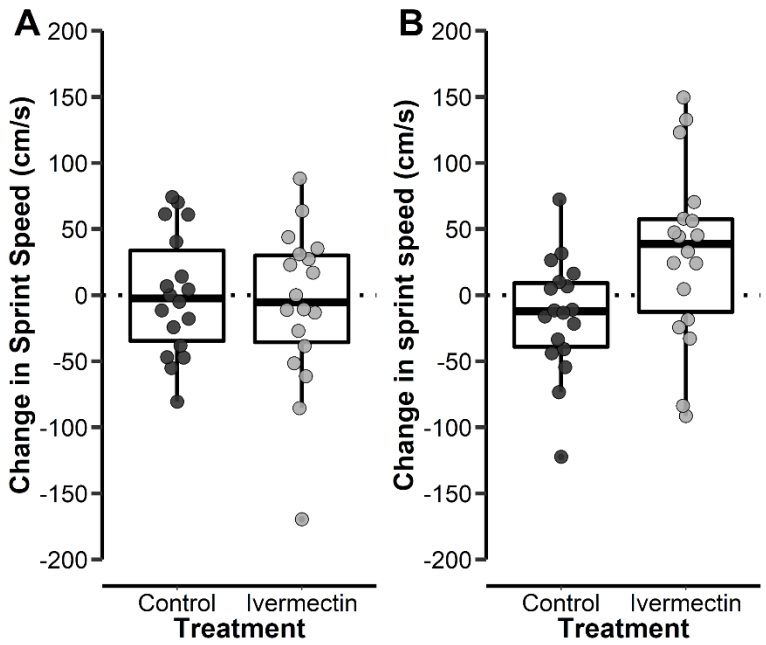

Figure 2. Change in sprint speed for adult males in the lab experiment from (A) 2 days pre-treatment to 2 days post-treatment, or (B) 2 days pre-treatment to 2 months post-treatment. Symbols represent individual males and box-and-whisker plots report medians (heavy line), upper and lower quartiles (boxes), and ranges (whiskers) in each treatment group. There was no short-term effect of either the vehicle (Control) or the drug (Ivermectin) on sprint speed (A). Parasite removal significantly increased sprint speed at 2 months post-treatment. This increase was significantly greater than the change in sprint speed for the control group (B). For simplicity, these figures depict change in sprint speed, whereas the analysis used a repeated-measures, mixed-effect linear model including both pre- and post-treatment measures.

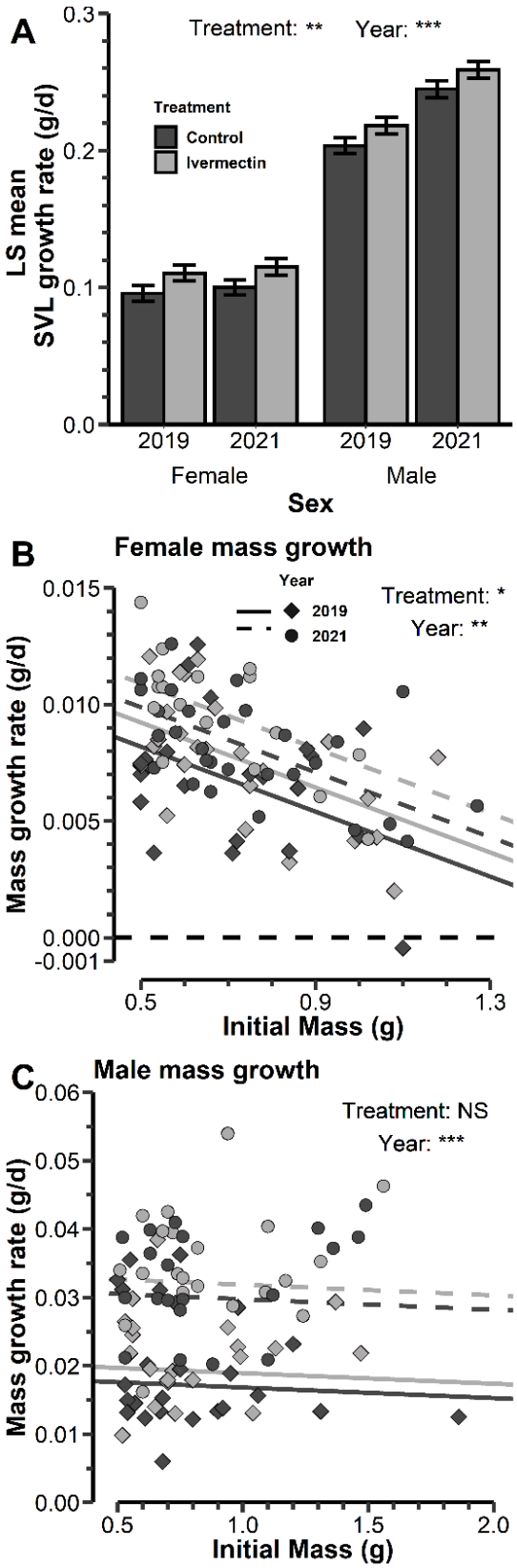

Figure 3. Growth rates of snout-vent length (SVL) and body mass for control and ivermectin treated juvenile males and females, from the period between July and October for the years 2019 and 2021. Parasite removal increased the growth rate of SVL for males and females (A), and the growth rate of mass for females (B,C). In A least square means for each sex were calculated at their respective means for initial SVL. The lines in B and C are from the slope and intercept of the linear regression of mass growth rate on initial mass with treatment and year as fixed effects, within each sex.

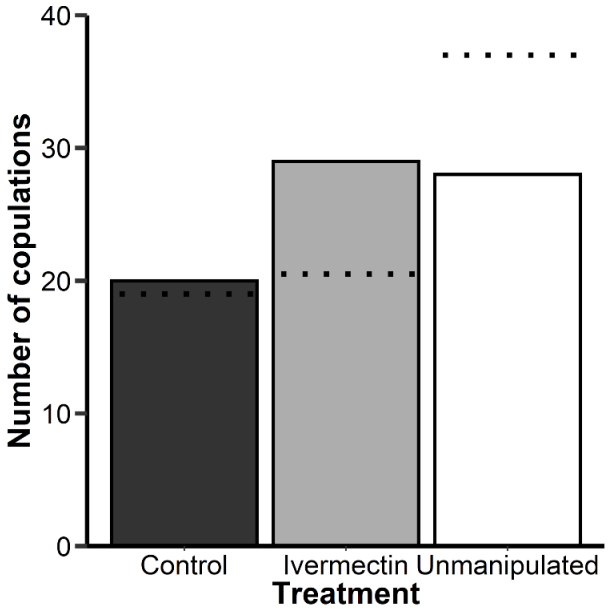

Figure 4. Bars give the total number of copulations of adult males in each treatment group, based on the number of females recaptured with fluorescent powder corresponding to that group. Dashed lines give the expected number of copulations (under the null hypothesis of no difference between groups) based on the total number of powdered males in that group and the total number of copulations detected across all groups. Ivermectin males had more copulations than expected while unmanipulated males had fewer copulations than expected.

Supplementary Tables and Figures

Table S1. Comparison of Cormack-Jolly-Seber models of estimated recapture probability and monthly survival probability rate for *Anolis sagrei* adults across three sampling occasions, as implemented in RMARK. The columns "Recapture Rate" and "Survival" indicate whether these parameters were estimated separately by different combinations of Sex, Treatment, and Time, or fixed at a single estimate (l). Sex (female, male), Treatment (ivermectin, control) and Time (March to May, May to July) are all factors with two levels. "Comparison" indicates the two models that are being statistically compared. "LRT" gives the likelihood ratio test statistic and is calculated as -2 times the log likelihood of the null model minus -2 times the log likelihood of the full model. The preferred model (indicated by bolded terms) estimated a single recapture probability for each Sex and Treatment and separate survival probabilities for the two time intervals, but not for each Sex or Treatment.

Model number	Recap. Rate	Survival	*n*Par	-2 * logLik	Comp.	df	LRT	p-value
1	1	1	2	824.99				
2	Sex	1	3	824.42	1 - 2	1	0.57	0.44
3	Treatment	1	3	824.68	1 - 3	1	0.31	0.57
4	Sex + Treatment	1	4	823.96	1 - 4	2	1.03	0.59
5	Sex * Treatment	1	5	823.66	1 - 5	3	1.33	0.72
6	**1**	**Time**	3	820.84	1- 6	1	4.15	0.04
7	1	Sex	3	823.52	1 - 7	1	1.47	0.22
8	1	Treatment	3	824.66	1 - 8	1	0.33	0.56
9	1	Sex + Treatment	4	823.16	1 - 9	2	1.83	0.40
10	1	Sex * Treatment	5	822.97	1 - 10	3	2.02	0.56
11	1	Sex + Time	4	819.21	6 - 11	1	1.62	0.20
12	1	Treatment + Time	4	820.47	6 - 12	1	0.36	0.54
13	1	Sex + Treatment + Time	5	818.82	6 - 13	2	2.01	0.36
14	1	Sex * Treatment + Time	6	818.61	6 - 14	3	2.22	0.52
15	1	Sex + Treatment * Time	6	818.78	6 - 15	3	2.05	0.56
16	1	Sex * Time + Treatment	6	818.42	6 - 16	3	2.41	0.49
17	1	Sex * Treatment * Time	9	817.78	6 - 17	6	3.05	0.80
18	Sex	Time	4	820.48	6 - 18	1	0.35	0.55
19	Treatment	Time	4	820.66	6 - 19	1	0.17	0.67
20	Sex + Treatment	Time	5	820.20	6 - 20	2	0.64	0.72
21	Sex * Treatment	Time	6	819.66	6 - 21	3	1.17	0.75

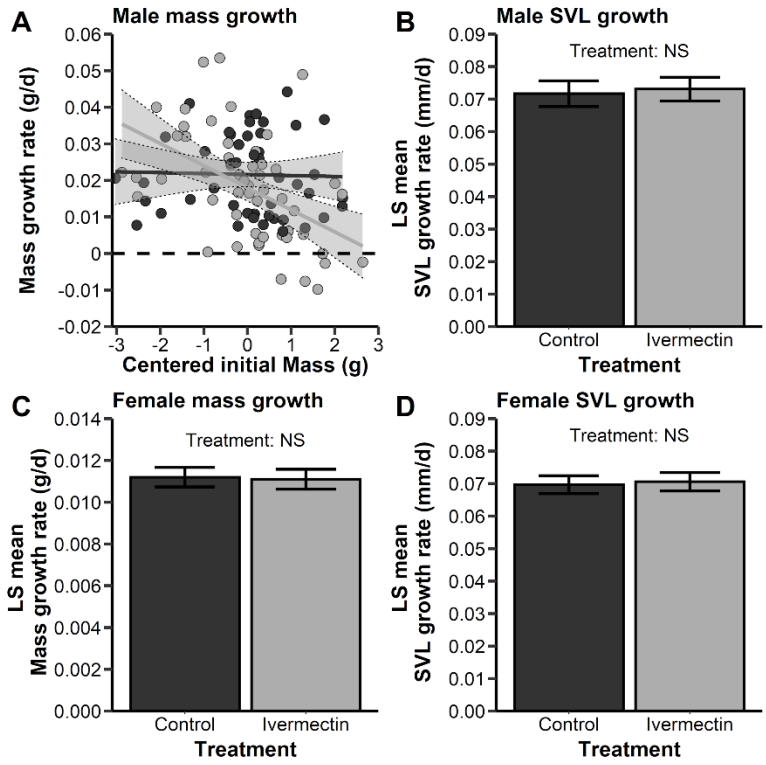

Figure S1. Effects of parasite removal on growth rate from March to May 2019 for body mass (g/d) (A, C) and SVL (mm/d) (B, D) for either adult males (A, B) or adult females (C, D). Panel A shows individual growth with best-fit least squares regression lines for each group with a 95% confidence interval band on the respective regression lines. There is a significant interaction between starting mass and treatment (control (black) and ivermectin (gray))for mass gain in adult males (A).Panels B, C and D give estimated marginal means for each treatment group, with error bars denoting ±1SE.

Chapter Four:

The influence of parasites on selection through their effect on the opportunity for selection, phenotypic variance, and the correlation between phenotypes and fitness

Abstract

Parasites can have dramatic effects on the phenotypes of their hosts and are known to reduce host fitness by reducing survival and reproductive success. While most work has focused on the effects of parasites on the means of host phenotypes and fitness, parasites can also influence selection by 1) influencing the variance and covariance of phenotypes, 2) increasing the variance in relative fitness (the opportunity for selection), and 3) shaping the correlation between phenotypes and fitness. To test whether parasites affect selection through these three mechanisms, I performed a capture-mark-recapture study paired with the experimental removal of nematode parasites in a wild population of brown anole lizards, *Anolis sagrei*. I measured body size, head width, head length, and the size and coloration of the dewlap, a sexually dimorphic ornament. I treated individuals for parasites at the end of their hatch year and recaptured them three times throughout the subsequent breeding season. I found that parasite removal significantly decreased the variance in female body size. However, parasite removal did not influence multivariate patterns of phenotypic covariance in males or females. While parasite removal did not influence overwinter survival, or survival early in the breeding season, it significantly increased survival for both males and females across the entire breeding season. This increase in survival reduced the opportunity for selection across the breeding season. Parasite removal significantly changed selection during the overwinter period, although differently in males and females. This was due to parasite removal changing the correlation between phenotypes and relative fitness not through changes in the variance of relative fitness. During the breeding season, most phenotypes were not under selection and selection estimates did not differ between treatment groups for the majority of the

phenotypes. For the selection estimates that did differ, the effect of parasite removal on both the opportunity for selection and the correlation between phenotype and relative fitness were important in structuring the difference. Through their effect on the variance in relative fitness, parasites increased the variance and magnitude of selection generated through random phenotype fitness associations, thereby increasing the potential for non-adaptive evolution. These results suggest that the effects of parasites on both the opportunity for selection and the correlation between phenotypes and fitness are important for the eco-evolutionary dynamics of their host populations.

Introduction

Nearly all organisms risk infection from a diverse array of parasites and pathogens. Parasites by definition reduce the fitness of their hosts, and have been shown to have a substantial negative effect on survival (reviewed by: Robar *et al.*, 2010, and Wittman & Cox, 2021), and to reduce mating success and offspring production (Webb & Hurd, 1999; Newey & Thirgood, 2004; Pioz *et al.*, 2008; Wittman *et al.*, 2022; Dyrcz *et al.*, 2005). While much attention has been given to the effects of parasites on mean fitness, their influence on the variance in fitness, which can influence the evolutionary dynamics of host populations, has often been overlooked. In addition to their effects on fitness, parasites are known to affect the expression of many host phenotypes, including behavior, growth, morphology, performance, and metabolism (Lafferty & Morris, 1996; Tierney *et al.*, 1996; Sandland & Goater, 2001; Forbes *et al.*, 2002; Binning *et al.*, 2017; Kelehear *et al.*, 2019; Wittman *et al.*, 2022). While the effects of parasites on mean host phenotypes have been well studied, their effects on variance and covariance in host phenotypes have received relatively less attention, other than for behavior (Poulin &

Thomas, 1999; Nakagawa *et al.*, 2015). The co-evolutionary dynamics between host resistance and parasite infectivity and the role of parasites in driving sexual selection for host resistance and signaling traits have been well studied, but their general role in shaping selection on host traits not directly involved in host-parasite interactions has received little attention (Dybdahl & Lively, 1998; Moller *et al*., 1999; Kerstes *et al.*, 2012; Gibson *et al*., 2020). Parasites, through their effects on 1) the variance in host phenotypes, 2) the variance in host relative fitness, and 3) the correlation between phenotypes and relative fitness, can moderate selection on all host phenotypes.

The evolutionary change in a quantitative phenotype due to selection can be predicted through the breeder's equation (Lande & Arnold 1983; Walsh & Lynch, 2018). Under this framework, evolutionary change is decomposed into a term for the change in phenotypic value within a generation, i.e., selection, and one for the transmission of that change to the next generation, i.e., inheritance (Robertson, 1966; Price, 1970; Lande & Arnold, 1983; Walsh & Lynch, 2018). The breeder's equation is given by

$$\Delta\bar{z}' = Cov(w, z) * V_A/V_P \qquad (Eqn.\ 1)$$

where z is a quantitative phenotype, w is relative fitness, an individual's absolute fitness divided by population mean absolute fitness, $\Delta\bar{z}'$ is the change in the mean phenotypic value across a generation, $Cov(w, z)$ is selection and describes the difference in the mean phenotypic value before selection and the fitness-weighed mean phenotypic value after selection, V_A is the additive genetic variance of z, and V_P is the phenotypic variance of z. The covariance component of *Eqn*. 1 is also known as the selection differential (*s*). The variance in relative fitness (V_w) (*Eqn*. 3), also termed the opportunity for selection (*I*),

defines the maximum amount of change in fitness possible within a generation (Crow, 1958; Arnold & Wade, 1984). The variance in relative fitness or opportunity for selection is given by

$$I = V_w = V_W/\bar{W}^2 \qquad (Eqn.\ 2)$$

where V_W is variance in absolute fitness, and $\bar{W}^2$ is mean absolute fitness. When a phenotype is standardized to unit variance the selection differential can be further decomposed into the product of the standard deviation in relative fitness and the correlation between relative fitness and phenotype. Parasites can influence selection through their action on either or both components.

For predicting evolutionary change in multiple correlated phenotypes, the multivariate extension of the breeder's equation is used. This formulation decomposes selection differentials into selection gradients (β) which give the change in relative fitness for a one unit change in a phenotype while holding other phenotypes constant (Lande & Arnold, 1983; Walsh & Lynch, 2018). The multivariate breeder's equation is given by

$$\Delta\bar{\mathbf{Z}}' = \mathbf{G}\mathbf{P}^{-1}\mathbf{s} \qquad (Eqn.\ 3a)$$

$$\Delta\bar{\mathbf{Z}}' = \mathbf{G}\boldsymbol{\beta} \qquad (Eqn.\ 3b)$$

where $\Delta\bar{\mathbf{Z}}'$ is now a vector of changes in phenotypic means across a generation, $\mathbf{s}$ is a vector of selection differentials, $\mathbf{P}$ is the phenotypic covariance matrix, $\mathbf{G}$ is the additive genetic covariance matrix, and $\boldsymbol{\beta}$ is the vector of selection gradients. In addition to determining what trait combinations selection can "see", the covariances contained within $\mathbf{P}$ give rise to indirect selection by transforming the action of direct selection into changes in phenotypic means of correlated phenotypes (*Eqn*. 3a).

Because parasites can influence both mean fitness and the variance in fitness, they should also influence I, and thereby the strength of selection. This relationship has been demonstrated in the Sonoran Desert Fruit Fly, *Drosophila nigrospiracula*. Across populations, variation in relative mating success was positively correlated with infection intensity of the parasitic mite, *Macrocheles subbadius*, as was selection against mite parasitism (Polak & Markow, 1995). For the special case of using binary measures of fitness (mating success, survival), the variance in relative fitness is equal to the probability of failure (mortality, not mating) divided by the probability of success (survival, mating). On average, parasitized hosts experience a 3.5-fold increase in the odds of mortality compared to unparasitized individuals, and thus parasites likely play a significant role in structuring s in natural populations (Fig.1) (Wittman *et al.* 2021, Chapter 2). While parasites are expected to reduce the mean value for non-binary components of fitness such as reproductive success, they do not necessarily increase V_w. However, because the distribution of parasites among hosts is rarely uniform, and individual hosts differ in the fitness costs of a given parasite burden (i.e., tolerance), parasites are generally expected to increase V_W and V_w. Despite this expectation, few studies have tested whether parasites actually increase the opportunity for selection, or the realized magnitude and pattern of selection on phenotypes

Another aspect of the host population through which parasites can affect evolutionary dynamics is the variance in host phenotypes (Poulin & Thomas 1999). Phenotypic variances and co-variances comprise the substrate that selection acts upon, and give rise to indirect selection by transforming the action of direct selection into changes in phenotypic means of correlated phenotypes (*Eqn*. 3a). Parasites are known to

influence the expression of a variety of phenotypes in their hosts, including behavior, body size, morphology, and coloration (Poulin & Thomas, 1999). Depending on the distribution of parasites in the host population and the magnitude of their effect on the phenotype, the phenotypic distribution of a parasitized population may have its mean shifted, have its variance increased or decreased, have its higher moments changed, or become bimodal (Poulin & Thomas, 1999). Parasites could also have important effects on phenotypic covariances. If the prevalence of a parasite in a host population is moderate and its distribution among hosts is random with respect to the values of the phenotypes involved, parasitism could mask phenotypic covariance, increase covariance, or change the direction of covariance, depending on how it affects the phenotypes involved.

The ecological agents of selection, such as climate, predation, and food availability, can jointly determine selection through their interactions (McAdam & Boutin, 2007;Calsbeek & Cox, 2010; MacColl, 2011; Marrot *et al.,* 2018; Cox *et al.*, 2022). Parasites have the potential to modify the magnitude, direction, and shape of selection caused by other agents of selection. If ecological agents of selection interact with the infection state of hosts, then the strength of selection across the whole of the population may be weakened by different patterns of phenotype fitness correlations within each subgroup. In this case, removing parasites from the population would reveal selection that was not apparent in the parasitized population. Alternatively, if parasites drive patterns of selection that reinforce those of other selective agents, then given the same variation in fitness, selection would be of greater magnitude in parasitized populations due to an increased correlation between phenotype and fitness. If parasitism has no effect on the correlation between phenotype and fitness, then the negative effect of

parasites on survival would increase the strength of selection by increasing the variance in relative fitness. Despite these many theoretical possibilities, I know of only one study that has tested whether parasites shape patterns of selection for phenotypes not directly related to host-parasite interactions (Brown & Brown, 2018).

Conceptually, selection is typically defined as nonrandom association between phenotype and fitness whereby phenotypic differences cause fitness differences (Endler, 1986; Kingsolver & Pfennig, 2007; Rice, 2004). In the absence of an environmental factor influencing both phenotype and fitness, assuming the phenotype is uncorrelated with other phenotypes, and assuming an infinite population size, a non-zero value of $Cov(w, z)$ is necessarily due to a causal relationship between phenotype and fitness. This is because random differences in fitness among individuals will be directionless with respect to phenotype, i.e., the expectation of s due to random processes is zero. In a finite population, s still describes the change in mean phenotype due to an association between phenotype and fitness, however a non-zero s is not necessarily due to a causal dependency of fitness on a phenotype (Rice, 2004; Okasha, 2006). In finite populations, random survival or reproduction with respect to phenotype can generate a non-zero value for the covariance, this is equivalent to drift (Rice 2004). In finite populations, the opportunity for selection also sets the upper limit on the potential magnitude of s generated through random events. By increasing the variance in relative fitness, parasites may increase the frequency and magnitude of random covariances between phenotypes and fitness, potentially leading a population to "randomly walk" with a greater rate than non-parasitized populations.

To test whether parasites influence selection acting on their hosts by 1) influencing the variance and covariance of phenotypes, 2) by increasing the variance in relative fitness (the opportunity for selection), and 3) by shaping the correlation between phenotypes and fitness, I performed a capture-mark-recapture study paired with the experimental removal of nematode parasites in a wild population of brown anole lizards, *Anolis sagrei*. I measured the following phenotypes: snout-vent length, body mass, head width, head length, dewlap area, dewlap hue, and dewlap brightness. Snout-vent length has been shown to be under selection in *A. sagrei* (Cox & Calsbeek, 2015), and the dewlap, an elaborated secondary sexual ornament, is predicted to signal condition and parasite resistance (Padilla et al., 2007; Vergara et al., 2012). Head width and head length were included as ecologically relevant phenotypes with no *a priori* predicted association with parasitism (Sanger et al. 2011). Specifically, I tested whether parasite removal decreases phenotypic variance, affects phenotypic covariance, decreases the opportunity for selection, affects the direction and magnitude of selection, and decreases the opportunity for stochastic evolution. I did not make directional predictions for the effect of parasite removal on phenotypic covariance, or the direction and shape of selection.

Methods

Lizard hosts and nematode parasites

I performed a capture-mark-recapture study of *Anolis sagrei*, paired with the experimental removal of their nematode parasites. I used a population of *A. sagrei* on a spoil island in the Guana Tolomato Matanzas National Estuarine Research Reserve (GTM NERR; 29°37’43” N, 81°12’42” W). This population has been surveyed extensively by my lab group as part of a long-term study on sexual conflict in the wild.

This island is well suited for capture-mark-recapture studies because lizards have not been known to emigrate between islands and the recapture rate of animals is high (Males > 95%, Females > 85%, Chapter 3, Unpublished data T.N. Wittman). Anoles on this island are parasitized by two species of nematodes, *Physaloptera* sp., which infects the mucosal lining of the gastric cavity, and *Cyrtosomum penneri*, which infects the cloaca and is exclusively sexually transmitted (Chapter 3). On an island close to my study site (<2km), the prevalence of *Physaloptera* infection is 56%, while all adults are infected with *C. penneri* (Chapter 3). Further background on *A. sagrei* and its parasites is given in Chapter 3. Field work was conducted under permits from the GTM NERR and all procedures involving lizards were approved by the University of Virginia Animal Care and Use Committee (protocol 3896).

I captured juvenile males and females at the end of the breeding season (October 2020) and treated all individuals above 0.5 grams body mass with an injection of either (1) a custom gelling formulation of Ivermectin (IVM, n = 177 males, n = 206 females), or (2) the gelling vehicle as a control (n = 174 males, n = 206 females). As shown in Chapter 3, this IVM gelling solution provides long-term control of nematode infections and is safe and effective in *A. sagrei*. Detailed information on the production of the gelling formulation of IVM is given in Chapter 3. Prior to treatment, I gave each lizard a unique toe clip for identification, measured its snout-vent length (SVL), to the nearest 1 mm using a ruler, and measured its body mass to the nearest 0.01 g using a digital balance (Ohaus Scout Pro: SP202). I then sorted individuals by these measures of size to create two size-matched treatment groups (IVM, control) within each sex. I did not measure head width, head length, dewlap area, or dewlap coloration for juveniles.

Treatment groups did not differ in mean or variance for initial size for males (SVL: t = 0.24, P = 0.81; mass: t = 0.029, P = 0.97) or females (SVL: t = -0.13, P = 0.89; mass: t = -0.21, P = 0.83). Within 24 h of capture and treatment, I released each animal at its site of initial capture.

I resampled the island in March, July, and October of 2021. During each recapture census, I measured SVL, body mass, head width, and head length of the lizards and took a photograph of their manually extended dewlaps. Head width and head length were measured to the nearest 0.1 mm using calipers. Head width was measured at the widest section of the head. Head length was measured from the tip of the snout to the back of the cranium. At each recapture, I re-treated the animals with IVM or control injections calibrated to their new body mass. Lizards first caught in March 2021 were added to the experiment and assigned treatment as described above (IVM: n = 35 males, n = 82 females; Control: n = 30 males, n = 78 females). I used R4.1.3 for all statistical analysis (R Core Team, 2019). Using the photographs of the dewlaps, I measured area, hue, brightness, and saturation. Detailed methods of the photography and measurement of dewlap phenotypes are given in Chapter 1.

Treatment effects on variance in phenotypes

I tested whether parasite removal significantly decreased variance in SVL and mass measured in March 2021 after accounting for the initial variance in the phenotypes measured in October 2020. I used these phenotypes because they were measured on the same individuals at both time points. I used a variance partitioning approach that allocates change in variance into within-individual change (i.e., growth) and change due

to selective loss of individuals via mortality (Rebke *et al.*, 2010; Zhang *et al.*, 2015). This allowed me to account for patterns of selection that may increase or decrease the variance of the phenotype measured in October for individuals alive in March. The variance in SVL was significantly less in March than October, while there was no difference across measurement period for mass. I tested whether the reduction in variance was significantly greater, or the increase in variance was significantly smaller, between October and March for IVM treatment groups than control groups. I used a randomization procedure for significance testing. Within each sex I generated 1000 data sets, each time randomly assigning treatment to an individual. For each dataset, within each randomly assigned treatment group, I computed the difference in variance between the phenotypes measured in October for those individuals recaptured in March, and the phenotypes measured in March. Then I computed the difference between the IVM and control groups for the difference in variance between October and March. I calculated *P* values by dividing the number of instances where the statistic was equal to or greater than the observed value by the total number of randomized datasets.

I tested whether IVM treatment influenced the phenotypic variance-covariance matrix for SVL, body condition, head width, head length, dewlap area, dewlap hue, dewlap brightness, and dewlap saturation using a model-comparison approach. The phenotypic measurements used were collected in March on individuals who were treated in October. I calculated body condition as the residuals from the regression of natural log (*ln*) transformed body mass on *ln*-transformed SVL within each sex and treatment group. I *ln* transformed SVL, head width, head length and dewlap area prior to analysis. I fit multivariate mixed-effect models using the package sommer v4.1.6 within R, with

separate models for males and females (Covarrubias-Pazaran, 2016). I either constrained the residual variance and covariance of the phenotypes to be the same across treatment groups or allowed them to vary between treatment groups. I then compared these two models using a likelihood ratio test. Because individuals first treated in March were used in subsequent survival and selection analysis, I additionally tested for an effect of first treatment period (October or March) on the residual covariance structure of the phenotypes. Using the model comparison approach described above, I tested for an effect of first treatment period (October or March) within each sex without regard for treatment group (IVM or Control). I additionally tested for an effect of first treatment period (October or March) within each treatment group. Importantly, to test whether the effect of first treatment period (October or March) differed with treatment group, I fit a model where the residual covariance was structured by a four-level factor (IVM October, IVM March, Control October, Control March), and compared it to one where the residual covariance was only structured by first treatment period (October or March).

Survival and variance in fitness

I used generalized linear models with a binomial error distribution and a logit link function to test for effects of sex, treatment, and their interaction on apparent survival (observed survival, uncorrected for the estimated probability of recapture) between October and March, between March and July, and between March and October. I used survival as a measure of fitness, in this population survival to the first breeding season and survival across the breeding season are positively related with reproductive success within that year (unpublished data: Cox, Kahrl, Reedy, Sears, Bhave, & Wittman). For each sampling period, I tested whether the opportunity for selection differed between

treatment groups by calculating the log odds ratio of mortality for the control to the IVM group and testing whether this differed from zero.

To estimate survival while also estimating and accounting for recapture probability between censuses in October, March, July, and October, I constructed Cormack-Jolly-Seber capture-mark-recapture models using the Rmark interface for the program MARK (Laake, 2013). I used a model comparison approach to test for the effects of treatment, sex, their interaction, and time on monthly survival rate and treatment, sex, and their interaction on recapture rate. I built all possible models from these terms and assessed their fit using the small sample size-corrected Akaike information criterion.

Selection analysis

I used survival as an estimate of fitness. Because SVL, head width, head length, and dewlap area are strongly correlated, I used size-corrected head width, head length, and dewlap area instead of the untransformed phenotypes. I calculated the size-corrected measures of head width, head length, and dewlap area as the residuals from the regression of the *ln*-transformed phenotype on *ln* SVL. I calculated the residuals within each sex and treatment group. I then standardized the phenotypes to a mean of zero and unit variance within each sex and treatment group (De Lisle & Svensson, 2017). Prior to standardization, I *ln*-transformed SVL. I relativized fitness within each sex and treatment group (see paragraph below). Different standardization methods can lead to different results from selection analysis depending on the relativization of fitness and standardization of phenotypes across or within comparison groups. However, because one of my main interests is to assess how parasites influence the strength of selection

through changes in the variance in relative fitness, relativizing fitness within each treatment group was necessary. Otherwise, the group with the higher average survival would have been mathematically predisposed to a higher variance in relative fitness. The phenotypic covariance matrices did not differ between treatment groups for phenotypes measured in March, the period when lizards born in 2020 are entering their first breeding season. Therefore, standardization of phenotypes within each treatment group and within each sex should not influence the outcome of selection analysis. For selection during the overwinter period (October to March) as juveniles, the distribution of initial phenotypic values was balanced between groups, so standardizing phenotypes within groups should not influence the conclusions.

Among the phenotypes measured in October and used in the selection analysis on juveniles, *ln* SVL exhibited a significant departure from normality for all sex and treatment groups (Table S1). The phenotypes measured in March and used in the selection analysis on adults exhibited significant departures from multivariate normality for all sex and treatment groups, and most phenotypes exhibited departure from univariate normality (Table S1-S2). While violations of normality and multivariate normality do not impact estimates of linear and nonlinear selection differentials (s and c), violations of multivariate normality do impact the estimation of linear and nonlinear selection gradients (β and γ). Because of the violations of multivariate normality, I used the method of Morrissey and Sakrejda (2013) to estimate β and γ, and for consistency s and c, in both adults and juveniles. This approach can be used for arbitrary fitness functions and uses the partial derivatives of the fitness landscape to estimate selection gradients. These gradients are appropriate for use in predicting evolutionary response

through the breeder's equation given multivariate normality of breeding values, while gradients estimated through linear regression require phenotypes to exhibit multivariate normality to work with the breeders equation (Geyer & Shaw, 2008; Morrissey & Sakrejda, 2013; Morrissey, 2014; Walsh & Lynch, 2018). I used generalized linear models with a binomial error distribution and logit link function to estimate the fitness function between phenotypes and survival. For s and c, I estimated the first and second derivatives of average fitness with respect to average phenotypic values and divided these estimates by mean population fitness, which in this case is average survival withing a sex and treatment group. For β and γ, I followed the same procedure but took the partial derivatives.

Selection analysis: juveniles

For overwinter survival for animals first treated as juveniles in October 2020, I estimated linear and non-linear standardized selection differentials (s and c respectively) on SVL and body condition. I estimated s and c using separate models, with s estimated from models with only linear terms and c from models with linear and quadratic terms. Standard errors for selection differentials were estimated using cases bootstrapping with 1000 iterations, and P-values were calculated using this distribution of estimated differentials. To test for treatment effects on the direction and magnitude of selection, I fit generalized linear models with a binomial error distribution and a logit link function with survival as a response, phenotype and treatment as main effects, and an interaction between phenotype and treatment. I then compared this to a reduced model which excluded the interaction effect using a likelihood ratio test. For testing whether parasites influence the form and magnitude of non-linear selection, I used the same approach as

above but added the additional terms of the squared phenotypic values and their interactions with treatment. I do not present estimates of β and γ for juveniles for the overwinter period (October 2020 to March 2021) because SVL and body condition have no phenotypic correlation, and I did not detect any evidence for correlational selection within any sex or treatment group.

Selection analysis: adults

I performed selection analysis on adults for two periods during the breeding season, from March to July, and from March to October. Importantly, survival from March to October was significantly improved by parasite removal, while survival from March to July was not. When using survival as an estimate of fitness, the variance in relative fitness mathematically increases with decreasing mean survival. Thus, performing selection analysis over both time periods therefore provides me with comparisons of treatment effects on selection over an episode when parasites increase variance in relative fitness and another episode when they do not. Few males survived to October and thus their fitness surfaces were poorly estimated. Due to the small number of males alive in October, I could not fit a full multivariate model to estimate selection gradients for that time period. Instead, I broke the phenotypes up into two separate modules, and estimated β and γ on those subsets of phenotypes separately. One group of phenotypes contained SVL, body condition, residual head width, and residual head length, while the other contained SVL, residual dewlap area, dewlap hue, dewlap brightness, and dewlap saturation. I also estimated multivariate selection for these subsets of phenotypes for females for the same survival period, as well as for all groups for survival between March and July. To test for treatment effects on the direction and

magnitude of selection differentials, I fit generalized linear models with a binomial error distribution and a logit link function with survival as a response, phenotype and treatment as main effects, and an interaction between phenotype and treatment. I then compared this to a reduced model which excluded the interaction effect using a likelihood ratio test. I used the same approach to test for treatment effects on multivariate selection, with the full model containing the phenotypes and treatment as main effects and terms for the interaction between each phenotype and treatment.

Magnitude and direction of multivariate selection

In addition to the model comparison tests for the effects of parasitism on selection differentials and gradients, I tested whether ivermectin treatment changed the overall magnitude of multivariate selection by comparing the length of the vector of selection gradients between treatment groups. The length of a vector in multivariate space is given by its norm and is a measure of its magnitude. I calculated the norm of each vector of β. I used randomization to test whether parasite removal significantly changed the overall magnitude of multivariate selection. First, I calculated the difference between the observed norm of the β vector in the control group and the observed norm of the β vector in the IVM group, then I generated 1000 simulated datasets where I randomly assigned treatments to individuals without replacement. Next, within each treatment group, I re-standardized the phenotypes, estimated the β vector, took the norm of the vector, and calculated the difference of the norm between control and IVM. Finally, I calculated the *P*-value as the number of times the randomized distribution had a difference between norms as large or larger than the observed value, divided by the total number of replicates. To test whether IVM treatment significantly changed the direction of

multivariate linear selection, I estimated the vector correlation between β vectors from control and IVM groups within each sex. The vector correlation describes the angle between two vectors and can be used as an overall measure of the similarity in multivariate space that is independent of magnitude. I used the same randomization procedure described above to test whether the vector correlation is significantly lower than would be expected by chance.

Random covariance between phenotype and fitness

I estimated the influence of parasitism on generating stochastic covariance between a phenotype and fitness by randomly permuting survival across individuals within a treatment group 1000 times, then calculating the covariance between relative fitness and a phenotype for each permuted dataset. For this analysis, due to computation time, I calculated the selection differential as the covariance between relative fitness, $w_i/\bar{W}$, and the standardized phenotype, *ln* SVL. I then calculated the variance among the estimated selection differentials and the difference in variance between control and IVM treatment groups within each sex and time period. To test the significance of the difference in variance, I used a double randomization procedure. First, I generated 1000 datasets where treatment was randomly permuted across individuals within a sex. Then for each of these 1000 datasets I randomly permuted survival across individuals within the treatment groups 1000 times. For each of these one million doubly permuted datasets, I calculated relative fitness, standardized *ln* SVL, and calculated the selection differential. For each of the 1000 treatment randomized datasets, I calculated the variance of standardized selection differentials and took the difference between treatment groups. This allowed me to generate a null expectation for the difference in variance of selection differentials. I

calculated *P* values as the number of treatment randomized datasets which had a difference equal to or greater than the observed difference divided by the total number of treatment randomized replicates.

Results

Effect on phenotypic variance and covariance

There was significantly less phenotypic variance for SVL measured in March than measured in October, while there was no difference in variance for mass (Table 1). IVM females had a significantly greater decrease in phenotypic variance for SVL than did control females, after accounting for the significant disruptive selection on SVL in IVM females (Table 1). There was no significant effect of treatment on the change in SVL from October to March in males, or for mass in males or females (Table 1.).

However, holistically, the phenotypic covariance matrices measured in March for animals treated in October did not differ between treatment groups in either sex (Table 2). There were significant differences in the phenotypic covariance matrices measured in March between animals that were first treated in October and those first treated in March, but this effect did not differ by treatment (Table 2). Further, there was no difference in the phenotypic covariance matrices between treatments when including all animals either first treated in October or March (Table 2). Thus, the difference in phenotypic covariance structure associated with when an individual was first treated should not affect the analysis of treatment differences in selection through phenotypic standardization. The variances, correlations, and means for the phenotypes are given in tables S3-S6.

Survival and variance in fitness

Juveniles

Overwinter survival did not differ between treatment groups for males or females, and males had significantly lower survival than females (Sex: $\chi^2_1 = 27.32$, $P < 0.0001$. Treatment: $\chi^2_1 = 0.21$, $P = 0.64$. Sex*Treatment: $\chi^2_1 = 0.99$, $P = 0.32$) (Table 3) (Fig. 2). Because fitness was measured as survival and parasite removal did not affect survival over this period, parasite removal did not increase the opportunity for selection (Odds mortality Control / Odds mortality IVM) (Males: Log Odds Ratio = 0.25, SE = 0.23. Females: Log Odds Ratio = -0.06, SE = 0.19) (Table 3). The influences of sex and treatment on survival over this time period were similar when using a Cormack-Jolly-Seber model to account for recapture probability (Tables 4-5).

Adults

For the period between March and July, parasite removal did not significantly increase survival in either males or females, and males had significantly lower survival than females (Sex: $\chi^2_1 = 6.26$, $P = 0.01$. Treatment: $\chi^2_1 = 0.75$, $P = 0.15$. Sex*Treatment: $\chi^2_1 = 1.76$, $P = 0.18$) (Fig.2). Because fitness was measured as survival and parasite removal did not affect survival over this period, it did not increase the opportunity for selection (Odds mortality control / Odds mortality IVM) (Males: Log Odds Ratio = -0.23, SE = 0.34. Females: Log Odds Ratio = 0.30, SE = 0.21) (Table 3). Parasite removal significantly increased survival across the entire breeding season (March to October), and there was no significant difference in the effect of treatment between males and females, although males had significantly lower survival than females (Sex: $\chi^2_1 = 6.18$, $P = 0.01$. Treatment: $\chi^2_1 = 8.03$, $P = 0.005$. Sex*Treatment: $\chi^2_1 = 0.22$, $P = 0.63$). IVM treatment significantly decreased the opportunity for selection in females (Log Odds Ratio = 0.93,

SE = 0.35) and while the effect in males was not significant (Log Odds Ratio = 0.56, SE = 0.72), the power to detect an effect was limited due to low survival (only 6 IVM males and 3 control males were alive in October 2021; Table 3; Fig. 2).

I found similar results when using a Cormack-Jolly-Seber capture-mark-recapture analysis that accounts for recapture probability. The best-fitting model in terms of AICc score contained the terms time, sex, treatment and an interaction between treatment and time for the survival estimate, and the term sex for recapture probability. Treatment was included in the terms for the survival estimate in three of the four models within Δ2AICc of the top model. I present the top ten models in terms of AICc values in Table 4. Recapture rates were high for both males (0.97) and females (0.87). However, the sex term in the model was not significant (Table 5). IVM treatment significantly increased survival for the time period between July 2021 and October 2021, and this effect did not differ significantly between males and females (Table 5).

Selection

Juveniles

I found no significant linear selection differentials on SVL or body condition for any sex or treatment group, and no estimates significantly differed between treatment groups (all $P > 0.4$) (Table 6). However, there was significant disruptive selection on SVL in IVM females and stabilizing selection on body condition in IVM males. Both estimates of nonlinear selection significantly differed between treatment groups within the respective sex (Table 6., Fig. 3). There were no other significant non-linear selection estimates (Table 6).

Adults

Selection measured between March and July

For the univariate selection analysis in males, the linear selection differential for SVL in control males was negative and significantly different than zero, but there were no other significant linear selection differentials (Table 7). Further, there was no significant difference between treatment groups within males for the estimated linear selection differential on any of the phenotypes ($P > 0.1$ for all phenotype-by-treatment interactions). For the univariate selection analysis in females, none of the estimated linear selection differentials were significantly different from zero for either treatment group (Table 7), and there were no significant differences between the treatment groups for the estimated linear selection differentials (all $P > 0.2$).

There were no significant nonlinear selection differentials for any of the phenotypes in any of the sex or treatment groups (Table 7). However, control and IVM males differed significantly in the nonlinear selection differential estimated for residual head width, which was positive in control males and negative in IVM males (Phenotype * Treatment: $\chi^2_1 = 6.64$, $P = 0.01$) (Table 7). There were no other significant differences in nonlinear selection differentials between treatment groups for either sex (all $P > 0.1$).

In the multivariate analysis of selection, I detected significant negative directional selection acting on dewlap hue and dewlap saturation in IVM males (Table 8). Both of these selection gradients were significant in the analysis using the subset of dewlap phenotypes and SVL, and in the analysis using all of the phenotypes. In control males, I detected significant negative directional selection on SVL, but this was only significant in

the module on the subset of phenotypes containing SVL, body condition, head length, and head width, although the estimated selection gradient was in the same direction and similar in magnitude in the other models (Table 8). However, the treatment groups did not significantly differ in the overall pattern of multivariate linear selection for all traits or either subset as assessed through model comparison (all $P > 0.16$). I did not detect any significant linear selection acting on any of the phenotypes in either treatment group for females, and the treatment groups did not differ in the overall pattern of multivariate linear selection for all traits or either subset as assessed through model comparison (all $P > 0.45$).

When comparing the overall magnitude of multivariate linear selection, I found that when using all phenotypes, IVM males had a significantly greater length of the selection vector than control males (Table 9). This was also true when using the module containing dewlap phenotypes, but not when using the module containing head width and head length (Table 9). There was no difference in the length of the vector of selection gradients between treatment groups in females (Table 9).

The direction of multivariate linear selection, as measured by the vector correlation between vectors of selection gradients, did not significantly differ between treatment groups in either sex (Table 10). This is likely due to the majority of selection gradients being near zero and having standard errors much larger than their magnitudes. The distribution of vector correlations from the randomization procedure was diffuse and covered most of the range from -1 to 1.

Multivariate non-linear selection models that included all phenotypes were unable to converge for either treatment group within males, thus I restricted my analysis to the

two modules of phenotypes. I found significant disruptive selection on SVL in control males, but only in the module containing SVL, body condition, head width, and head length (Table 11). There were no significant non-linear selection gradients in IVM males for either module. There were no significant non-linear selection gradients in control females for either module. In IVM females, I detected significant correlational selection between SVL and body condition, and between head with and head length (Table 12). The treatment groups did not differ in the overall pattern of multivariate non-linear selection for either sex for all traits or either subset as assessed through model comparison (all $P > 0.3$).

Selection measured between March and October

Across all sex and treatment groups, I found two significant linear selection differentials: positive linear selection for residual dewlap area in control females, and positive linear selection for residual head length in IVM females. These selection differentials also differed significantly between control and IVM females (Table 13, residual dewlap area * Treatment: $P = 0.02$, residual head length * Treatment: $P = 0.02$). There were no other significant linear or non-linear selection differentials, or differences between treatment groups for any selection differential (Table 13). The only significant linear selection gradient among all phenotypes and sex and treatment groups was positive directional selection on residual head length in IVM females (Table 14). The treatment groups did not differ in the overall pattern of multivariate linear selection for either sex for all traits or either subset as assessed through model comparison (all $P > 0.19$).

Multivariate non-linear selection was inestimable in males. I could not achieve model conversion and thus estimates and their errors are unstable. In females, there were no significant non-linear selection gradients in either treatment group (Table 15) and multivariate non-linear selection did not differ between treatment groups (all $P > 0.3$).

I did not find any significant differences in the norm of the vector of linear selection gradients between treatment groups (Table 9). While the norm of the vector in control males was 2 to 3.6 times as large as that in IVM males, these estimates contained substantial error and thus the null distribution under randomization had a large span.

The direction of multivariate selection, as measured by the vector correlation between vectors of selection gradients, was not significantly lower between control and IVM groups in either sex than expected between groups created by random treatment assignment within each sex (Table 10). This is likely due to the majority of selection gradients being near zero and having standard errors much larger than their magnitudes. The distribution of vector correlations from the randomization procedure was diffuse and covered most of the range from -1 to 1.

Random covariance between phenotype and fitness

For the period between March and July, when the opportunity for selection did not differ between treatments, there was no difference between control and IVM groups in the variance of estimates of s generated by randomly permuting survival records with respect to phenotypic values (Males: variance ratio (control/IVM) = 0.98, $P = 0.67$. Females: variance ratio (control/IVM) = 1.35, $P = 0.21$) (Table 16). However, for the period between March and October, when the opportunity for selection was greater for control groups, I found that control females had 2.8 times more variance in estimates of s than

IVM females ($P = 0.01$). While control males had 3.5 times more variance in s than IVM males, this was ratio was not significant ($P = 0.11$).

Discussion

I found that IVM treatment to remove parasites from brown anoles decreased the variance of SVL in females, but not in males or for other phenotypes, and had no overall effect on patterns of phenotypic covariance. I further found that IVM treatment significantly increased survival to the end of the breeding season, and thus decreased the opportunity for selection, although there was no effect of IVM treatment on survival for the overwinter period in juveniles or early in the breeding season for adults. IVM treatment changed some patterns of selection for the over overwinter period in juveniles, generating significant disruptive selection on SVL in females and stabilizing selection on condition in males (Fig.3). However, the multivariate fitness landscape of adults was poorly characterized, with few significant selection gradients and large standard errors relative to the estimates. The few significant selection gradients I found should be interpreted with caution due to the number of statistical tests performed. For the period where the opportunity for selection did not differ between treatment groups, I found that the overall magnitude of directional selection was greater in IVM males than control males. This was driven by significant negative directional selection on dewlap hue and saturation in IVM males. When the opportunity for selection was greater in control groups, there was a trend for the magnitude of directional selection to be greater in the control group compared to the IVM group within both males and females, but the treatments were not significantly different. There was no clear pattern of multivariate nonlinear selection differing between the groups. Eigenvalues from the canonical rotation

of the matrices of nonlinear selection gradients indicated that there was no overall pattern of multivariate stabilizing or disruptive selection. Further, the distribution of eigenvalues, which ranged from positive to negative, and the magnitude of the leading and trailing eigenvalues, were each similar between treatment groups. I found that by increasing survival, IVM treatment decreased the variance in estimated selection differentials under random phenotype and fitness associations. Overall, I found little support for the effect of parasites on eco-evolutionary dynamics acting through phenotypic variation, but that parasites in some cases can potentially have important effects through their influence on variance in fitness and the correlation between phenotype and fitness.

The effects of parasites on the growth and development of their hosts are ultimately what would structure their effects on the variance in body size. Thus, the effect of IVM treatment on decreasing the variance in SVL in females should come about through homogenization of the growth rate among individuals at a certain size, by increasing growth at small starting size or decreasing it at larger starting sizes. While variance in the residuals from the regression of SVL growth on initial SVL was larger for control females, and the estimated effect of initial SVL was greater for IVM females, neither of these differences were significant (Chapter 3, $P = 0.15$, $P = 0.51$). I found no effect of parasites on general patterns of variance and covariance among the phenotypes measured in March. I did not have starting values for phenotypes other than SVL and mass, thus if patterns of non-linear selection acting on the other phenotypes differ between treatment groups it could have masked any within individual effects on changes in the phenotypic variances and covariances, as I saw for SVL in females. The effect of parasites on structuring patterns of phenotypic variance and covariance may be more

important in host-parasite systems where the incidence of parasitism is moderate to high and their effects on development are more substantial (Johnson *et al.*, 1999; Miura *et al.*, 2006; Johnson & Hartson, 2009; Laciny, 2021). While I do not have data on the prevalence of *Physaloptera* in juvenile *A. sagrei*, the nematode *C. penneri* is sexually transmitted and thus juveniles do not harbor this parasite. Given that *Physaloptera* is environmentally transmitted through insect prey items, an individual's cumulative risk of infection is expected to increase across time and thus the prevalence of infection is likely to be lower in juveniles compared to adults.

Ivermectin treatment did not increase survival for juveniles or adults early in the breeding season, but it had a dramatic effect on survival across the whole breeding season. Females who were treated with IVM had 2.5 times greater odds of surviving until October relative to control females, while IVM treated males had 1.8 times greater odds of survival for the same time period relative to control males. This contrasts with a previous study which found no survival benefit to parasite removal on adult *A. sagrei*, but in that study survival was only measured until July, where I also found no survival benefit to IVM treatment (Chapter 3). There are reasons to suspect that the survival costs of parasitism may accumulate across the breeding season (Leivesley *et al.*, 2019; Albery *et al.*, 2021). First, reproductive activity can have high energetic costs and is known to reduce survival (Clutton-Brock *et al.*, 1989; Stearns, 1989; Zera & Harshman, 2001; Cox & Calsbeek, 2009b; Cox *et al.*, 2010), and parasites may exacerbate the survival cost of reproduction by utilizing host resources (Careau *et al.*, 2013; Leivesley *et al.*, 2019; Albery *et al.*, 2021). Second, reproduction is associated with decreased immune function (Fedorka *et al.*, 2004), which may magnify the detrimental effects of parasites (Cox *et al.*,

2010; Knutie *et al.*, 2017). The energetic demands of reproductive activity in brown anoles are large. Females produce a single-egg clutch, approximately 10% of their body mass, every 1-2 weeks across the breeding season, and males engage in intersexual signaling and aggressive intrasexual displays and interactions (Cox & Calsbeek, 2009b).

Patterns of non-linear selection acting on juveniles differed significantly between treatment groups for SVL in females and body condition in males. Because there was no difference in the opportunity for selection between treatment groups during this period, this indicates that parasites influence the correlation between phenotype and fitness rather than variance in fitness itself. Selection was significant in the IVM treatment groups but not the control groups, suggesting the presence of an agent of selection which either acts in the absence of parasites, or interacts with parasites in a way that masks its effect. The only other study to investigate whether parasites shape patterns of phenotypic selection on phenotypes not directly related to host-parasite interactions found that selection acting on nestling body mass was stabilizing in the presence of parasites and positive and directional in their absence (Brown & Brown, 2018). While the effect in this study was different from the pattern that I found, it nonetheless suggests that the influence of parasites on selection may be common. Through their effect on phenotype fitness correlations parasites likely have an important role in structuring the evolutionary trajectory of host populations.

For selection acting during the earlier part of the breeding season (March to July), where the opportunity for selection did not differ between treatment groups, the difference in the correlation between phenotypes and fitness is more important in structuring differences in selection between the treatment groups. For that period, the

largest correlation between phenotype and fitness was for SVL in control males (r = -0.24). This was the strongest and only significant selection differential. For multivariate selection gradients, the relationship among phenotypes became more important in structuring the strength of selection. The two strongest selection gradients in this period were acting on dewlap hue and dewlap saturation in IVM males, despite these two phenotypes showing weak correlations with fitness (r = -0.108, r = -0.045). While there were significant differences between treatment groups for selection on various phenotypes, given the large number of tests I performed, most of these results may be due to spurious associations rather than causal effects. Thus, I view the multivariate summary comparisons, which carry over uncertainty through the randomization procedures, as a more appropriate test for treatment effects on selection. The overall strength of multivariate selection was greater for IVM males than control males (Table 9). This shows that parasites can affect the strength of multivariate selection through their influence on patterns of correlation between phenotypes and fitness.

The effect of parasites on survival across the entire breeding season increased the opportunity for selection. In both males and females, the square root of the opportunity for selection was 1.6 times greater for the control group than for the IVM group. Thus, for this period, linear and non-linear selection differentials are structured both by the correlation between phenotypes and fitness and by the opportunity for selection, with the additional effect of phenotypic correlations for selection gradients. However, I found no significant differences between treatment groups for any selection estimates during this time period. The selection differentials and gradients were estimated with a large amount of error due to low survival. While some estimates were quite large, they were all well

below the limit imposed by the opportunity for selection (Table 3, Table 13). Given that there was a large difference in the opportunity for selection between treatment groups, and no difference in the phenotypic covariance matrices, the lack of a difference in the realized strength of selection between treatment groups must have been due to random variation in the correlations between phenotypes and fitness between the treatment groups. While some of the results are suggestive, such as the difference in vector norms between treatment groups within males for both periods of the breeding season, I do not find strong support for the role of parasites in structuring the causal relationships between phenotypes and fitness (selection) or their strength for adults. The effect of parasites on selection arising through variance in another component of fitness or lifetime reproductive success may differ from selection arising through variance in survival. In this population of anoles, across multiple years, there is no selection on body size when fitness is measured as survival, however there is consistent positive linear selection on body size in males and females when fitness is measured as reproductive success (Unpublished data: Cox, Reedy, Kahrl, Sears, Bhave & Wittman).

I found that parasites can increase the role of drift in evolution by increasing the variance in the strength of selection differentials generated through random associations between phenotype and fitness. Selection differentials and selection gradients comprise both random events (fitness differences not causally related to phenotypic differences) and true “selection”, a causal relationship between phenotype and fitness (Rice, 2004). Non-significant selection differentials and gradients that are numerically greater than zero may still represent some level of covariance between phenotype and fitness in the sampled population. In finite populations, the majority of a population can be sampled

and the covariance between fitness and a phenotype known. While statistical tests aimed at inferring causality and using infinite population assumptions may find the covariance non-significant, or likely due to chance, the covariance is still acting to change phenotypic values in that population. Regardless of the generating mechanism, any covariance between phenotype and fitness will result in evolutionary change if the phenotype is heritable and assumptions of environmental independence of breeding values are met (Walsh & Lynch, 2018). In other words, random fitness-phenotype associations drive non-adaptive evolutionary change in finite populations. In this study we sampled a large majority of the population and thus many non-significant selection estimates are likely greater than zero for this population but generated through random processes. As the upper bound of any fitness phenotype association is set by the variance in relative fitness, processes that influence the opportunity for selection may influence both adaptive and non-adaptive evolution. Using survival from March to October, I found that in females, IVM treatment significantly decreased the variance in selection differentials generated from randomized fitness phenotype associations, thus decreasing the potential for non-adaptive phenotypic change and evolution. The mean absolute value of the randomly generated selection differentials for IVM females was approximately half that of control females ($s = 0.13$, $s = 0.24$). In males, the variance in selection differentials was greater in control males than in IVM males, but this difference was not significant due to the low survival and high opportunity for selection in both groups. This shows that parasites can potentially affect the evolutionary dynamics of host populations by influencing the likelihood and magnitude of random fitness-phenotype associations.

My results suggest that the effect of parasites on selection are unlikely to come about through their effect on the phenotypic covariances of their hosts. Parasites can have a strong effect on selection for at least some traits and selective episodes, for the overwinter period in juveniles there was a significant treatment effect on non-linear selection in both males and females. Parasite removal also increased the magnitude of multivariate linear selection acting on male early in the breeding season. The opportunity for selection did not differ between treatment groups for both periods, suggesting that parasite removal increased the correlation between phenotype and fitness for at least some phenotypes. Although parasite removal had a large effect on the opportunity for selection through survival differences across the breeding season, this did not result in consistent differences between treatment groups in the strength of selection. However, the effect of parasites on the opportunity for selection increased the potential for random phenotype fitness associations to affect evolution. Overall, my results show the effect of parasites on selection are variable and may be context dependent. Further, the effects of parasites on selection may more often arise through changes in the correlation between phenotypes and fitness than through changes in the opportunity for selection.

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Figures

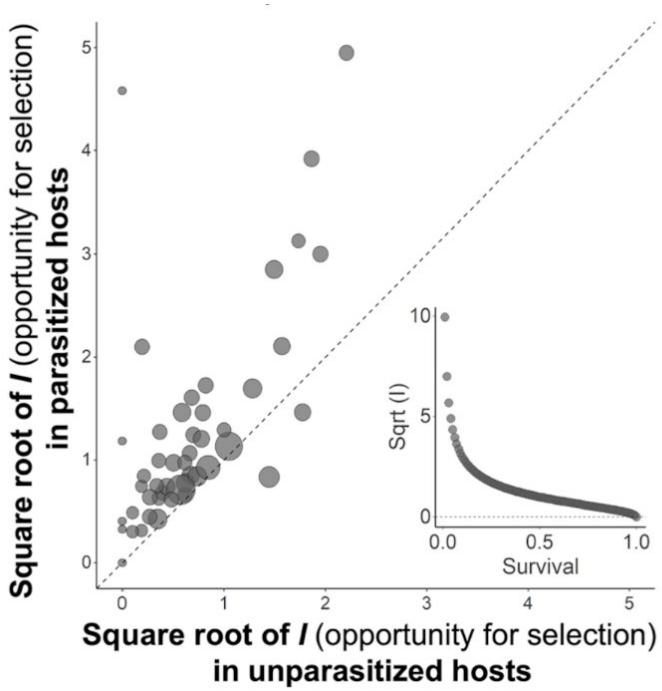

Figure 1. Reanalysis of data used in Chapter 2. Each point gives the square root of the opportunity for selection, Sqrt(*I*), calculated from survival for parasitized and unparasitized individuals within a study. The size of the points is inversely proportional to the sampling variance of the associated odds ratio. Nearly all points fall above the 1:1 line indicating a greater opportunity for selection in the parasitized groups. The inset shows the relationship between survival probability and the square root of the opportunity for selection.

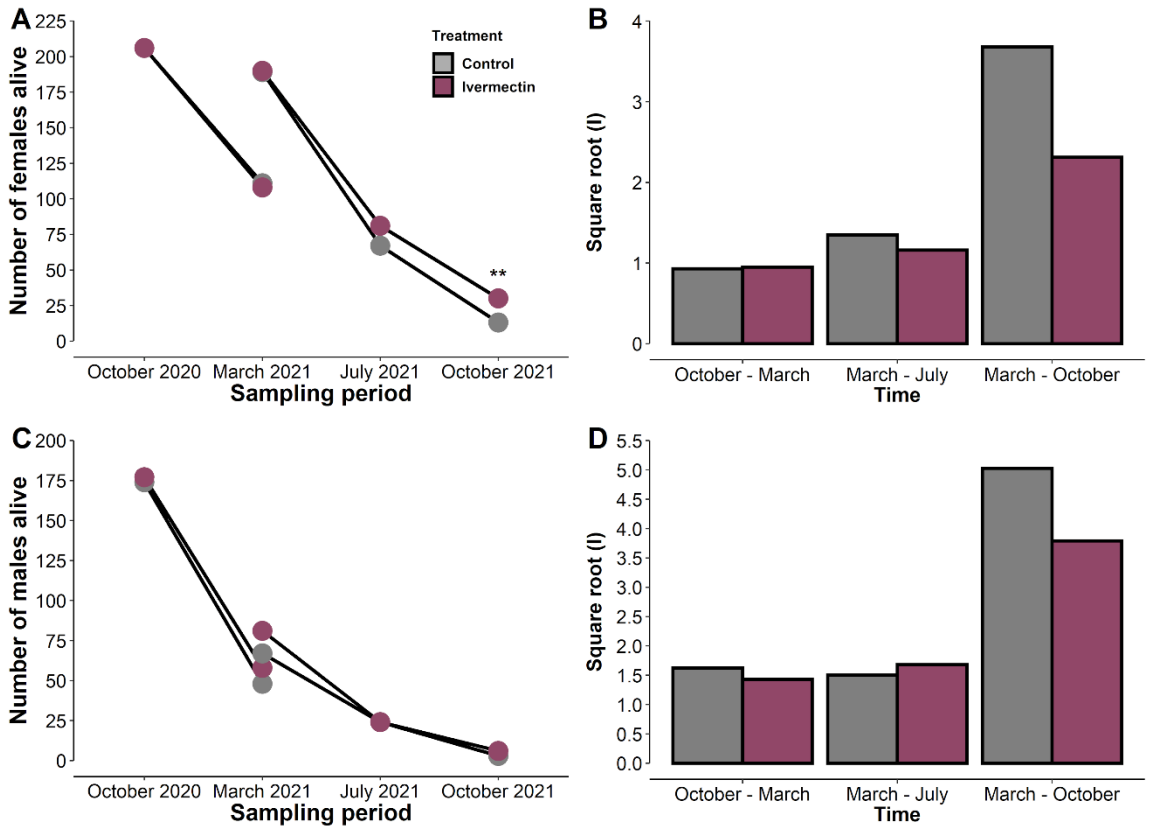

Figure 2. The left panels show the number of individuals alive within each treatment group at the start of each time interval. Two values are given for March 2021, the points connected by the line to October 2020 indicate the number of animals alive from within the group treated in October 2020, while the other points give the total number of animals alive after treating additional newly caught animals. The panels on the right give the square root of the opportunity for selection for the time intervals given on the x-axis. The square root of the opportunity for selection gives the upper limit to the strength of selection.

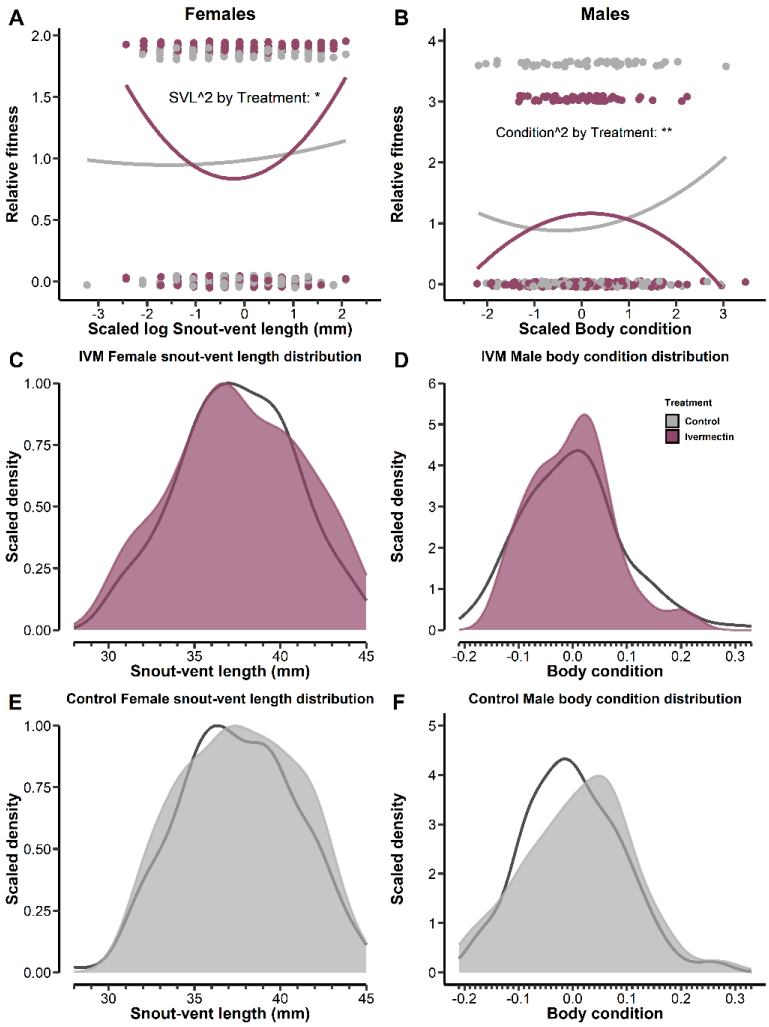

Figure 3. Panels A and B show the estimated quadratic fitness surface for snout-vent length in juvenile females and body condition in juvenile males. In panels C and D the distribution indicated by the solid black line shows the distribution of phenotypic values for the ivermectin treatment group before section, and the shaded red distribution shows the distribution of phenotypic values after selection. In panels E and F the distribution indicated by the solid black line shows the distribution of phenotypic values for the control treatment group before section, and the shaded grey distribution shows the distribution of phenotypic values after selection.

Tables

Table 1. Variances estimates of SVL and Mass within a given sex and treatment group. Variances were estimated from traits measured either in October 2020 or March 2021. The column Oct. Recap are variance estimates of the traits measured in October for a subset of individuals that were recaptured in March. The difference of the Oct. Recap variance estimates and the Oct. variance estimates is the change in variance due to selective effects. The difference in Oct. Recap variance estimates and March variance estimates is the within individual component of the total change in variance. P values were calculated using the respective randomization procedure described in the methods.

		Control				Ivermectin					
		Variance			Difference	Variance			Difference		
Sex	Trait	Oct.	Oct. Recap	March	Oct. Recap – March	Oct.	Oct. Recap	March	Oct. Recap – March	Diff. IVM – Diff Control	*P*
F	SVL	11.05	10.95	5.73	5.22	11.12	14.45	4.57	9.88	4.66	0.01
F	Mass	0.076	0.072	0.105	-0.015	0.073	0.087	0.103	-0.033	0.017	0.22
M	SVL	51.05	49.99	18.8	31.19	51.24	59.09	14.84	44.25	13.06	0.14
M	Mass	0.887	1.017	1.194	-0.177	0.859	0.902	0.964	-0.062	0.239	0.26

Table 2. Likelihood ratio test for multivariate mixed models with different constraints on the residual covariance structure. Treatment did not affect the phenotypic variance covariance matrix. The phenotypic variance covariance matrices of individuals first treated in March 2021 were significantly different from individuals first treated in October 2020. Importantly this effect did not differ between treatment groups. DF is the difference in the number of parameters between the focal model and the null model with no residual covariance structure

Sex	First Treatment	Treatment	Residual Covariance Structure Comparison	LRT Statistic	DF	*P*
Female	1	—	Null vs Treatment	36.01	36	0.46
	1 & 2	—	Null vs first treatment (1, 2)	253.00	36	< 0.001
	1 & 2	Ivermectin	Null vs first treatment (1, 2)	87.72	36	< 0.001
	1 & 2	Control	Null vs first treatment (1, 2)	68.21	36	< 0.001
	1 & 2	—	First treatment (1, 2) vs First Treatment by Treatment	68.72	72	0.58
	1 & 2	—	Null vs Treatment	27.90	36	0.83
Male	1	—	Null vs Treatment	45.68	36	0.13
	1 & 2	—	Null vs first treatment (1, 2)	91.73	36	< 0.001
	1 & 2	Ivermectin	Null vs first treatment (1, 2)	102.26	36	< 0.001
	1 & 2	Control	Null vs first treatment (1, 2)	39.19	36	0.33
	1 & 2	—	First treatment (1, 2) vs First Treatment by Treatment	86.63	72	0.12
	1 & 2	—	Null vs Treatment	41.71	36	0.24

Table 3. Number of individuals that were alive at the end of the associated time and the number of animals who presumably died during that interval, for each sex and treatment group. The total number of individuals in a row is the number of individuals within the respective sex and treatment group who were known to be alive at the beginning of the respective time interval. Trip number 1 was the first sampling period of the experiment in October 2020, trip number 2 was in March 2021, trip number 3 was in July 2021 and trip number 4 was in October 2021. The standard error was calculated as the square root of the probability of survival times the probability of mortality divided by the sample size. The opportunity for selection (*I)* was calculated by dividing the number of lizards that died by the number of lizards alive.

Sex	Treatment	Interval: trip # to trip #	Number Alive	Number Dead	Survival Probability	SE	Square root of *I*
M	Ivermectin	1-2	58	119	0.328	0.035	1.43
M	Control		48	126	0.276	0.034	1.62
F	Ivermectin		108	98	0.524	0.035	0.95
F	Control		111	95	0.539	0.035	0.93
M	Ivermectin	2-3	24	68	0.261	0.046	1.68
M	Control		24	54	0.308	0.052	1.50
F	Ivermectin		81	109	0.426	0.036	1.16
F	Control		67	122	0.354	0.035	1.35
M	Ivermectin	2-4	6	86	0.065	0.026	3.79
M	Control		3	76	0.038	0.022	5.03
F	Ivermectin		30	160	0.158	0.026	2.31
F	Control		13	176	0.069	0.018	3.68
M	Ivermectin	3-4	6	18	0.250	0.088	1.73
M	Control		3	21	0.125	0.068	2.65
F	Ivermectin		30	51	0.370	0.054	1.30
F	Control		13	54	0.194	0.048	2.04

Table 4. Top 10 Cormack-Jolly-Seber capture-mark-recapture models from among all candidate models. Survival probability gives the terms included in the model for structuring the survival probability, recapture probability is the terms included in the same model for structuring the recapture probability. Npar is the number of parameters in the model. AICc is the small sample size-corrected Akaike information criterion score, DeltaAICc gives the difference in AICc score between the top model and the respective model, and -2LnL gives -2 times the log likelihood of the model.

Survival probability	Recapture probability	npar	AICc	DeltaAICc	-2LnL
~Treatment * time + time * Sex	~Sex	11	2057.97	0.00	2035.79
~Treatment + Sex * time	~Sex	9	2058.99	1.03	2040.87
~Treatment * time + time * Sex	~Treatment + Sex	12	2059.61	1.64	2035.40
~Treatment + Sex + time	~Sex	7	2059.90	1.93	2045.83
~Sex * time	~Sex	8	2059.91	1.95	2043.82
~Treatment * time + time * Sex	~Treatment * Sex	13	2060.12	2.15	2033.87
~Treatment * time + time * Sex	~1	10	2060.54	2.57	2040.39
~Treatment + Sex * time	~Treatment + Sex	10	2060.83	2.86	2040.68
~Sex + time	~Sex	6	2060.86	2.90	2048.81
~Sex * time	~Treatment + Sex	9	2060.90	2.93	2042.78

Table 5. Parameter estimates from the top supported Cormack-Jolly-Seber capture-mark-recapture model. The first column gives the aspect of the model the terms in the row apply to. The terms give the associated linear and interaction terms that were included in the model, the reference at the intercept is Control Females between the first and second trip. The term Time is the time between sampling trips (Trip 1 = October 2020, Trip 2 = March 2021, Trip 3 = July 2021, Trip 4 = October 2021). Estimates gives the logit scale parameter estimates from the model, SE gives the associated standard error. Z gives the z-scores calculated by dividing an estimate by its standard error, and *P* gives the *P*-value for the terms in the model and was calculated using the z-scores.

Survival probability or Recapture probability	Terms	Estimates	SE	*Z*	*P*
Survival probability	Intercept	0.285	0.144		
Survival probability	Treatment_Ivermectin	0.066	0.161	0.41	0.68
Survival probability	Time (2-3)	-0.706	0.209	-3.38	<0.001
Survival probability	Time (3-4)	-1.672	0.329	-5.09	< 0.001
Survival probability	Sex_Male	-1.139	0.170	-6.68	< 0.001
Survival probability	Treatment_Ivermectin * Time (2-3)	0.056	0.254	0.22	0.82
Survival probability	Treatment_Ivermectin * Time (3-4)	0.870	0.394	2.21	0.02
Survival probability	Time (2-3) * Sex_Male	0.574	0.267	2.14	0.03
Survival probability	Time (3-4) * Sex_Male	0.529	0.457	1.16	0.24
Recapture probability	Intercept	1.903	0.257	7.41	<0.001
Recapture probability	Sex+_Male	1.741	1.043	1.67	0.09

Table 6. Estimates of univariate linear and nonlinear selection in juveniles for the time period between October 2020 and March 2021. Treatment gives the treatment of the group the associated estimates of selection were measured for, I = Ivermectin, C = Control. Beta and gamma give the linear and non-linear selection estimates for the associated trait. The c^2 and *P* column give the Chi-square test statistic and associated p value. They were generated using a generalized linear model with a binomial error distribution and a logit link function. Randomization p value Ivermectin – Control gives a significance test for the difference between treatment groups for the associated selection estimate. We only performed this randomization test for selection estimates that were significant within a given sex and treatment group.

Sex	Treatment	Trait	β	γ	SE	χ^2	*P*	*P*: Ivermectin - Control
F	C	SVL	0.04		0.06		0.49	
	C	SVL		0.04	0.10		0.74	
	I	SVL	0.03			0.20	0.65	
	I	SVL		0.27	0.07	8.81	0.003	0.037
	C	Cond	-0.03		0.06	0.23	0.63	
	C	Cond		0.09	0.08	1.16	0.28	
	I	Cond	-0.10		0.07	2.29	0.13	
	I	Cond		0.03	0.08	0.20	0.65	
M	C	SVL	0.09		0.12	0.65	0.42	
	C	SVL		0.18	0.23	0.65	0.42	
	I	SVL	0.001		0.11	0	0.99	
	I	SVL		0.27	0.21	1.66	0.19	
	C	Cond	0.13		0.12	1.06	0.30	
	C	Cond		0.17	0.17	0.87	0.35	
	I	Cond	-0.02		0.10	0.04	0.85	
	I	Cond		-0.33	0.12	5.75	0.016	0.008

Table 7. Linear (*s*) and non-linear (*c*) selection differentials, their standard errors and associated *P*-values for all sex and treatment groups, estimated using survival between March and July.

	Female											
	Control						*Ivermectin*					
Trait	*s*	SE	*P*	*C*	SE	*P*	*s*	SE	*P*	*C*	SE	*P*
SVL	-0.03	0.11	0.74	-0.04	0.13	0.69	0.02	0.09	0.78	-0.11	0.11	0.39
Condition	0.03	0.11	0.79	0.16	0.15	0.25	0.06	0.09	0.49	-0.08	0.12	0.60
Res. Head Width	0.11	0.11	0.36	0.02	0.14	0.95	-0.04	0.09	0.66	-0.25	0.11	0.08
Res. Head Length	-0.02	0.12	0.94	0.10	0.14	0.44	0.17	0.09	0.08	-0.15	0.12	0.28
Res. Dew. Area	0.02	0.11	0.91	-0.03	0.13	0.71	0.02	0.09	0.78	0.02	0.10	0.80
Hue	-0.18	0.11	0.09	-0.25	0.15	0.09	-0.09	0.09	0.31	-0.07	0.11	0.56
Brightness	-0.13	0.11	0.25	-0.10	0.11	0.22	-0.09	0.09	0.36	-0.05	0.09	0.56
Saturation	0.06	0.11	0.56	-0.03	0.13	0.77	0.02	0.10	0.84	-0.06	0.10	0.59

	Male											
	Control						*Ivermectin*					
Trait	*s*	SE	*P*	*C*	SE	*P*	*s*	SE	*P*	*C*	SE	*P*
SVL	-0.33	0.15	0.04	0.51	0.30	0.06	-0.005	0.19	0.99	0.47	0.34	0.16
Condition	0.20	0.19	0.25	0.20	0.27	0.51	-0.022	0.18	0.93	-0.14	0.23	0.61
Res. Head Width	0.07	0.18	0.66	0.32	0.22	0.18	0.13	0.18	0.50	-0.47	0.13	0.07
Res. Head Length	-0.04	0.18	0.88	-0.08	0.21	0.75	-0.12	0.18	0.51	-0.24	0.19	0.33
Res. Dew. Area	0.05	0.18	0.82	-0.09	0.24	0.74	0.07	0.18	0.71	0.27	0.22	0.24
Hue	0.22	0.17	0.22	0.33	0.28	0.23	-0.20	0.19	0.28	0.30	0.24	0.20
Brightness	0.23	0.19	0.21	0.01	0.26	0.88	0.06	0.19	0.71	-0.23	0.20	0.33
Saturation	-0.17	0.19	0.32	-0.10	0.13	0.57	-0.07	0.19	0.73	0.08	0.18	0.50

Table 8. Linear selection gradients (*b*), their standard errors and associated *P*-values for all sex and treatment groups estimated using survival between March and July. The linear selection gradients were estimated using either all traits or the subset of traits contained in modules 1 and 2. The modules contained the associated traits in the Trait column.

		Female						Male					
		Control			*Ivermectin*			*Control*			*Ivermectin*		
Module	Trait	*β*	SE	*P*	*β*	SE	*P*	*β*	SE	*P*	*β*	SE	*P*
1	SVL	-0.13	0.11	0.24	0.01	0.10	0.95	-0.29	0.23	0.20	0.35	0.27	0.14
	Res. Dew. Area	-0.07	0.14	0.55	0.01	0.12	0.97	0.10	0.21	0.62	0.28	0.22	0.17
	Hue	-0.18	0.13	0.19	-0.08	0.11	0.45	0.20	0.19	0.30	**-0.51**	**0.27**	**0.03**
	Brightness	-0.09	0.12	0.41	-0.07	0.10	0.48	0.08	0.19	0.64	0.15	0.19	0.40
	Saturation	-0.02	0.14	0.94	-0.02	0.12	0.82	0.05	0.28	0.81	**-0.72**	**0.34**	**0.04**
2	SVL	-0.03	0.11	0.83	0.02	0.09	0.78	**-0.34**	**0.15**	**0.03**	-0.003	0.19	0.99
	Condition	0.01	0.11	0.89	0.03	0.09	0.74	0.26	0.22	0.20	0.006	0.22	0.99
	Res. Head Width	0.11	0.11	0.35	-0.08	0.09	0.38	-0.05	0.20	0.81	0.15	0.19	0.36
	Res. Head Length	-0.02	0.11	0.86	0.17	0.09	0.06	-0.12	0.18	0.52	-0.15	0.20	0.45
All	SVL	-0.08	0.11	0.47	0.01	0.09	0.94	-0.24	0.24	0.35	0.279	0.27	0.24
	Condition	0.01	0.11	0.91	0.02	0.09	0.79	0.26	0.22	0.24	-0.002	0.20	0.95
	Res. Head Width	0.09	0.11	0.42	-0.07	0.09	0.39	0.02	0.21	0.91	0.23	0.19	0.21
	Res. Head Length	-0.04	0.11	0.77	0.16	0.10	0.09	-0.08	0.19	0.66	-0.19	0.20	0.36
	Res. Dew. Area	-0.06	0.14	0.68	-0.01	0.11	0.92	0.06	0.22	0.77	0.32	0.22	0.10
	Hue	-0.16	0.14	0.22	-0.06	0.11	0.53	0.19	0.20	0.29	**-0.55**	**0.26**	**0.01**
	Brightness	-0.10	0.11	0.35	-0.05	0.10	0.67	0.15	0.19	0.38	0.08	0.19	0.71
	Saturation	0.01	0.13	0.96	-0.01	0.11	0.87	0.02	0.27	0.92	**-0.67**	**0.33**	**0.03**

Table 9. Norm of the vector of linear selection gradients for each sex and treatment group, estimated for both time periods using either all traits or the specified module of traits. Module 1 contains SVL, residual dewlap area, dewlap hue, dewlap brightness, and dewlap saturation, module 2 contains SVL, body condition, residual head width and residual head length. *P*-values are derived from the randomization procedure described in the methods.

		Female			Male		
Period	Module	Control Norm	Ivermectin Norm	*P*	Control Norm	Ivermectin Norm	*P*
March - July	All traits	0.25	0.20	0.75	0.41	1.02	0.004
	1	0.25	0.10	0.80	0.38	1.00	0.008
	2	0.12	0.19	0.10	0.44	0.21	0.19
March - October	1	0.62	0.45	0.27	2.75	1.31	0.18
	2	0.46	0.80	0.12	2.67	0.74	0.06

Table 10. Vector correlations between the vectors of linear selection gradients from ivermectin and control treatment groups, estimated using either all traits or the specified module of traits. Module 1 contains SVL, residual dewlap area, dewlap hue, dewlap brightness, and dewlap saturation, module 2 contains SVL, body condition, residual head width and residual head length. *P*-values are derived from the randomization procedure described in the methods.

		Female		Male	
		Control $\boldsymbol{\beta}$ *and Ivermectin* $\boldsymbol{\beta}$		*Control* $\boldsymbol{\beta}$ *and Ivermectin* $\boldsymbol{\beta}$	
Period	Module	Vector Correlation	*P*	Vector Correlation	*P*
March - July	All traits	0.04	0.82	-0.29	0.39
	1	-0.56	0.35	0.13	0.62
	2	0.76	0.98	-0.52	0.27
March - October	1	-0.06	0.42	0.88	0.95
	2	0.28	0.07	0.09	0.80

Table 11. Estimates of non-linear selection gradients for both modules of traits, estimated using survival between March and July for control and ivermectin males. The diagonal gives stabilizing/disruptive selection, with the standard error in the parenthesis, the lower triangle gives estimates of correlational selection with their standard errors in parenthesis. The upper triangle gives the *P*-values of the respective correlational selection gradients. The column *P* Diag gives the *P*-values of the selection estimates from the diagonal of the matrix.

		Control Males						*Ivermectin Males*						
Module		SVL	Res. D. A.	Hue	Bright.	Sat.	*P* Diag		SVL	Res. D. A.	Hue	Bright.	Sat.	*P* Diag
1	SVL	0.22 (1.08)	0.09	0.13	0.26	0.35	0.85	SVL	0.35 (0.52)	0.21	0.75	0.51	0.61	0.48
	Res. D. A.	-0.69 (0.36)	-0.29 (0.45)	0.96	0.85	0.14	0.7	Res. D. A.	0.51 (0.33)	0.28 (0.32)	0.36	0.86	0.92	0.36
	Hue	-0.73 (0.39)	0.006 (0.29)	0.76 (0.41)	0.96	0.21	0.11	Hue	-0.10 (0.32)	0.29 (0.23)	0.20 (0.51)	0.72	0.98	0.67
	Bright.	-0.81 (0.64)	0.06 (0.29)	0.03 (0.32)	-0.59 (0.63)	0.08	0.41	Bright.	-0.25 (0.27)	0.04 (0.20)	0.13 (0.27)	-0.38 (0.27)	0.78	0.42
	Sat.	0.87 (0.75)	0.67 (0.35)	0.49 (0.36)	1.12 (0.57)	-1.03 (0.73)	0.37	Sat.	-0.34 (0.56)	0.09 (0.36)	-0.10 (0.48)	-0.09 (0.36)	-0.26 (0.81)	0.98

	Control Males					*Ivermectin Males*					
		SVL	Cond.	Res. H.W.	Res H.L.	*P* Diag		SVL	Cond.	Res. H.W.	Res H.L.	*P* Diag
2	SVL	0.93 (0.53)	0.37	0.19	0.62	0.04	SVL	0.35 (0.52)	0.21	0.75	0.51	0.61
	Cond.	-0.22 (0.37)	0.31 (0.70)	0.97	0.26	0.46	Cond.	0.51 (0.33)	0.28 (0.32)	0.36	0.86	0.92
	Res. H.W.	0.37 (0.42)	0.02 (0.55)	0.08 (0.53)	0.82	0.67	Res. H.W.	-0.10 (0.32)	0.29 (0.23)	0.20 (0.51)	0.72	0.98
	Res. H.L.	0.16 (0.43)	-0.25 (0.40)	0.07 (0.32)	-0.09 (0.44)	0.83	Res. H.L.	-0.25 (0.27)	0.04 (0.20)	0.13 (0.27)	-0.38 (0.27)	0.78

Table 12. Estimates of non-linear selection gradients for both modules of traits, estimated using survival between March and July for control and ivermectin females. The diagonal gives stabilizing/disruptive selection, with the standard error in the parenthesis, the lower triangle gives estimates of correlational selection with their standard errors in parenthesis. The upper triangle gives the *P*-values of the respective correlational selection gradients. The column *P* Diag gives the *P*-values of the selection estimates from the diagonal of the matrix.

		Control Females					
Module		SVL	Res. D. A.	Hue	Bright.	Sat.	*P* Diag
1	SVL	0.09 (0.16)	0.19	0.11	0.43	0.34	0.44
	Res. D. A.	0.25 (0.20)	0.25 (0.26)	0.82	0.14	0.81	0.37
	Hue	-0.21 (0.15)	0.06 (0.19)	-0.33 (0.22)	0.41	0.82	0.12
	Bright.	-0.12 (0.16)	0.23 (0.16)	0.13 (0.15)	-0.24 (0.14)	0.09	0.07
	Sat.	-0.16 (0.17)	-0.03 (0.22)	-0.02 (0.20)	-0.23 (0.14)	-0.12 (0.26)	0.71

	Ivermectin Females					
	SVL	Res. D. A.	Hue	Bright.	Sat.	*P* Diag
SVL	-0.02 (0.12)	0.39	0.24	0.04	0.06	0.88
Res. D. A.	0.08 (0.12)	0.36 (0.22)	0.30	0.45	0.09	0.10
Hue	0.12 (0.15)	0.20 (0.16)	-0.09 (0.16)	0.13	0.37	0.32
Bright.	-0.23 (0.13)	-0.13 (0.15)	0.20 (0.16)	-0.18 (0.14)	0.57	0.16
Sat.	-0.32 (0.17)	-0.25 (0.16)	0.11 (0.16)	0.09 (0.14)	0.26 (0.22)	0.19

		Control Females				
		SVL	Cond.	Res. H.W.	Res H.L.	*P* Diag
2	SVL	0.02 (0.21)	0.57	0.27	0.41	0.98
	Cond.	0.07 (0.15)	0.15 (0.18)	0.68	0.37	0.49
	Res. H.W.	-0.15 (0.13)	-0.06 (0.13)	-0.03 (0.16)	0.12	0.80
	Res. H.L.	0.09 (0.14)	0.11 (0.12)	0.15 (0.10)	0.05 (0.15)	0.76

	Ivermectin Females				
	SVL	Cond.	Res. H.W.	Res H.L.	*P* Diag
SVL	0.05 (0.13)	**0.004**	0.53	**0.01**	0.75
Cond.	**0.28 (0.09)**	-0.02 (0.13)	0.24	0.30	0.73
Res. H.W.	-0.06 (0.08)	0.08 (0.08)	**-0.25 (0.13)**	0.67	**0.04**
Res. H.L.	**-0.29 (0.11)**	0.09 (0.10)	-0.04 (0.09)	-0.07 (0.14)	0.40

Table 13. Linear (*s*) and nonlinear (*c*) selection differentials and their standard errors for each of the eight traits estimated within each sex and treatment group for the period between March and October.

	Female											
	Control			*Control*			*Ivermectin*			*Ivermectin*		
Trait	*s*	SE	*P*	*C*	SE	*P*	*s*	SE	*P*	*C*	SE	*P*
SVL	-0.31	0.21	0.15	0.07	0.23	0.31	-0.21	0.16	0.21	-0.08	0.14	0.75
Condition	0.11	0.28	0.71	-0.25	0.48	0.65	0.12	0.17	0.51	-0.06	0.26	0.89
Residual Head Width	-0.26	0.27	0.33	0.12	0.50	0.68	0.02	0.18	0.86	-0.36	0.20	0.22
Residual Head Length	-0.02	0.28	0.99	0.25	0.39	0.36	**0.70**	**0.17**	**0.001**	0.17	0.21	0.45
Residual Dewlap Area	**0.54**	**0.26**	**0.04**	-0.002	0.53	0.89	-0.18	0.16	0.31	-0.14	0.25	0.54
Hue	-0.07	0.28	0.82	-0.06	0.49	0.99	0.08	0.16	0.70	-0.44	0.22	0.12
Brightness	-0.22	0.24	0.37	-0.11	0.43	0.99	-0.04	0.17	0.85	-0.15	0.18	0.49
Saturation	0.43	0.28	0.16	-0.15	0.43	0.81	-0.02	0.17	0.92	-0.46	0.24	0.13

	Male											
	Control			*Control*			*Ivermectin*			*Ivermectin*		
Trait	*s*	SE	*P*	*C*	SE	*P*	*s*	SE	*P*	*C*	SE	*P*
SVL	1.16	1.18	0.41	1.64	2.81	0.56	0.56	0.54	0.30	0.38	0.96	0.46
Condition	2.37	0.91	0.06	4.29	2.42	0.12	0.46	0.37	0.24	-0.16	0.60	0.81
Residual Head Width	1.16	0.57	0.12	1.56	1.29	0.09	0.39	0.36	0.30	-0.28	0.46	0.59
Residual Head Length	0.64	0.60	0.35	0.94	0.76	0.23	0.27	0.36	0.52	-0.24	0.52	0.72
Residual Dewlap Area	-0.19	0.61	0.81	0.51	0.85	0.34	0.28	0.39	0.51	0.08	0.53	0.76
Hue	0.48	0.43	0.35	0.37	0.79	0.64	-0.81	0.50	0.13	1.38	0.96	0.12
Brightness	0.35	0.68	0.62	0.06	1.05	0.95	-0.59	0.36	0.14	0.34	0.35	0.25
Saturation	0.41	0.78	0.60	0.46	0.85	0.59	0.54	0.53	0.35	0.45	0.75	0.26

Table 14. Linear selection gradients (*b*) for all sex and treatment groups estimated using survival between March and October. The linear selection gradients were estimated using the subset of traits contained in modules 1 or 2. The modules contained the associated traits in the Trait column.

		Female						Male					
		Control			*Ivermectin*			*Control*			*Ivermectin*		
Module	Trait	*β*	SE	*P*	*β*	SE	*P*	*β*	SE	*P*	*β*	SE	*P*
1	SVL	-0.29	0.23	0.25	-0.26	0.16	0.10	2.33	1.50	0.25	0.6	0.63	0.46
1	Res. Dew. Area	0.43	0.32	0.22	-0.30	0.21	0.22	0.33	0.66	0.69	0.37	0.40	0.47
1	Hue	0.15	0.28	0.59	0.01	0.18	0.94	0.87	0.44	0.19	-0.88	0.55	0.21
1	Brightness	-0.18	0.26	0.55	-0.07	0.19	0.69	1.09	0.74	0.32	-0.52	0.35	0.22
1	Saturation	0.26	0.34	0.49	0.19	0.22	0.44	-0.25	0.96	0.81	-0.38	0.71	0.74
2	SVL	-0.28	0.20	0.21	-0.23	0.15	0.16	1.41	1.08	0.32	0.58	0.54	0.36
2	Condition	0.16	0.27	0.62	-0.02	0.18	0.92	2.16	1.05	0.17	0.33	0.39	0.44
2	Res. Head Width	-0.25	0.26	0.37	-0.13	0.16	0.49	0.63	0.73	0.56	0.33	0.37	0.40
2	Res. Head Length	-0.04	0.26	0.91	0.76	0.18	<0.001	0.27	0.47	0.69	0.04	0.40	0.95

Table 15. Estimates of non-linear selection gradients for both modules of traits, estimated using survival between March and October, for control and ivermectin females. The diagonal gives stabilizing/disruptive selection, with the standard error in the parenthesis, the lower triangle gives estimates of correlational selection with their standard errors in parenthesis. The upper triangle gives the *P*-values of the respective correlational selection gradients. The column *P* Diag gives the *P*-values of the selection estimates from the diagonal of the matrix. For the traits Res. H.W. is residual head width, Res. H. L. is residual head length, and Res. D. A. is residual dewlap area.

		Control Females					
Module		SVL	Res. D. A.	Hue	Bright.	Sat.	*P* Diag
1	SVL	0.32 (0.40)	0.80	0.40	0.97	0.92	0.24
	Res. D. A.	0.09 (0.38)	-0.54 (0.83)	0.93	0.95	0.85	0.93
	Hue	-0.26 (0.47)	0.09 (0.53)	1.28 (1.38)	0.88	0.86	0.91
	Bright.	-0.07 (0.42)	-0.16 (0.45)	0.1 (0.59)	-0.12 (0.68)	0.32	0.90
	Sat.	0.09 (0.39)	0.38 (0.56)	0.62 (0.78)	-0.57 (0.48)	-0.21 (0.82)	0.80

	Ivermectin Females					
	SVL	Res. D. A.	Hue	Bright.	Sat.	*P* Diag
SVL	0.04 (0.33)	0.46	0.87	0.44	0.29	0.49
Res. D. A.	0.13 (0.28)	0.07 (0.53)	0.89	0.32	0.61	0.96
Hue	-0.06 (0.35)	0.09 (0.39)	-0.10 (0.67)	0.98	0.38	0.85
Bright.	0.20 (0.35)	0.33 (0.42)	0.02 (0.45)	0.02 (0.51)	0.60	0.65
Sat.	-0.24 (0.37)	0.24 (0.44)	0.22 (0.36)	-0.19 (0.36)	-0.51 (0.62)	0.42

		Control Females				
		SVL	Cond.	Res. H.W.	Res H.L.	*P* Diag
2	SVL	0.82 (0.62)	0.66	0.92	0.14	0.16
	Cond.	0.22 (0.39)	-0.04 (0.66)	0.65	0.48	0.78
	Res. H.W.	0.07 (0.36)	-0.14 (0.40)	-0.35 (0.49)	0.34	0.77
	Res. H.L.	0.55 (0.34)	0.23 (0.41)	0.41 (0.38)	0.20 (0.53)	0.60

	Ivermectin Females				
	SVL	Cond.	Res. H.W.	Res H.L.	*P* Diag
SVL	-0.05 (0.29)	0.23	0.34	0.10	0.99
Cond.	0.17 (0.19)	-0.09 (0.27)	0.10	0.70	0.66
Res. H.W.	0.20 (0.19)	0.39 (0.22)	-0.48 (0.32)	0.48	0.23
Res. H.L.	-0.33 (0.25)	0.08 (0.24)	0.13 (0.18)	0.18 (0.38)	0.48

Table 16. Estimates of variance in the standardized selection differential estimated for random associations between survival and traits value. Variance ratio gives the ratio of control to ivermectin variance in *s* when survival values are randomly permuted across individuals within the treatment. The *P*-value is calculated from a double randomization procedure described in the methods.

	Female				Male			
	Control	Ivermectin			Control	Ivermectin		
Survival Period	Variance in *s*	Variance in *s*	Variance ratio	*P*	Variance in *s*	Variance in *s*	Variance ratio	*P*
March – July	0.011	0.008	1.34	0.21	0.031	0.032	0.98	0.67
March - October	0.088	0.031	2.85	0.01	0.54	0.156	3.46	0.11

Supplementary Tables

Table S1. The results of Shapiro-Wilk tests of normality for the phenotypic traits measured on juveniles in October 2020 and on adults in March 2021. Within the Trait column, Res. H.W. is residual head width, Res. H. L. is residual head length, and Res. D. A. is residual dewlap area. Most traits exhibit significant departures from normality.

		Female				Male			
		Control		*Ivermectin*		*Control*		*Ivermectin*	
Period	Trait	Shapiro -Wilk	*P*	Shapiro -Wilk	*P*	Shapiro -Wilk	*P*	Shapiro -Wilk	*P*
Oct. 20	Ln SVL	0.981	0.009	0.981	0.007	0.927	<0.001	0.928	<0.001
	Condition	0.991	0.21	0.985	0.03	0.989	0.23	0.983	0.03
Mar. 21	Ln SVL	0.896	<0.001	0.915	<0.001	0.867	<0.001	0.853	<0.001
	Condition	0.995	0.79	0.987	0.11	0.971	0.08	0.992	0.85
	Res. H. W.	0.988	0.15	0.977	0.004	0.986	0.56	0.993	0.89
	Res. H. L.	0.977	0.004	0.966	<0.001	0.993	0.955	0.989	0.64
	Res. D. A.	0.959	<0.001	0.984	0.04	0.987	0.626	0.975	0.08
	Hue	0.965	<0.001	0.911	<0.001	0.918	<0.001	0.917	<0.001
	Saturation	0.909	<0.001	0.938	<0.001	0.984	0.49	0.958	0.005
	Brightness	0.986	0.07	0.988	0.13	0.887	<0.001	0.811	<0.001

Table S2. The results of Mardia tests for multivariate skew and kurtosis for groups of phenotypic traits measured in March 2021 and used in multivariate selection analysis for adults throughout the breeding season. Within the Trait module column, Res. H.W. is residual head width, Res. H. L. is residual head length, and Res. D. A. is residual dewlap area. All trait groups show significant departures from multivariate normality for all sex and treatment groups.

Trait Module	Female							
	Control				Ivermectin			
	Skew	*P*	Kurtosis	*P*	Skew	*P*	Kurtosis	*P*
All traits	346.2	<0.001	8.4	<0.001	443.8	<0.001	10.9	<0.001
SVL, Res. D. A., hue, brightness saturation	214.3	<0.001	9.8	<0.001	284.3	<0.001	12.8	<0.001
SVL, condition, Res. H.W., Res. H. L.	127.0	<0.001	8.9	<0.001	90.8	<0.001	6.8	<0.001
	Male							
	Control				Ivermectin			
	Skew	*P*	Kurtosis	*P*	Skew	*P*	Kurtosis	*P*
All traits	215.2	<0.001	2.3	0.02	253.1	<0.001	3.5	<0.001
SVL, Res. D. A., hue, brightness saturation	117.8	<0.001	4.3	<0.001	155.7	<0.001	6.6	<0.001
SVL, condition, Res. H.W., Res. H. L.	40.5	0.004	0.4	0.66	54.1	<0.001	0.4	0.69

Table S3. Variances of and correlations between the phenotypes in control females measured in March 2021 and used in the selection analysis on adults. The diagonal gives the variance of each trait with its standard error in parenthesis. The lower triangle gives correlations with their associated standard errors in parenthesis. The Column Mean (SE) gives the trait means and their standard errors.

Phenotypic correlation matrix: Control Females									Mean (SE)
	Ln SVL	Cond.	Ln H.W.	Ln H.L.	Ln Dew. Area	Hue	Bright.	Sat.	
Ln SVL	0.0043 (0.0008)								3.803 (0.005)
Cond.	0.01 (0.09)	0.0060 (0.0006)							-0.008 (0.006)
Ln H.W.	0.70 (0.05)	0.14 (0.08)	0.0037 (0.0006)						1.87 (0.0045
Ln H.L.	0.88 (0.02)	0.11 (0.08)	0.67 (0.06)	0.0028 (0.0005)					2.54 (0.004)
Ln Dew. Area	0.57 (0.06)	0.11 (0.08)	0.39 (0.08)	0.54 (0.06)	0.116 (0.013)				2.38 (0.025)
Hue	-0.24 (0.07)	-0.07 (0.07)	-0.31 (0.07)	-0.26 (0.08)	-0.46 (0.06)	22.18 (2.89)			19.36 (0.35)
Bright.	-0.09 (0.07)	0.03 (0.06)	0.06 (0.07)	-0.09 (0.06)	-0.17 (0.07)	0.32 (0.06)	63.05 (11.89)		53.85 (0.59)
Sat.	0.17 (0.07)	0.12 (0.06)	0.09 (0.07)	0.16 (0.07)	0.54 (0.06)	-0.39 (0.06)	-0.28 (0.08)	69.69 (9.15)	63.79 (0.62)

Table S4. Variances of and correlations between the phenotypes in ivermectin females measured in March 2021 and used in the selection analysis on adults. The diagonal gives the variance of each trait with its standard error in parenthesis. The lower triangle gives correlations with their associated standard errors in parenthesis. The Column Mean (SE) gives the trait means and their standard errors.

Phenotypic correlation matrix: Ivermectin Female									Mean (SE)
	Ln SVL	Cond.	Ln H.W.	Ln H.L.	Ln Dew. Area	Hue	Bright.	Sat.	
Ln SVL	0.0042 (0.0006)								3.806 (0.005)
Cond.	0.08 (0.08)	0.006 (0.0006)							0.007 (0.006)
Ln H.W.	0.78 (0.04)	0.14 (0.08)	0.0039 (0.0005)						1.88 (0.005)
Ln H.L.	0.87 (0.02)	0.19 (0.08)	0.74 (0.04)	0.0029 (0.0004)					2.55 (0.004)
Ln Dew. Area	0.55 (0.06)	0.03 (0.09)	0.42 (0.06)	0.52 (0.06)	0.112 (0.012)				2.39 (0.025)
Hue	-0.14 (0.08)	-0.13 (0.08)	-0.15 (0.09)	-0.20 (0.08)	-0.36 (0.07)	26.69 (4.88)			19.38 (0.38)
Bright.	-0.15 (0.07)	-0.1 (0.07)	-0.08 (0.06)	-0.18 (0.06)	-0.20 (0.07)	0.39 (0.06)	64.62 (9.56)		54.18 (0.59)
Sat.	0.20 (0.06)	0.03 (0.08)	0.16 (0.07)	0.19 (0.06)	0.55 (0.06)	-0.23 (0.08)	-0.30 (0.08)	75.64 (9.27)	63.49 (0.64)

Table S5. Variances of and correlations between the phenotypes in control males measured in March 2021 and used in the selection analysis on adults. The diagonal gives the variance of each trait with its standard error in parenthesis. The lower triangle gives correlations with their associated standard errors in parenthesis. The Column Mean (SE) gives the trait means and their standard errors.

Phenotypic correlation matrix: Control Males									Mean (SE)
	Ln SVL	Cond.	Ln H.W.	Ln H.L.	Ln Dew. Area	Hue	Bright.	Sat.	
Ln SVL	0.0128 (0.003)								4.02 (0.013)
Cond.	0.09 (0.12)	0.0013 (0.002)							0.001 (0.01)
Ln H.W.	0.92 (0.02)	0.26 (0.11)	0.0152 (0.003)						2.12 (0.014)
Ln H.L.	0.96 (0.01)	0.18 (0.12)	0.90 (0.02)	0.0103 (0.002)					2.73 (0.01)
Ln Dew. Area	0.91 (0.02)	0.21 (0.12)	0.83 (0.04)	0.90 (0.02)	0.216 (0.049)				4.81 (0.05)
Hue	-0.19 (0.11)	-0.17 (0.11)	-0.24 (0.10)	-0.25 (0.11)	-0.29 (0.11)	7.58 (1.69)			13.45 (0.31)
Bright.	-0.45 (0.09)	-0.27 (0.12)	-0.5 (0.09)	-0.44 (0.10)	-0.45 (0.08)	0.08 (0.11)	48.08 (7.13)		46.04 (0.79)
Sat.	0.54 (0.08)	0.27 (0.13)	0.52 (0.08)	0.59 (0.07)	0.68 (0.06)	-0.41 (0.10)	-0.23 (0.11)	19.76 (5.82)	87.59 (0.51)

Table S6. Variances of and correlations between the phenotypes in ivermectin males measured in March 2021 and used in the selection analysis on adults. The diagonal gives the variance of each trait with its standard error in parenthesis. The lower triangle gives correlations with their associated standard errors in parenthesis. The Column Mean (SE) gives the trait means and their standard errors.

Phenotypic correlation matrix: Ivermectin Males									Mean (SE)
	Ln SVL	Cond.	Ln H.W.	Ln H.L.	Ln Dew. Area	Hue	Bright.	Sat.	
Ln SVL	0.0149 (0.003)								4.02 (0.013)
Cond.	-0.08 (0.10)	0.0069 (0.0009)							-0.001 (0.009)
Ln H.W.	0.94 (0.01)	0.01 (0.10)	0.0177 (0.003)						2.12 (0.014)
Ln H.L.	0.97 (0.01)	0.03 (0.10)	0.93 (0.02)	0.0116 (0.002)					2.74 (0.011)
Ln Dew. Area	0.93 (0.02)	-0.04 (0.10)	0.84 (0.04)	0.91 (0.02)	0.296 (0.084)				4.80 (0.057)
Hue	-0.26 (0.12)	0.01 (0.10)	-0.21 (0.12)	-0.32 (0.11)	-0.33 (0.12)	8.73 (1.86)			13.58 (0.31)
Bright.	-0.35 (0.08)	0.02 (0.09)	-0.28 (0.08)	-0.35 (0.09)	-0.32 (0.09)	0.01 (0.10)	47.41 (9.95)		46.59 (0.72)
Sat.	0.59 (0.11)	-0.03 (0.10)	0.52 (0.11)	0.62 (0.10)	0.73 (0.09)	-0.63 (0.09)	-0.07 (0.10)	35.32 (10.96)	87.48 (0.70)