Journal of Evolutionary Biology, 2024, **37**, 28–36
https://doi.org/10.1093/jeb/voad011
Advance access publication 7 December 2023
Research Article

JOURNAL OF
Evolutionary Biology

Inbreeding depression in a sexually selected weapon and the homologue in females

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Abstract

Theory predicts that traits with heightened condition dependence, such as sexually selected traits, should be affected by inbreeding to a greater degree than other traits. The presence of environmental stress may compound the negative consequences of inbreeding depression. In this study, we examined inbreeding depression across multiple traits and whether it increased with a known form of environmental stress. We conducted our experiment using both sexes of the sexually dimorphic leaf-footed cactus bug, *Narnia femorata* (Hemiptera: Coreidae). Adult male cactus bugs have enlarged hind legs used as weapons in male–male contests; these traits, and their homologue in females, have been previously found to exhibit high condition dependence. In this study, we employed a small developmental group size as an environmental stress challenge. Nymph *N. femorata* aggregate throughout their juvenile stages, and previous work has shown the negative effects of small group size on survivorship and body size. We found evidence of inbreeding depression for survival and seven of the eight morphological traits measured in both sexes. Inbreeding depression was higher for the size of the male weapon and the female homolog. Additionally, small developmental group size negatively affected survival to adulthood. However, small group size did not magnify the effects of inbreeding on morphological traits. These findings support the hypothesis that traits with heightened condition dependence exhibit higher levels of inbreeding depression.

Keywords: animal weapons, Coreidae, genetic stress, heightened condition dependence, juvenile density

Introduction

Inbreeding is surprisingly common in nature (Crnokrak & Roff, 1999; Keller & Waller, 2002) often with deleterious effects on plants and animals (Niskanen et al., 2020; O’Grady et al., 2006; Sasson & Ryan, 2016). Non-adaptive inbreeding is also becoming more frequent as humans degrade the landscape. For example, habitat fragmentation can reduce population numbers and consequently lead to inbreeding and its negative effects (Bouzat, 2010; Niskanen et al., 2020; O’Grady et al., 2006). Another consequence of landscape degradation is that developmental environments can become more stressful, lowering individual fitness (Martínez-Mota et al., 2007; Suorsa et al., 2003). Together, the result is that populations are often facing greater genetic stress through inbreeding and environmental stress through habitat deterioration. Researchers often assume that the negative effects of inbreeding will increase when individuals develop under stressful environmental conditions (reviewed in Armbruster & Reed, 2005; Reed et al., 2012), but empirical results are mixed (Fox et al., 2011; Howie et al., 2019; Keller & Waller, 2002; Kristensen et al., 2008; Marr et al., 2006; Waller et al., 2008). Nonetheless, Fox and Reed (2011) found that the magnitude of realistic fitness reducing stress positively correlates with the magnitude of inbreeding depression. Furthermore, the work highlights the value of using biologically realistic levels of stress when examining possible synergies.

Life-history traits such as fertility, survival, and growth rates (Armbruster & Reed, 2005; Charlesworth & Willis, 2009; Fox & Reed, 2011) have been the most common targets of inbreeding research. This spotlight on life-history traits has left our understanding of how inbreeding affects morphological traits relatively limited (Curik et al., 2003; DeRose & Roff, 1999). Moreover, very few studies have examined the consequences of inbreeding for sexually selected morphological traits (but see Bellamy et al., 2013; Hooper & Bonduriansky, 2022; Howie et al., 2019; Zajitschek & Brooks, 2010). This is a surprising omission because the expression of sexually selected ornaments and weapons can be critical to reproductive success. Furthermore, through negative effects on sexually selected traits, inbreeding has the potential to reduce the effective population size, leading to even greater inbreeding (Niskanen et al., 2020; O’Grady et al., 2006).

The weapons used in same-sex contests over mates are some of the most variable morphological structures within and across species (Emlen, 2008; Emlen & Nijhout, 2000). Their size and expression are typically highly sensitive to environmental factors during development. The heightened condition dependence of sexually selected traits has been relatively well documented through experiments that manipulate environmental quality (e.g., diet; Allen & Miller, 2017; Bonduriansky, 2007; Bonduriansky & Rowe, 2005; Cotton et al., 2004; Miller et al., 2016). Links between heightened condition dependence and genetic quality are often suggested (Rowe & Houle, 1996), but experimental manipulations to test these links are scarce (but see Hooper & Bonduriansky, 2022; Howie et al., 2019). Inbreeding can lead to greater levels of homozygosity and developmental abnormalities. Thus, in many cases, inbred individuals have lower genetic quality

Received April 21, 2021; revised October 25, 2023; accepted November 22, 2023

(Charlesworth & Willis, 2009). For this reason, inbreeding should have pronounced morphological consequences for many sexually selected traits. Furthermore, the consequences of inbreeding for sexually selected traits may not be static, but instead vary according to environmental (including social) factors experienced during development. We used group size to test for the effect of a stressful social environment during the juvenile stages on inbreeding depression, as developmental density has been reported to increase the magnitude of inbreeding depression in other organisms (Fox & Reed, 2011; Yun & Agrawal, 2014). Yet, these hypotheses remain largely untested. Our aim was to address this knowledge gap first to evaluate the effect of inbreeding on traits with heightened condition dependence and second to evaluate these effects across two biologically relevant social contexts during development.

We used the sexually dimorphic leaf-footed cactus bug, *Narnia femorata* Stål (Hemiptera: Coreidae), as our focal species; females have overall larger bodies than males, but males have disproportionately larger hind legs (Allen & Miller, 2017; Miller et al., 2016). Male *N. femorata* establish territories on *Opuntia* spp. cactus pads and defend their territories against other conspecifics using their hind legs as weapons; males signal to each other before, during, and after aggressive interactions; they lift a hind leg in the direction of the opponent and sometimes kick into the air (Nolen et al., 2017). Larger males, which also possess larger hind legs—due to the positive allometry (*slope* values above 1) of the hind leg traits (Allen & Miller, 2017, 2020; Miller et al., 2016)—are more likely to be dominant over smaller ones when defending their territories (Nolen et al., 2017; Procter et al., 2012). Previous work has shown that the male hind legs of *N. femorata* exhibit a higher level of condition dependence than is seen for other morphological traits in this species (Allen & Miller, 2017; Gillespie et al., 2014; Miller et al., 2016; Sasson et al., 2016). Female hind leg traits also exhibit higher condition dependence than other traits, but to a lesser extent than male hind leg traits (Allen & Miller, 2017; Miller et al., 2016). We predicted that inbreeding should most negatively impact male hind legs, followed by the female hind legs, and then other morphological traits. Thus, we tested for the effects of inbreeding on a range of morphological traits for both sexes and used relevant social contexts during development to test our hypotheses. In the wild female *N. femorata*, eggs are laid in groups (range = 1–46 eggs, mean ± *SD* = 7.70 ± 5.40 egg per clutch, n = 804 clutches [75 females]; unpublished data), and nymphs are commonly found aggregating in groups of varying size (1 to over 30 on a single cactus pad; Allen & Miller, 2020). Previous research has shown that developing in small groups is stressful (e.g., due to thermoregulatory constraints and/or lack of feeding facilitation) and has negative effects on survival to adulthood (Allen & Miller, 2020). Thus, we predicted that social stress, in this case small group size (=low density) during development, would exacerbate inbreeding depression, affecting male sexually selected morphological traits more than other traits.

Materials and methods

Parental generation (F_p)

Fifth-instar nymphs were collected from the Ordway-Swisher Biological Station (Melrose, FL) in September 2014. Individual nymphs were kept in plastic deli containers with topsoil and a cactus pad (*Opuntia mesacantha*) with ripe fruit attached to it. This rearing protocol works for all life stages in this species (Allen et al., 2018). After nymphs moulted into adults and reached sexual maturity (2 weeks after moulting), we randomly paired males and females.

Parental pairs (F_p)

Male–female pairs were allowed to mate freely, and ripe fruit was provided (one per cup). After eggs were laid, we removed them and placed them in a new container with cactus and no fruit (first-instar nymphs do not feed). We selected the first 15 eggs from each of the 32 females over a 2-week period.

Founding generation nymphs (F_0)

Once the eggs hatched and the nymphs reached the second instar, each female's nymphs (15) were split equally into three cups. We supplied two ripe fruits per cup. After nymphs moulted into adults and reached sexual maturity (2 weeks after moulting), we paired them as described below.

Founding generation adults (F_0)

To create inbred and outbred lines, we used the "block" design proposed by Roff (Fox & Reed, 2011; Hooper & Bonduriansky, 2022; Joseph et al., 2016; Marsh et al., 2017; Roff, 1998; Vega-Trejo et al., 2015). A block consisted of two (A + B) randomly paired families from the 32 F_p pairs we had

Table 1. Generalized linear mixed models testing for the effects of sex (fixed factor), genetic status (Gen, fixed factor), and group size (Grp, fixed factor [social stress]) effects, and their interactions on beak length (BL), head length (HL), and hind tibia area (HTA), using body size as a covariate and block as a random factor.

Factor[a]		Body size	Sex	Gen[b]	Grp	Sex × Gen	Sex × Grp	Gen × Grp	Sex × Gen × Grp
BL	F	**1916.2**	**98.0**	**10.5**	**6.85**	0.01	<0.001	0.42	0.47
	p	**<.0001**	**<.0001**	**.0015**	**.009**	.91	.95	.52	.50
HL	F	**736.5**	**25.2**	1.79	0.33	**4.37**	0.07	0.05	0.68
	p	**<.0001**	**<.0001**	.184	.57	**.037**	.78	.83	.41
HTA	F	**1394.5**	**150.6**	**7.23**	0.540	0.266	0.001	0.627	0.006
	p	**<.0001**	**<.0001**	**.008**	.46	.61	.97	.43	.94

Note. Statistically significant effects are highlighted in bold. Sample sizes: inbred females 2-nymph treatment—37, inbred females 10-nymph treatment—84, inbred males 2-nymph treatment—30, inbred males 10-nymph treatment—94, outbred females 2-nymph treatment—34, outbred females 10-nymph treatment—119, outbred males 2-nymph treatment—50, outbred males 10-nymph treatment—112.

[a] df = 1,396 for all factors except genetic status.

[b] df = 1,142 for genetic status.

available, therefore creating 16 blocks. From each family, we haphazardly chose two females and two males; we crossed offspring from the two families to create two outbred lines (♀A/♂B and ♀B/♂A) and crossed offspring within each family to create two inbred lines (♀A/♂A and ♀B/♂B). This design ensured that inbred and outbred families were created from the same set of alleles, providing an advantage over other experimental designs (Fox, 2005). Male–female pairs were allowed to mate freely, and ripe fruit was provided as needed. Eggs from all the pairs were extracted and set up separately, using the same methodology as in the *Parental pairs* section (see also Allen et al., 2018 for more details). Within each block, we obtained 30 eggs per cross (new F_1 families).

The inbred/outbred generation (F_1)

From each block ($N = 16$), we obtained 60 inbred nymphs (30/cross) and 60 outbred nymphs (30/cross). After moulting to the second instar, nymphs from each cross were separated equally into 2-nymph (5 cups) and 10-nymph groups (2 cups). Two-nymph groups got one fruit/cup and 10-nymph groups five fruits/cup. After individuals moulted into adults and reached sexual maturity (2 weeks after moulting), they were frozen.

Insect measurements

The measuring protocol follows the same methodology used by Procter et al. (2012). A digital camera (Canon EOS 50D) attached to a dissecting microscope (Leica M165 C) was used to photograph all the extremities and body; the software ImageJ (Rasband, 2011) was used for the linear and area measurements. The morphological traits chosen were beak length (=rostrum [mouthparts]), head length, pronotum width, front femur length, hind femur length, and hind femur width. Legs and body were separated to facilitate the measuring procedure. Area measurements were taken of the hind tibia and femur, as these are the most enlarged trait part of the male weapon and the most condition-dependent traits (Allen & Miller, 2017). We used pronotum width as a proxy for body size; in this species, it is highly correlated with overall body size (Allen & Miller, 2017; Gillespie et al., 2014; Miller et al., 2016). Where applicable, means of paired body parts were used for analyses. For the purposes of this study, all male hind leg traits were considered to be sexually selected traits because of their involvement in signalling and fighting (Nolen et al., 2017; Procter et al., 2012); in contrast, female hind leg traits were referred to as the female homologous traits.

Statistical analysis

All analyses were conducted using R (R Core Team, 2022). In total, we created 16 blocks; however, we only used 14 blocks in our morphological trait analyses because two blocks produced too few outbred individuals for analysis (i.e., ≤1). Each block consisted of two inbred and two outbred families. From these blocks, we obtained 596 adults, 581 that could be measured (an average of 5.2 adults per block per genetic status [inbred/outbred] treatment per group size per sex). For all analyses of morphological traits, we used 560 individuals for which we got values for all eight traits; sample sizes for all analyses are presented in Table 1.

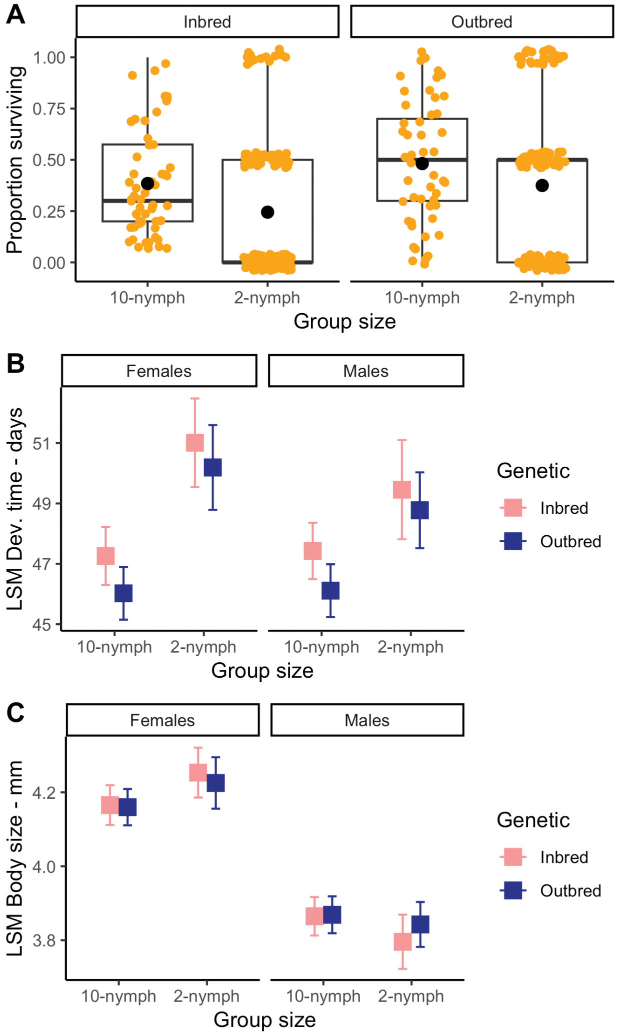

Figure 1. (A) Boxplots and raw data of the proportion of nymphs per cup (yellow circles) that survived to adulthood under the four genetic/group size (social stress) treatments. Black circles represent means. Inbreeding and small developmental group size had negative effects on *Narnia femorata* survivorship from second-instar nymph to adulthood. (B) Least-square means (±*SE*; GLMM) for developmental time; number of days to complete development to adulthood from the beginning of second instar for both sexes under the four genetic/group size treatments. Only small developmental group size had a negative effect on development rates. Sample sizes are shown in Table 1. (C) Least-square means (±*SE*; GLMM) for body size (=pronotum width) for both sexes under the four genetic/group size (social stress) treatments. Body size was sexually dimorphic, as females had overall larger bodies. We found no evidence of an effect of genetic status or developmental group size on body size. Main factor interactions were not statistically significant.

We used a binomial logistic regression to test for genetic status (fixed factor) and group size (fixed factor) effects and their interaction on survivorship to adulthood, using block as a random factor. All 16 blocks were used for this analysis. The survivorship analysis was done without separating by sex, as it was not possible to assign sex to the nymphs that died during development. This was done using the function *glmer* on the R-package *lme4*.

To test for sex (fixed factor), genetic status (fixed factor), and group size (fixed factor) effects and their interactions on development time and body size (pronotum width), we used

Table 2. Generalized linear mixed models for each sex, testing for the effects of genetic status (Gen, fixed factor), and group size (Grp, fixed factor) effects, and their interaction on front femur length (FFL), hind femur length (HFL), hind femur width (HFW), and hind femur area (HFA), using body size as a covariate and block as a random factor.

Females[a]						Males[c]					
Factor		Body size	Gen[b]	Grp	Gen × Grp	Factor		Body size	Gen[d]	Grp	Gen × Grp
FFL	*F*	**928.7**	**4.43**	<0.01	0.13	FFL	*F*	**1,191.4**	**5.37**	0.39	0.41
	p	**<.0001**	**.038**	.98	.72		*p*	**<.0001**	**.02**	.53	.52
HFL	*F*	**1,293.66**	**9.60**	0.21	0.14	HFL	*F*	**1,870.1**	**4.46**	1.8	1.10
	p	**<.0001**	**.0025**	.65	.71		*p*	**<.0001**	**.0369**	.18	.29
HFW	*F*	**972.6**	**4.43**	2.39	0.83	HFW	*F*	**1,265.8**	2.445	1.82	1.54
	p	**<.0001**	**.0378**	.12	.36		*p*	**<.0001**	.12	.18	.22
HFA	*F*	**1,595.96**	**7.38**	0.24	0.13	HFA	*F*	**2,386.7**	**6.638**	1.686	0.26
	p	**<.0001**	**.0078**	.63	.72		*p*	**<.0001**	**.011**	.20	.61

Note. Statistically significant effects are highlighted in bold. Sample sizes are shown in Table 1.
[a]Females: df = 1,156 for all factors except genetic status.
[b]Females: df = 1,100 for genetic status.
[c]Males: df = 1,157 for all factors except genetic status.
[d]Males: df = 1,111 for genetic status.

generalized linear mixed models [GLMM] using block as a random factor, with rearing cup (=container) nested within each block. To test for sex (fixed factor), genetic status (fixed factor), and group size (fixed factor) effects and their interactions on the other morphological traits ($n = 7$), we used GLMMs using body size as a covariate and block as a random factor, with rearing cup nested within each block. This was done using the function *lme* on the R-package *nlme*. First, we tested for interactions between the main factors (sex, genetic status, and group size) on the scaling relationships (*allometric slopes*) between body size and the seven other morphological traits (ANCOVAs—slope homogeneity test). We found statistically significant body size × sex interactions for four of the traits (Supplementary Table S1): front femur length (FFL), hind femur length (HFL), hind femur width (HFW), and hind femur area (HFA). These significant interactions illustrate how these male traits exhibit steeper positive allometries (*slope* values above 1) than their female counterparts. Female hind leg traits also display *slope* values above 1, which is also evidence of the higher condition dependence of these traits for both sexes, in which individuals with larger body sizes also possess larger traits (Supplementary Figures S1–S4; Bonduriansky 2007). Thus, we performed a second set of ANCOVAs (*slope* homogeneity test) for these four traits (FFL, HFL, HFW, and HFA) separating the analyses by sex (females—Supplementary Table S2; males—Supplementary Table S3).

Finally, we investigated whether male hind leg traits (sexually selected weapon; Nolen et al., 2017; Procter et al., 2012) exhibited higher inbreeding depression than the female homologue (female hind legs), and non-hind leg traits by performing a two-way analysis of variance using type of trait (hind leg vs. non-hind leg) and sex as fixed factors and including their interaction, to determine differences in magnitude. The least-square means obtained from the GLMMs (see Figure 1C and Tables 1 and 2) were used to calculate Inbreeding Depression for all traits and all sex/group size combinations. Inbreeding depression was measured as follows: $[\mathrm{LSMean}_{\mathrm{outbred}} - \mathrm{LSMean}_{\mathrm{inbred}}]/\mathrm{LSMean}_{\mathrm{outbred}}$ (Fox & Reed, 2011), it represents a proportional reduction in performance

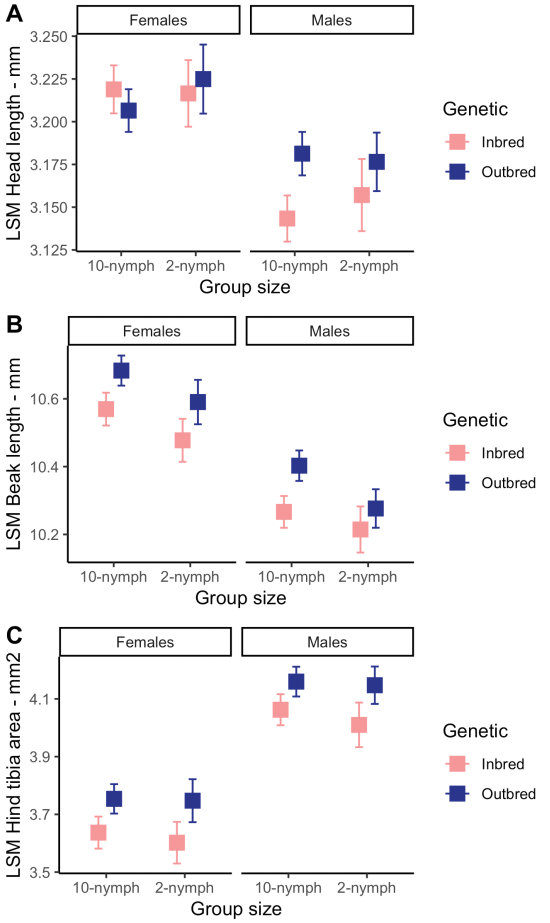

Figure 2. Least-square means (±*SE*; GLMM with body size as covariate) for (A) beak length, (B) head length, and (C) hind tibia area for both sexes under the four genetic/group size treatments. Inbreeding had a negative effect on beak length and hind tibia area, but not on head length (Table 1). Small developmental group size only affected beak length negatively for both sexes. There was sexual dimorphism for the three traits; females developed proportionally longer heads and beaks in relation to body size, but males developed larger hind tibias. Sample sizes are shown in Table 1.

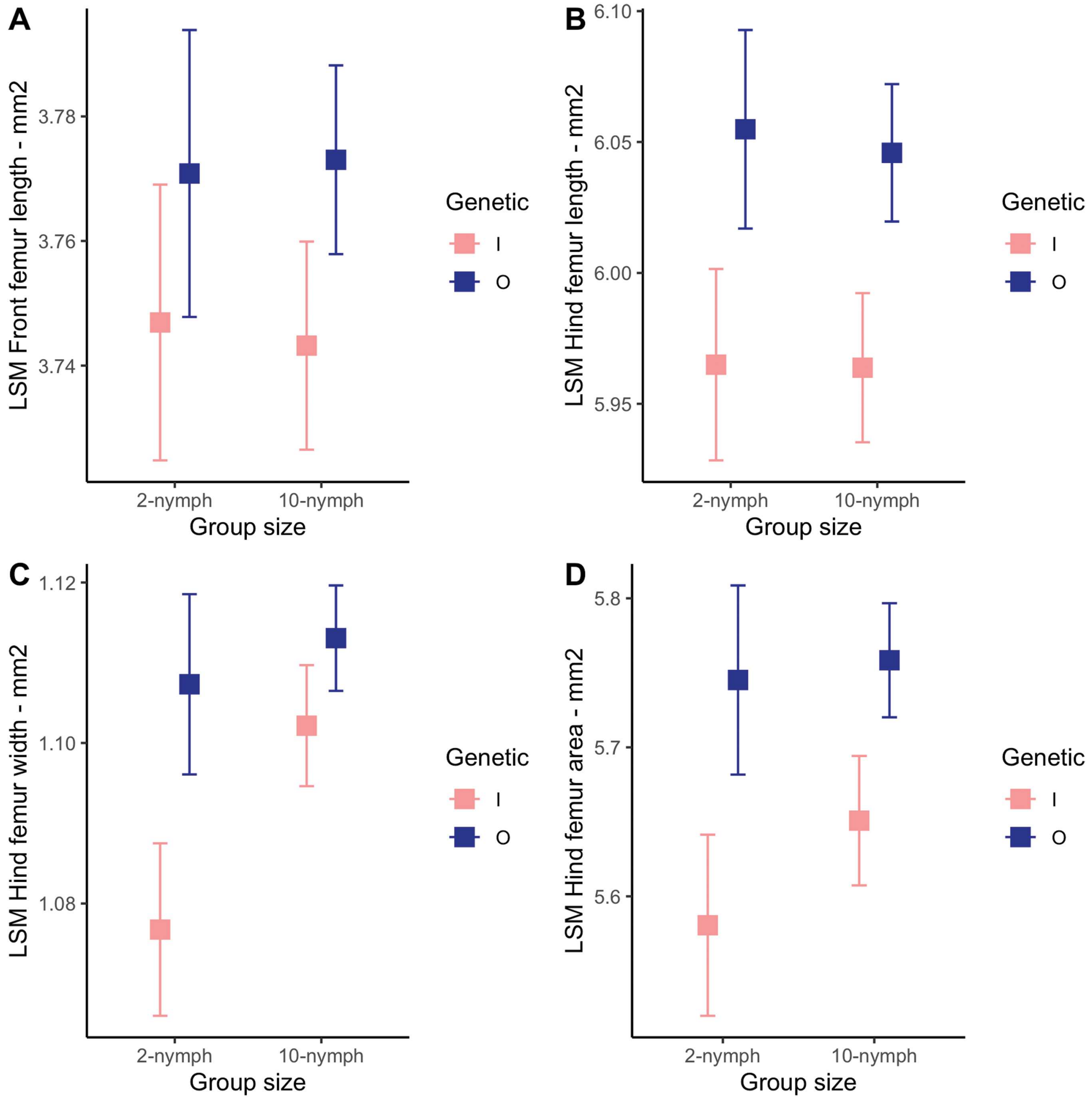

Figure 3. Least-square means (±*SE*; GLMM with body size as covariate) for (A) front femur length, (B) hind femur length, (C) hind femur width, and (D) hind femur area for females under the four genetic/group size treatments. Inbreeding had a negative effect on all traits; developmental group size had no statistically significant effect on any trait (Table 2). Genetic status: I = inbred, O = outbred. Sample sizes are shown in Table 1.

due to inbreeding (positive value). A negative ID value indicated the inbred lines performed better (larger trait size) than outbred lines within a block.

Results

Life-history traits

As expected, both inbreeding (logistic regression; $\chi^2 = 3.222$, df = 1, $p = .0013$) and small developmental group size ($\chi^2 = 4.17$, df = 1, $p < .0001$) negatively affected survival to adulthood (Figure 1A), with no interaction ($\chi^2 = -1.002$, df = 1, $p = .317$). Survivorship was twice as high for outbreds in the larger nymphal groups as for inbreds in the smaller groups. Individuals in small groups took longer to reach adulthood (Figure 1B; $F_{1,425} = 6.8$, $p = .009$), but inbreeding had no effect on developmental time ($F_{1,149} = 1.07$, $p = .30$), with no sex differences ($F_{1,425} = 1.72$, $p = .19$), and no statistically significant interactions.

Effects of inbreeding and group size on morphological traits

We found that inbreeding did not affect body size ($F_{1,142} < 0.0001$, $p = .984$; Figure 1C); furthermore, as expected, body size was found to be sexually dimorphic with females on average having larger bodies ($F_{1,397} = 122.7$, $p < .0001$), but developmental group size did not have an effect on body size ($F_{1,397} = 0.205$, $p = .65$), and there were no statistically significant interactions. Thus, to determine the effects of the main factors on other morphological traits, we used pronotum width (our metric of body size) as a covariate (Supplementary Tables S1–S3). The results of the GLMMs for head length (HL), beak length (BL), and hind tibia area (HTA) are presented in Table 1. The results of the GLMMs for front femur length (FFL), hind femur length (HFL), hind femur width (HFW), and hind femur area (HFA) are presented in Table 2, separated by sex.

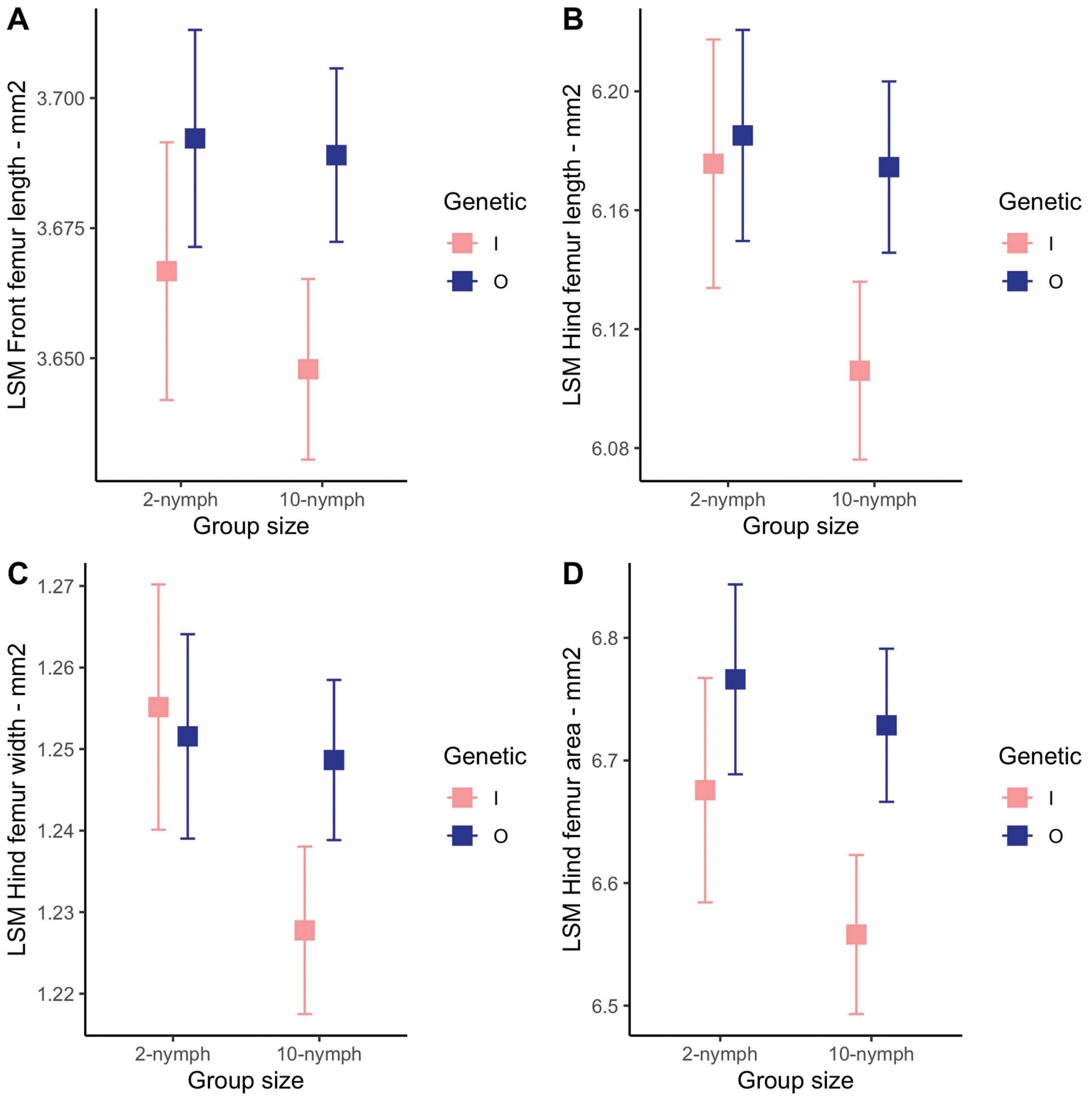

Figure 4. Least-square means (±*SE*; GLMM with body size as covariate) for (A) front femur length, (B) hind femur length, (C) hind femur width, and (D) hind femur area for males under the four genetic/group size treatments. Inbreeding had a negative effect on all traits except hind femur width; developmental group size had no statistically significant effect on any trait (Table 2). Genetic status: I = inbred, O = outbred. Sample sizes are shown in Table 1.

Most morphological traits, including all hind leg traits, were negatively affected by inbreeding (Figures 2–4), but not our proxy for overall body size (Figure 1C); thus, inbred insects were not smaller in body size, but the length of their head and growth of their appendages were often stunted relative to their body size. Head length (Figure 2A) was negatively affected by inbreeding but only in males; we found a statistically significant sex-*by*-genetic status interaction (Table 1). Surprisingly, hind femur width (Figures 3C and 4C) was negatively affected by inbreeding, but only in females; male HFW also trended toward a negative inbreeding effect. We found no effect of developmental group size on hind leg traits for either sex (Tables 1 and 2). In fact, beak length was the only trait affected by developmental group size (Table 1; Figure 2B); nymphs that developed as part of the larger groups grew longer beaks in relation to body size (Figure 2B).

Inbreeding depression across trait types

Inbreeding depression was higher for hind leg traits (ANOVA: $F_{1,28} = 16.7$, $p = .0003$; Figure 5), with no mean differences across sexes (ANOVA: $F_{1,28} = 0.11$, $p = .74$). We did find a significant *trait type-by-sex* interaction (ANOVA: $F_{1,28} = 4.835$, $p = .0363$), as female hind leg traits exhibited the highest levels of inbreeding. Inbreeding depression was the lowest overall (across males and females) for female non-hind leg traits (Figure 5).

Discussion

It has been well documented that inbreeding negatively affects fitness-related life-history traits, such as survival and fecundity (Charlesworth & Willis, 2009; Niskanen et al., 2020), but much less is known about their effects on morphological traits (DeRose & Roff, 1999). In this study,

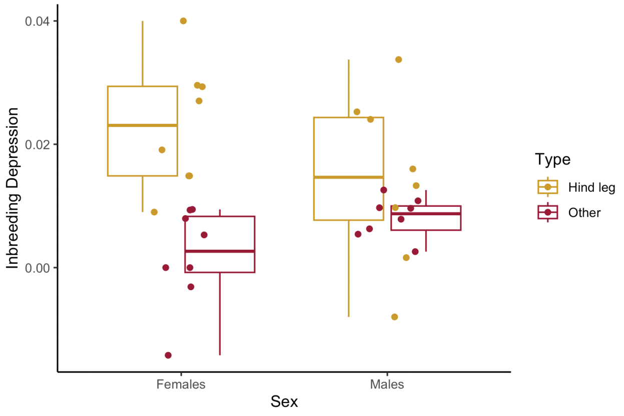

Figure 5. Box plots and violin plots of inbreeding depression across trait types and sexes. Inbreeding depression was higher for hind leg traits (e.g., male weapons and female homologues) than for the other traits, with no mean difference across sexes. Hind leg traits: hind femur length, hind femur width, hind femur area, and hind tibia area. Other traits: pronotum width, head length, beak length, and front femur length. Each mean represents eight data points, as each trait provides two values, one for each group size (social stress) treatment.

on top of the expected deleterious inbreeding effects on survival, we also found negative effects of inbreeding on morphology. The negative inbreeding effects on morphological traits did not vary across the two social group contexts, but inbreeding depression was higher for hind leg traits for both sexes (Figure 5); which in the case of males serve as weapons during male–male interactions (Nolen et al., 2017; Procter et al., 2012). Surprisingly, the magnitude of the negative effects of inbreeding was slightly higher for female hind leg traits. Furthermore, it has been argued that environmental stress (including social stress) should lead to an increase in inbreeding depression (reviewed in Armbruster & Reed, 2005; Reed et al., 2012), but empirical studies often fail to find this pattern (Waller et al., 2008). Our study also does not support the hypothesis that environmental stress increases mean inbreeding depression (Reed et al., 2012; Waller et al., 2008).

We included both sexual traits and those not closely linked to sexual selection in our study; we concluded that in this species inbreeding negatively affects sexually selected traits and their homologues in females more than other morphological traits (Figure 5). Across both social stress treatments, inbreds of both sexes exhibited smaller hind legs relative to body size. At a broad scale, the evidence on morphological traits, including sexually selected traits (DeRose & Roff, 1999; Curik et al., 2003; Bolund et al., 2010; Bellamy et al., 2013; Niskanen et al., 2020; Figures 2–5), indicates that inbreeding reduces mean trait size relatively little when compared with life-history traits (reviewed in DeRose & Roff, 1999; Figure 1A). This does not mean that this smaller effect on morphological traits is not biologically significant, just that life-history traits may be especially sensitive to inbreeding.

The size of the hind legs has implications for male–male competition (Nolen et al., 2017; Procter et al., 2012). Males of *N. femorata* use their hind legs to signal while also kicking, pouncing upon, or squeezing their opponent (Nolen et al., 2017; C. W. Miller unpublished videos). Thus, it would be valuable to measure the fighting biomechanics, skill, and injury resistance of inbred versus outbred males. Furthermore, the performance of inbred *versus* outbred individuals in important life-history traits (e.g., lifetime egg production, offspring viability) and mate choice remains to be seen; these comparisons would allow us to identify additional negative consequences of inbreeding in this species. Studies using the same inbreeding experimental design we employed here, but with mosquitofish (Vega-Trejo et al., 2015, 2017), failed to detect mean difference in body size and a sexually selected trait (gonopodium), but the researchers found that inbred males produced 50% fewer offspring than their outbred counterparts under a competitive scenario (Vega-Trejo et al., 2017).

Interestingly, we found here that inbreeding affected both sexes of *N. femorata*, but with a slightly larger negative effect on female hind leg traits (Figure 5), contrary to what we had originally predicted. Why female legs should have heightened condition dependence and heightened sensitivity to inbreeding is unclear. Resource allocation trade-offs have been detected between male hind legs and their testes (Joseph et al., 2018; Miller et al., 2021)—a weapons–testes trade-off as assumed by sperm competition theory (Parker, 1970). Similarly, resource allocation trade-offs exist between female hind legs and female ovaries (Miller et al., 2019), though female legs lack a clear reproductive function. Female *N. femorata* use their legs primarily for locomotion. While females can be attacked by males on occasion (Li and Miller, in preparation), they have not been witnessed using their hind legs in defense or offense. The similarities in expression and allocation in male and female hind legs may simply be a result of intersexual genetic correlations. Dissimilar patterns have been found in stalk-eyed flies. There, inbreeding negatively affects male eye-stalks more than the eye-stalks of females (Bellamy et al., 2013).

Rowe and Houle's (1996) concept of condition dependence was largely discussed in the context of genetic quality, though many studies that have measured condition dependence in sexually selected traits have done so through manipulations of nutrition. However, the work in this article establishes that inbreeding disproportionately affects traits that exhibit higher levels of condition dependence, in this case, the hind femur weapons of male *N. femorata* and the female homologues. We have previously shown that the hind femur weapons are exquisitely sensitive to nutrition in their expression, with female hind leg traits also being more condition-dependent traits than other morphological traits (Allen & Miller, 2017; Miller et al., 2016). Thus, altogether, these studies suggest that the heightened condition dependence revealed in some traits with an environmental stressor also materializes when the stressor is genetic in origin.

Conclusion

It has been argued that inbreeding and environmental stress should act synergistically to increase inbreeding depression, but empirical results are mixed (Armbruster & Reed, 2005; Reed et al., 2012). Our goal with this study was to elucidate how contemporary novel scenarios, with both genetic and ecologically relevant stressors, can affect sexually selected traits. Our main findings were that inbreeding had negative effects on most morphological traits, and social stress did not increase the negative effects of inbreeding for any morphological trait measured. Furthermore, inbreeding depression was higher (on average 3.75 times higher) for the hind leg traits than for other traits, across both sexes. In conclusion, we provide evidence of more pronounced negative effects of inbreeding on traits that exhibit higher levels of condition dependence, providing a link between inbreeding and the expression of a trait already known to have nutritional heightened condition-dependent expression. It will be interesting in future work to establish the extent to which traits showing exquisite responsiveness to one environmental stressor will also show it to another.

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Data availability

The data underlying this article are available in the Dryad Digital Repository at https://doi.org/10.5061/dryad.2ngf1vhvw.

Author contributions

Pablo Allen (Conceptualization [equal], Data curation [lead], Formal analysis [lead], Methodology [equal], Project administration [lead], Writing—original draft [lead], Writing—review & editing [equal]) and Christine Miller (Conceptualization [equal], Formal analysis [supporting], Funding acquisition [lead], Methodology [equal], Project administration [supporting], Writing—original draft [supporting], Writing—review & editing [equal])

Funding

The U.S. National Science Foundation (grants IOS-2226881 and IOS-1553100 to Christine W. Miller), and the University of Florida Agricultural Experiment Station and the National Institute of Food and Agriculture, U.S. Department of Agriculture, HATCH under FLA-ENY-7005707, supported this work.

Acknowledgments

The authors thank Paul Joseph, Jessica Gunson, Jamie Capps, Linh Nguyen, and Paige Carlson for help in the laboratory. The authors also thank Daniel Alexander Sasson and three anonymous reviewers for helpful comments on earlier versions of this manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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