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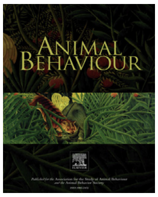

Effects of inbreeding on life-history traits and sexual competency in decorated crickets

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Although inbreeding depression in life-history traits has been well characterized, inbreeding effects on mating behaviour and sexually selected traits have been less well studied. Here, we assess levels of inbreeding depression in a number of fitness-related reproductive parameters of female decorated crickets, *Gryllodes sigillatus*. We predicted that due to direct negative effects of inbreeding and a potential trade-off between reproduction and current survival, as suggested by effects of inbreeding on immunity, inbred females would show significantly reduced reproductive output compared with outbred females. We also examined sex-specific effects of inbreeding on mating competency, focusing specifically on the female's decision to mount a male and the male's ability to transfer a spermatophore. We predicted that any inbreeding depression in sexual competency should be more evident in the success of spermatophore transfer than in female mounting propensity because of the tighter link between mating success and fitness in males than in females. Inbred females produced fewer offspring with longer development times compared with outbred females, results consistent with theory, as inbreeding depression is expected to be more severe for traits more tightly coupled with fitness. Inbreeding also had sex-specific effects on sexual competency. Inbred females were less likely to mount outbred males than were outbred females to mount inbred males. Inbred males were significantly less likely than outbred males to transfer a spermatophore regardless of female inbreeding status. These results reveal that inbreeding may have unexpected consequences for sexual competency and highlight the importance of considering mating behaviour when assessing effects of inbreeding within populations.

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Inbreeding depression is the decline in fitness that ensues when genetic relatives mate (Charlesworth & Charlesworth, 1987). It occurs either as a consequence of the exposure of deleterious alleles in the homozygous condition (partial dominance hypothesis), or as a result of the overall decrease in heterozygosity that accompanies inbreeding, leading to the elimination of heterozygous advantage at specific loci (overdominance hypothesis) (summarized in Roff, 2002). Inbreeding depression in life-history traits has been widely documented in plants and animals (Crnokrak & Roff, 1999). In general, inbreeding depression is expected to be more severe for traits that are more tightly coupled with fitness (Falconer, 1981; Roff, 1998a, b), and this is evidenced by the greater inbreeding depression observed in life-history traits of animals compared with their morphological traits (DeRose & Roff, 1999).

Although inbreeding depression in life-history traits has been well characterized, inbreeding effects on mating behaviour and sexually selected traits have been less well studied (Drayton, Hunt, Brooks, & Jennions, 2007; Janicke, Vellnow, Lamy, Chapuis, & David, 2014). Ala-Honkola, Uddström, Diaz Pauli, and Lindström (2009) showed that after only one generation of inbreeding, male least killifish, *Heterandria formosa*, perform fewer gonipodial thrusts and consequently experience reduced reproductive success compared with outbred males. Similarly, inbreeding alters the cuticular hydrocarbon profile of female leaf beetles (*Phaedon cochleariae*), rendering them more male-like and reducing their mating success (Müller, Lachenicht, & Müller, 2018). We might expect that because mating success is more tightly connected with fitness in males than

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it is in females (Bateman, 1948; Trivers, 1972), sexually selected traits of males that enhance male competitiveness or attractiveness to females should exhibit higher levels of inbreeding depression than mating behaviours of females (Drayton et al., 2007; Janicke et al., 2014). In support of this hypothesis, inbreeding resulted in a significant decline in male courtship intensity and ornamentation in guppies (Mariette, Kelley, Brooks, & Evans, 2006; Sheridan & Pomiankowski, 1997; van Oosterhout et al., 2003; Zajitschek & Brooks, 2010). In contrast, inbreeding in mosquitofish, *Gambusia holbrooki*, appears to have little effect on male attractiveness (Marsh, Vega-Trejo, Jennions, & Head, 2017; but see Vega-Trejo, Head, Keogh, & Jennions, 2017, for conflicting evidence). Inbreeding leads to a decrease in beak coloration, a lower song rate and an overall decrease in sexual attractiveness in male zebra finches, *Taeniopygia guttata* (Bolund, Martin, Kempenaers, & Forstmeier, 2010), and similar effects have recently been documented in canaries (de Boer, Eens, & Müller, 2016). Inbreeding can also lead to an alteration of call structure in insects that use acoustic signals to attract mates, including the courtship song of male *Drosophila* (Aspi, 2000) and the long-range advertisement calls of male crickets (Drayton et al., 2007), effects that can reduce male attractiveness.

In addition to its effects on male secondary sexual traits, inbreeding can also influence mating success through its effects on female mating preferences. These may occur either because inbreeding depression favours the avoidance of matings with close relatives or because outbred individuals are of higher quality than inbred males and are thus preferred as mates (Pilakouta & Smiseth, 2017). In burying beetles (*Nicrophorus vespilloides*), for example, inbred females prefer outbred males, but outbred females exhibit no such preference (Pilakouta & Smiseth, 2017). Similarly in house mice, *Mus domesticus*, females prefer the odours of outbred males to inbred males, but this preference is more pronounced in inbred females (Ilmonen, Stundner, Thoß, & Penn, 2009), leading the authors to conclude that this preference functions to enhance the heterozygosity of offspring, and hence, fitness.

Studies in crickets (Orthoptera: Gryllidae) have revealed significant inbreeding depression in an array of life-history traits including fecundity, hatching success, developmental rate, nymphal mass and life span (Archer, Zajitschek, Sakaluk, Royle, & Hunt, 2012; Drayton et al., 2007; Roff, 1998b, 2002). However, only one study has explicitly addressed inbreeding effects on sexually selected traits. Drayton et al. (2007) found that although inbreeding significantly reduced hatching success, nymphal survival and adult life span, effects on sexually selected traits were equivocal. There was no difference in calling effort between inbred and outbred males, and, although there were some effects on several finer-scale acoustic parameters, previous playback experiments (Bentsen, Hunt, Jennions, & Brooks, 2006) suggest that these effects would be unlikely to diminish call attractiveness of inbred males.

In contrast to previous studies showing inbreeding depression in a number of life-history traits in crickets, a comparison of immune defences of inbred and outbred individuals in the decorated cricket, *Gryllodes sigillatus*, revealed that inbred crickets actually exhibit stronger immune responses than outbred individuals (Duffield et al., 2019; Gershman et al., 2010a). Inbred crickets exhibited increased encapsulation ability, higher antibacterial activity and greater numbers of circulating haemocytes compared with outbred individuals, but there was no difference in two other components of insect immunity, phenoloxidase activity and lytic activity (Duffield et al., 2019; Gershman et al., 2010a). These findings do not appear to be an anomaly, as a similar study in another cricket species, *Teleogryllus commodus*, found a significant increase in the number of haemocytes of inbred individuals (Drayton & Jennions, 2011), cells critical to the success of the cellular immune response (Lawniczak et al., 2006).

Given the inbreeding depression shown in other life-history traits, why might inbred crickets show more robust immune responses than outbred crickets? One possibility is that inbred crickets may be trading reproduction for increased survival (Gershman et al., 2010a; but see Adamo, 2004a, b). In support of this possibility, experimentally induced immune responses in male *G. sigillatus* resulted in reduced reproductive effort, reflected in the smaller nuptial food gifts synthesized by inbred males, whereas an experimental increase in reproductive effort resulted in lower immunity (Kerr, Gershman, & Sakaluk, 2010). This same trade-off has been documented in a number of inbred lines in *G. sigillatus* (Gershman et al., 2010b); moreover, inbred males were found to synthesize smaller nuptial food gifts than outbred males (Gershman et al., 2010b).

Here, we assess levels of inbreeding depression in a number of fitness-related reproductive parameters of female decorated crickets, including fecundity, hatching success, development time and offspring survival. We predicted that if inbred female crickets are trading off reproduction for survival (i.e. increased immunity), inbred female crickets should show significantly reduced reproductive output compared with that of outbred females. In a companion experiment, we also examined sex-specific effects of inbreeding on mating competency, focusing specifically on the propensity of females to mount actively courting males and the ability of males to successfully transfer a spermatophore once mounted. For copulation in decorated crickets to ensue, the female must voluntarily mount the male (i.e. females cannot be forced to copulate). Thus, this aspect of the mating process is primarily under female control and provides an unambiguous signal of a female's mating preference. In contrast, once the female has mounted the male, the transfer of the spermatophore is largely dependent on the male's efforts (Sakaluk, 1987; Sakaluk, Duffield, Rapkin, Sadd, & Hunt, 2019). Thus, we further predicted that any inbreeding depression in sexual competency should be more evident in the success of spermatophore transfer than in female mounting propensity because of the tighter link between mating success and fitness in males than in females (Bateman, 1948; Trivers, 1972).

METHODS

Experimental Animals

Experimental *G. sigillatus* were the descendants of approximately 500 adult crickets collected in Las Cruces, New Mexico, U.S.A. in 2001, and used to initiate a laboratory colony maintained at a population size of approximately 5000 and allowed to breed randomly. Nine inbred lines used in this study were created by subjecting crickets, randomly selected from the large, panmictic population described above, to 23 generations of full-sib mating followed by multiple generations of panmixia within lines thereafter (Ivy, Weddle, & Sakaluk, 2005). Crickets were held in 55-litre plastic storage bins in an environmental chamber maintained at 31 °C on a 16:8 h light:dark cycle. Crickets were provisioned with Flukers® cricket chow (a commercial diet formulated expressly for rearing crickets and comprising a mixture of grain products, plant and animal protein and vitamins, among other ingredients), water provided in 40 ml plastic tissue culture flasks plugged with cotton dental rolls, and egg cartons to provide shelter and to increase the rearing surface area. Moistened peat moss provided in small plastic containers was made available both as an oviposition substrate and as a source of additional water.

Experiment 1: Inbreeding Depression in Life-history Traits

We assessed the level of inbreeding depression by comparing a number of fitness-related reproductive parameters of crickets from the outbred population with crickets derived from the nine inbred lines. We established three mating pairs per inbred line, yielding a total of 27 inbred pairs, and 27 mating pairs from adults randomly selected from the outbred source colony. Each pairing was established with newly eclosed adult crickets (adult day 0) to ensure their virginity prior to experimental pairing. Each experimental pair was held in a 710 ml plastic food storage container ventilated with a screened lid and provided with food, water, a small section of egg carton and an oviposition dish (a small shallow cup filled to a depth of 2 cm with moistened peat moss). The male was removed 10 days later, allowing sufficient time for copulations to occur and ensuring adequate insemination of the female (see Sakaluk, 1987). The female was allowed to oviposit for 30 days before her removal from the container, and survival of females was monitored daily. Although females in the laboratory typically survive 2 months or longer (Burpee & Sakaluk, 1993), their average life span under natural conditions in the field is approximately 3 weeks and does not often extend beyond 30 days (Sakaluk, Schaus, Eggert, Snedden, & Brady, 2002); hence, our measures of reproductive output are not unduly influenced by senescence over the time period they were assessed. Experimental containers were maintained inside an environmental chamber at 31 °C on a 16:8 h light:dark cycle.

Experimental containers were monitored for newly hatched nymphs daily for 30 days following removal of the female, and the total number of nymphs produced in this period recorded. The first 20 nymphs produced were reared to adulthood in 6-litre screened plastic bins provisioned as above. We recorded hatching time as the number of days elapsed from the time at which a pair was established to the time at which nymphs first appeared. We also recorded the number of nymphs surviving to sexual maturity (expressed as percentage of survival), development time as the number of days from hatch until the first offspring had undergone adult eclosion, the mass of each male and female upon the adult moult and the sex ratio of newly emerged adults. To compare levels of inbreeding depression among reproductive parameters, we calculated δ, the standardized coefficient of inbreeding (Lande & Schemske, 1985), which measures the percentage of change in a trait as a result of inbreeding, calculated as the difference in fitness of inbred and outbred progeny (computed as outbred minus inbred) divided by the fitness of outbred progeny.

Experiment 2: Effects of Inbreeding on Mating Success

To identify any sex-specific effects of inbreeding on mating success, we staged four kinds of mating trials: (1) outbred males paired with inbred females, (2) inbred males paired with outbred females, (3) inbred males paired with inbred females and (4) outbred males paired with outbred females. The first three mating combinations were replicated 20 times in each of the nine inbred lines, for a total of 540 mating trials (9 lines × 3 mating combinations × 20 replicates), whereas 36 mating trials involving outbred individuals of both sexes were staged. Experimental individuals were randomly selected from mixed-sex bins containing outbred crickets or inbred crickets from specific lines of unknown age and mating experience (presumably nonvirgin). These were held separately by sex approximately 48 h before mating trials to ensure a high degree of sexual motivation (Burpee & Sakaluk, 1993; Sakaluk, Avery, & Weddle, 2006).

The mating trials were conducted in a room maintained at 30–31.5 °C. Experimental matings were staged during the dark portion of the photoperiod in small Plexiglas mating chambers (8 × 3 cm and 6 cm high) lined with outdoor carpeting and illuminated by red light. Males were introduced first into the mating chambers followed by the female. After the male initiated courtship, the pair was given 30 min in which to complete a mating. During courtship, the male flattens his body against the substrate and makes repeated attempts to back under the female while producing a soft, distinctive courtship song. If the female is sexually responsive, she dorsally mounts the male, at which point the male attempts to transfer his spermatophore, which is secured outside the female's body at the base of her ovipositor (reviewed in Sakaluk et al., 2019). During mating trials, we recorded whether females mounted males and, if so, whether males succeeded in transferring a spermatophore.

Ethical Note

Our study adheres to the ASAB/ABS Guidelines for the use of animals in research, the legal requirements of the U.S.A. and all institutional guidelines at Illinois State University.

Data Analysis

All analyses were conducted using SAS statistical software (SAS 9.4; SAS Institute, Cary, NC, U.S.A.), and all tests were two tailed ($\alpha = 0.05$). All means reported are least-squares means (± 1 SE). Inbred line was included as a random effect in all analyses to account for the statistical nonindependence of subjects within inbred lines. In all mixed models, degrees of freedom were calculated using the Satterthwaite method, which often results in noninteger denominator degrees of freedom.

We used a Cox regression (PROC PHREG) to assess the effect of inbreeding on the survivorship of adult females used in the inbreeding depression study. The EXACT option was specified in the model statement to handle ties, instances in which different females had the same longevity, because this option assumes that longevities are continuous and ordered, assumptions that are likely to be met by our data. Females that lived for the entire 30-day egg laying period were included as right-censored observations. We accounted for nonindependence of females within inbred lines by invoking the robust variance estimator in PROC PHREG, which accounts for nonindependent observations similar to a mixed model (Allison, 2010).

To assess the effects of inbreeding on hatching time, total number of offspring produced, development time of male and female offspring and adult mass of male and female offspring, we used a general linear mixed model (PROC MIXED), with treatment (inbred or outbred) as a main effect and inbred line as a random effect. To examine the effect of inbreeding on survivorship of offspring from hatching to sexual maturity, we used a generalized linear mixed model (PROC GLIMMIX), with the number of offspring that survived as the dependent variable and the initial number of offspring as the binomial denominator (i.e. events/trials syntax); we included treatment (inbred or outbred) as the main effect and family as a random effect. Similarly, to assess the effect of inbreeding on the sex ratio of offspring that survived to sexual maturity, we used a generalized linear mixed model (PROC GLIMMIX), with the number of male offspring produced as the dependent variable and the number of sexed offspring as the binomial denominator (i.e. events/trials syntax).

To identify any sex-specific effects of inbreeding on the likelihood that a female would mount an actively courting male during mating trials, we used a generalized linear mixed model (PROC GLIMMIX) with a binary response (mounted or did not mount), with treatment (corresponding to the four mating combinations: inbred ♂ / inbred ♀, inbred ♂ / outbred ♀, outbred ♂ /

inbred ♀, outbred ♂ / outbred ♀) as the main effect and inbred line as a random effect. Similarly, to identify any sex-specific effects of inbreeding on the likelihood that the male would succeed in transferring a spermatophore once he was mounted by the female, we used a generalized linear mixed model (PROC GLIMMIX) with a binary response (mated or did not mate), with treatment as the main effect and inbred line as a random effect. Pairwise comparisons between treatments were made using *t* tests after controlling for multiple comparisons using the Tukey adjustment.

RESULTS

Experiment 1: Inbreeding Depression in Life-history Traits

Ninety three per cent of the outbred females (25/27) survived the entire 30-day egg laying period, whereas only 67% of inbred females (18/27) did so. Survival analysis revealed that the longevity of outbred females was significantly longer than that of inbred females (Wald (Sandwich) $\chi^2 = 4.1047$, $P = 0.0428$).

On average, nymphs of outbred females hatched about 2.5 days sooner than nymphs of inbred females, a statistically significant difference (Fig. 1a, Table 1). All of the outbred pairs produced offspring, whereas nine of 27 of the inbred pairs failed to do so, a statistically significant difference (Fisher's exact test: $P = 0.0043$). Outbred females produced five times more offspring than inbred females, which was also highly significant (Fig. 1b, Table 1). Even when excluding the reproductive failures, outbred females produced significantly more offspring than inbred females (number of offspring produced: outbred = 1159 ± 71; inbred = 326 ± 102; $F_{1,18.5} = 44.8$, $P < 0.0001$).

The time taken by both male and female offspring to attain sexual maturity (i.e. developmental time) was significantly shorter for those produced by outbred females than those produced by inbred females (Fig. 1c, Table 1). There was, however, no significant difference in the survival of offspring produced by outbred females and those produced by inbred females (Table 1; outbred survival = 84.2 ± 0.02%; inbred survival = 87.0 ± 0.03%), nor was there any difference between outbred and inbred groups in the adult mass attained by male and female offspring (Fig. 1d, Table 1). There was also no significant difference between inbred and outbred females in the sex ratio of offspring produced (Table 1; proportion of males produced: outbred = 0.487 ± 0.024; inbred = 0.563 ± 0.029).

Experiment 2: Effects of Inbreeding on Mating Success

There were significant differences among mating combinations in the propensity of females to mount courting males ($F_{3,150.7} = 3.45$, $P = 0.0181$; Fig. 2a). This result seemed to stem mainly from the reluctance of inbred females to mount outbred males. Inbred females were less likely to mount outbred males than were outbred females to mount inbred males ($t = -3.03$, $P_{\text{Tukey}} = 0.015$; Fig. 2a). No other pairwise comparison was significant, although the propensity of inbred females to mount outbred males was marginally higher than their propensity to mount inbred males ($t = 2.36$, $P_{\text{Tukey}} = 0.089$; Fig. 2a).

There were also significant differences among mating combinations in the likelihood that males would succeed in transferring a spermatophore once they had been mounted by receptive females ($F_{3,150.7} = 5.21$, $P = 0.0016$; Fig. 2b). Outbred males were significantly more likely to transfer a spermatophore than inbred males, regardless of the inbreeding status of the female (Fig. 2b).

DISCUSSION

We found clear evidence of inbreeding depression in multiple life-history traits in decorated crickets. Specifically, we found that inbred females suffered a shorter life span and produced fewer offspring with longer development times compared with outbred females. Additionally, we found sex-specific effects of inbreeding on both the propensity for females to mount males and the sexual competency of males as reflected in their ability to successfully transfer a spermatophore to a mounted female. Specifically, there was an asymmetry between inbred and outbred females in their affinity for males of the opposite inbreeding condition: inbred females were significantly less likely to mount outbred males than were outbred females to mount inbred females. With respect to their sexual competency, inbred males were less successful at transferring a spermatophore once mounted, independent of female inbreeding status.

The strongest evidence for inbreeding depression was revealed by the total number of offspring produced, with inbred pairs producing significantly fewer offspring than outbred pairs. As offspring production is arguably the trait most closely associated with fitness, this is consistent with theoretical expectations that inbreeding depression should be most evident in fitness-related traits (Roff, 1998a). The observed decrease in offspring production of inbred pairs relative to outbred pairs could be due to several factors, including differences in fertilization, fecundity, hatching success or a combination (Roff & DeRose, 2001). While the design of our experiment does not allow us to distinguish between these causes, results from other studies suggest that all can be important. Inbreeding can reduce male gametic quality, and thus fertilization (Losdat, Chang, & Reid, 2014), although negative consequences for fertilization may only manifest under sperm competition (Michalczyk, Martin, Millard, Emerson, & Gage, 2010). Even with successful fertilization, female fecundity or egg viability may be affected by inbreeding. In crickets, females of outbred *G. sigillatus* (Archer et al., 2012) and *Gryllus firmus* (Roff, 1998b) had higher fecundity compared with inbred females and, in *T. commodus*, eggs produced by outbred females had higher hatching success than those laid by inbred females (Drayton et al., 2007). These manifestations of inbreeding depression in females are similar to those that have been documented in disparate taxa (reviewed in Keller & Waller, 2002). For example, inbred Glanville fritillary butterflies, *Melitaea cinxia*, experienced reduced egg hatching rate and larval survival (Saccheri et al., 1998), and so too did inbred burying beetles (*N. vespilloides*) (Mattey, Strutt, & Smiseth, 2013). Inbreeding similarly leads to reduced hatching success in birds (de Boer, Eens, & Müller, 2018; Kempenaers, Adriaensen, Van Noordwijk, & Dhondt, 1996).

In addition to the number of progeny produced, we found evidence for inbreeding depression in the posthatching development of inbred offspring (i.e. time between hatching and sexual maturity). The time to hatching was also longer for offspring of inbred pairs relative to outbred pairs. This could be indicative of embryological developmental time and would mirror the finding for postnatal development. However, we cannot rule out that the first eggs of inbred females were laid later than those of outbred females, potentially as a consequence of differential propensities to mate between the groups (see Experiment 2: Effects of Inbreeding on Mating Success). Perhaps surprisingly, inbreeding status did not affect adult offspring mass of either sex. Thus, despite a longer postnatal developmental time, inbred offspring were still only able to attain similar body mass as outbred individuals. Drayton et al. (2007) also found that offspring from full-sib matings in the cricket *T. commodus* did not differ in mass from outbred offspring, but, in contrast to our findings, they found no effect of inbreeding

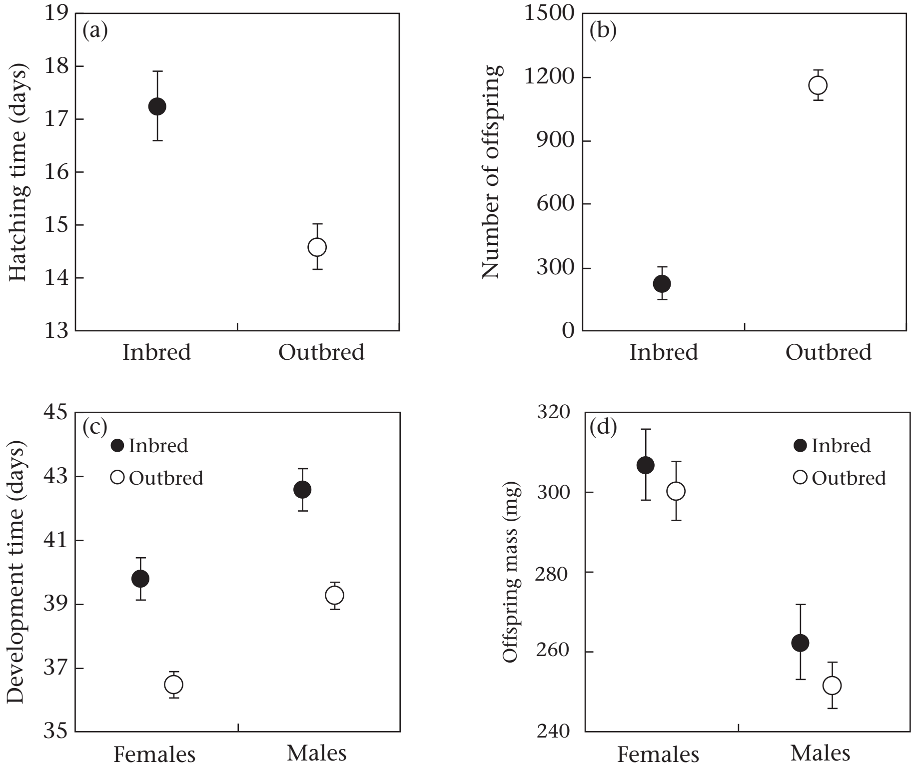

Figure 1. Comparison of fitness traits between inbred and outbred *G. sigillatus*: (a) hatching time of nymphs, (b) number of offspring produced, (c) development time of male and female progeny and (d) mass of male and female adult progeny. Points represent least-squares means (±SE).

on the time to hatching or maturity. Overall, our results of the effects of inbreeding on offspring traits are consistent with theoretical predictions, as life-history traits (e.g. development time), which are more tightly linked to fitness, are expected to be more susceptible to inbreeding depression than morphological traits (e.g. body size) (DeRose & Roff, 1999; Falconer, 1981; Roff, 1998b).

Inbreeding also had sex-specific effects in the context of sexual behaviour and courtship. Specifically, we found significant sex-specific effects of inbreeding on mating behaviour at two critical points of the mating process: (1) when females mounted courting males, a decision that is determined entirely by the female, and (2) when the male transferred his spermatophore to the female, which is determined largely by the male, but might also be influenced by the female. Specifically, inbred females appeared more likely to mount inbred males than outbred males, whereas outbred females exhibited no such difference, mounting outbred and inbred males with the same likelihood. Inbred males were significantly less likely to transfer a spermatophore regardless of female inbreeding status, resulting in a 30% reduction in mating success compared with outbred males. Although sex-specific effects of inbreeding on courtship intensity, ornamentation and mating success have been reported in other taxa (de Boer et al., 2016; Janicke et al., 2014; Sheridan & Pomiankowski, 1997), we know of no other study that has succeeded in separately parsing the effects of inbreeding on the male and female behavioural elements that operate in close concert to promote successful copulation.

The reluctance of inbred females to mount outbred males to which they were unrelated seems unusual given the fitness costs associated with inbreeding depression demonstrated here, and by extension, the implied benefits of outbreeding. While many studies have found evidence for inbreeding avoidance to avoid these costs (Pusey & Wolf, 1996), other studies have paradoxically found preferences for related individuals, even when inbreeding depression is apparent (Szulkin, Stopher, Pemberton, & Reid, 2013). For example, Townsend et al. (2019) found that the American crow, *Corvus brachyrhynchos*, preferred mating with relatives despite evident inbreeding depression. They argued that inbreeding preference (or tolerance) may be favoured by selection due to both genetic and material benefits of mating with kin (Townsend et al., 2019). However, the potential benefits of any preference of inbred females for male relatives from their own inbred line over unrelated outbred males seems paradoxical, given that inbred males are seemingly less capable of transferring a spermatophore. One potential explanation is that the range of acceptable cuticular hydrocarbon (CHC) profiles is narrower in inbred than outbred females, as has been reported in *Drosophila* (Loyau, Cornuau, Clobert, & Danchin, 2012). CHCs are waxy compounds produced on the adult cuticle of many insects and they play an important role in kin recognition and sexual selection (Nagamoto, Aonuma, & Hisada, 2005; Ryan & Sakaluk, 2009; Thomas & Simmons, 2008, 2009; Tregenza & Wedell, 1997; Weddle, Hunt, & Sakaluk, 2013; Weddle, Mitchell, Bay, Sakaluk, & Hunt, 2012; Weddle, Steiger, et al., 2013). Importantly, we know from previous studies that CHC profiles in *G. sigillatus* are significantly heritable (Weddle, Steiger, et al., 2013), and so it follows that the heterogeneity of CHC profiles experienced by outbred females during development and over generations will necessarily be greater than the profiles experienced by inbred females. On one hand, this could lead to differential selection on the CHC preferences of inbred and outbred females over evolutionary time, or, alternatively, the CHC profiles experienced by females during development could influence CHC preference for mates during adulthood. Coevolution between male CHC attractiveness and female preference within inbred lines might be driven not by inbreeding, but by genetic drift and/or

Table 1
The effect of inbreeding on life-history traits in decorated crickets, *G. sigillatus*

Trait	*ndf, ddf*	*F*	*P*	δ
Hatching time	1, 25.3	11.6	**0.0022**	-18.2
Number of offspring	1, 13.8	82.5	**0.0001**	80.2
Developmental time of daughters	1, 32.1	19.1	**0.0001**	-9.1
Developmental time of sons	1, 32.9	19.1	**0.0001**	-8.5
Adult mass of daughters	1, 43	0.34	0.56	-2.2
Adult mass of sons	1, 32.5	0.94	0.34	-4.3
Survival of offspring to sexual maturity	1, 25.78	0.34	0.56	-3.3
Sex ratio of adult offspring	1, 1	4.10	0.29	-15.5

Bold values designate significant differences between inbred and outbred groups.

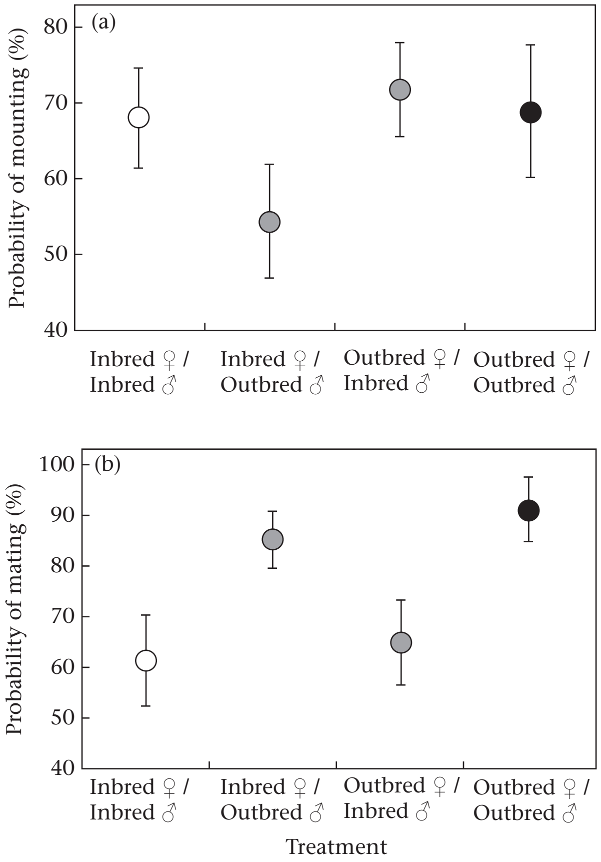

Figure 2. The effect of inbreeding on mating success in *G. sigillatus*. (a) Probability of females mounting actively courting males. (b) Probability of spermatophore transfer of males mounted by sexually receptive females. Points represent least-squares means (±SE).

selection. Another possibility is that inbred females prefer to mount inbred males over outbred males, irrespective of their relatedness to either male, because of assortative mating between low-quality mates (Holveck & Riebel, 2010; Riebel, Holveck, Verhulst, & Fawcett, 2010). Future studies should explore these possibilities and particularly the importance of social context (i.e. CHC profiles) experienced during development on mate choice.

The relatively modest, albeit curious, effect of inbreeding on the propensity of females to mount males stands in contrast to the more pronounced effect of inbreeding on the success of males in transferring the spermatophore once mounted. These results are consistent with our prediction that any inbreeding depression in sexual competency should be more evident in the success of spermatophore transfer than in female mounting propensity because of the tighter link between mating success and fitness in males than in females (Bateman, 1948; Trivers, 1972). Once mounted, inbred males were less likely to transfer a spermatophore than outbred males, irrespective of the inbreeding status of the female. This suggests that inbred males suffer from some behavioural and/or morphological dysfunction as a result of inbreeding that limits successful spermatophore transfer. Copulation in crickets is a complex process involving several coordinated movements by the male that elicit chain reactions necessary for complete spermatophore transfer (Kumashiro & Sakai, 2001). As such, any alterations to male behaviour or the morphology of the structure of the phallic complex, including the muscles and innervation of these structures, could significantly reduce a male's ability to transfer a spermatophore. This could, in turn, decrease reproductive success of inbred males relative to outbred males. A more detailed investigation into alterations of the reproductive physiology of males within our inbred lines might determine whether such inbreeding anomalies exist.

While we found significant effects of inbreeding on female mate choice and male sexual competency, there are two reasons why these are not likely to have contributed fully to the inbreeding depression in reproductive success that we report. First, inbred females were just as likely to mount inbred males as outbred females were to mount outbred males, thus ruling out any effects of female choice between inbred and outbred pairs. Second, even if male dysfunction relating to spermatophore transfer decreased mating frequency of inbred pairs, this would not fully account for the differences. Male dysfunction in spermatophore transfer may be responsible for some inbred pairs producing no offspring, but inbred pairs produced fewer offspring than outbred pairs even when these cases of reproductive failure were excluded. Where successful copulation was obviously achievable, the mating period of 10 days is more than enough time to achieve the number of copulations required for adequate insemination (Ivy & Sakaluk, 2005).

Evidence for effects of inbreeding on mating preferences across taxa is somewhat equivocal; while many studies report clear evidence for inbreeding avoidance, others find tolerance of, or even preference for, inbreeding (Keller & Waller, 2002). We found evidence for significant inbreeding depression in various life-history traits (i.e. reduced life span, reduced offspring production, longer offspring development time), as well as sex-specific effects of inbreeding on mate choice and the sexual competency of decorated crickets. Surprisingly, we found that inbred females preferentially mounted inbred males, despite inbred males being less successful at transferring spermatophores than outbred males. These results reveal that inbreeding may have unexpected consequences for mate choice and highlight the importance of considering sexually selected traits and reproductive performance when assessing the effects of inbreeding within populations.

Data Archiving

Data are archived in the Mendeley Data Repository: https://doi.org/10.17632/b36vrhs7kf.1.

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