Testing the role of same-sex sexual behaviour in the evolution of alternative male reproductive phenotypes

Jack G. Rayner, Nathan W. Bailey
School of Biology, University of St Andrews, St Andrews, Fife KY16 9TH, UK

Correspondence
JGR: jr228@st-andrews.ac.uk
NWB: nwb3@st-andrews.ac.uk

Key words: Alternative reproductive tactics, behavioural syndrome, field cricket, non-adaptive behaviour, same-sex sexual behaviour, SSB, *Teleogryllus oceanicus*

Author contributions: JGR conceived the study; JGR & NWB designed experiments; JGR performed experiments and analysed data; JGR wrote the manuscript with input from NWB

Abstract

Male same-sex sexual behaviour (SSB), where males court or attempt to mate with other males, is common among animal taxa. Recent studies have examined its fitness costs and benefits in attempts to understand its evolutionary maintenance, but the evolutionary consequences of SSB are less commonly considered. One potential impact of SSB might be to facilitate the evolution of traits associated with less sexually dimorphic males, such as alternative reproductive tactics, by diverting costly aggression from other males. To test this, we capitalised on the recent rapid spread of a silent male morph of field cricket, *Teleogryllus oceanicus*, which is unable to produce characteristic male acoustic signals, benefits from satellite mating behaviour, and exhibits feminised appearance and cuticular hydrocarbon profiles. We tested the prediction that interactions involving these non-signalling, less sexually dimorphic male morphs would show heightened rates of SSB, which could reduce the strength of male-male competition and permit greater access to females. We found no evidence that SSB was more common in trials involving silent males. Instead, SSB was predicted by courtship of females presented during a pre-trial treatment. Our results provide evidence supporting the view that SSB represents a spillover of sexually selected courtship behaviour in a non-adaptive context, but do not support a strong role for SSB in the evolution of less ornamented males in this system.

Introduction

Same-sex sexual behaviour (SSB), where individuals court or attempt to mate with members of the same sex, is taxonomically widespread (Bailey and Zuk, 2009). Recent studies have tested various adaptive and non-adaptive explanations offered for the evolutionary origins and persistence of these behaviours. These have provided some support for non-adaptive hypotheses of SSB resulting from mistaken identity (Harari, Brockmann, & Landolt, 2000; Sales et al., 2018), with influences of social environment (Bailey and French, 2012; Han and Brooks, 2015; Han, Santostefano, & Dingemanse, 2016) and mating system (MacFarlane, Blomberg, Kaplan, & Rogers, 2007). However, SSB might also play important roles in mediating male competition (Lane, Haughan, Evans, Tregenza, & House 2016; Kuriwada 2017) and increasing relative fitness under sexual selection of males that express it (McRobert and Tompkins, 1988; Steiner, Steidle, & Ruther, 2005; Preston-Mafham, 2006; Bierbach, Jung, Hornung, Streit, & Plath, 2013). Despite these research efforts, little is known about the influence SSB might have upon evolutionary change of other traits (Bailey and Zuk, 2009; Scharf and Martin, 2013; Hoskins, Ritchie, & Bailey, 2015).

Often viewed as evolutionarily counter-intuitive or costly (Maklakov and Bonduriansky, 2009; Scharf and Martin, 2013; Boutin, Harrison, Fitzsimmons, McAuley, & Bertram, 2016), the prevalence of SSB across taxa nevertheless suggests it could exert a substantial influence on evolution, for example by affecting the social selection pressures individuals experience. One way in which it has been suggested to do so is by altering the fitness consequences of same-sex encounters (Lane et al., 2016). For example, same-sex female pairs of a female-biased population of Laysan albatross exhibit cooperative breeding (Young, Zaun, &

VanderWerf, 2008), increasing their fitness and suggesting a role for SSB in facilitating the expression of alternative reproductive strategies (Young and VanderWerf, 2013). In males, SSB is generally expected to reduce the strength of aggressive interaction (Peschke, 1985; Preston-Mafham, 2006; Bailey and Zuk, 2009; Kuriwada, 2017), though evidence for this is mixed (Ruther and Steiner, 2008; Bailey and French, 2012; Lane et al., 2016).

Perhaps the most intuitive evolutionary consequence that SSB could exert, at least among invertebrates, arises from its well-supported link to “mistaken identity” (Harari, Brockmann, & Landolt, 2000; Dukas, 2010; Bailey and French, 2012; Scharf and Martin, 2013; Macchiano, Razik, & Sagot, 2018). In mating systems characterised by scramble competition, individuals that court or attempt to mate with a member of the same sex may do so because they have mistaken them for a member of the opposite sex. If mistaken identity is an important factor contributing to the incidence of male SSB, interactions involving less sexually dimorphic males should have a heightened likelihood of SSB (Preston-Mafham, 2006; Steiner et al., 2005), conceivably to their benefit (Peschke, 1985). For example, Norman et al. (1999) reported field-based observations that small, female-like males of the giant cuttlefish (*Sepia apama*) seem to avoid attack by mate-guarding males; while Dukas (2010) found immature male fruit flies (*Drosophila melanogaster*) are subject to heightened levels of SSB, apparently due to the ambiguity of their incompletely developed cuticular sex pheromones. These observations suggest an evolutionarily important role for SSB in facilitating the evolution of less sexually dimorphic males, through benefits arising from mistaken sex. Such benefits might as a consequence promote the evolution of alternative reproductive tactics, but this role for SSB in facilitating the spread of less sexually dimorphic males does not appear to have been evaluated.

We tested the prediction that interactions involving less sexually dimorphic males should show an increased incidence of SSB, by capitalising on the recent evolutionary spread of an adaptive, songless male morph of Hawaiian field cricket, *Teleogryllus oceanicus*. Male calling and courtship songs are an important determinant of mating success in field crickets (Balakrishnan and Pollack, 1996; Bailey and Zuk, 2008; Rebar, Bailey, & Zuk, 2009). However, 'flatwing' male morphs are rendered silent by genetically determined female-like wing morphology, which spread rapidly under selection from a parasitoid fly that locates males via their song (Zuk, Rotenberry, & Tinghitella, 2006). Loss of song also has important consequences for male-male interactions. For example, aggressive song plays an important role in agonistic contests (Logue et al., 2010). As well as feminised wing morphology, flatwing males exhibit cuticular hydrocarbon profiles more similar to those of females, compared with more sexually dimorphic 'normal-wing' males (Pascoal et al., 2018a), and their neural transcriptomes are feminised (Pascoal et al., 2018b). Importantly, flatwing males benefit from satellite mating strategies (Zuk et al., 2006; Zuk, Bailey, Gray, & Rotenberry, 2018), and may thus profit from heightened levels of mistaken identity in male-male interactions. Increased incidence of SSB in interactions involving these less sexually dimorphic males could therefore have facilitated their recent and rapid evolution, by reducing the levels of aggression they experience, and enabling access to females.

To test these predictions, we conducted trials involving normal-wing and silent flatwing males, and a mixture of both, and recorded the incidence of SSB across treatments. We predicted that interactions involving less sexually dimorphic flatwing males would exhibit heightened levels of SSB, which could potentially benefit them and thereby have facilitated their rapid spread.

Methods

Stocks and rearing

Crickets used in experiments were taken from a mixed-morph laboratory stock population, derived from eggs laid by females from a population on Kauai in 2014 (Pascoal et al., 2016). The stock population has since been maintained at >100 individuals with approximately equal proportions of normal-winged (Nw) and flatwing (Fw) males. Populations were reared in 20L plastic containers, with Burgess Excel Junior and Dwarf rabbit pellets and water available *ad libitum*, at 25C under a 12:12 photo-reversed light-dark cycle.

Males were removed from the mixed stock population as mature adults less than 4 weeks post-eclosion. For a sufficient sample size, the stock population was sampled over 4 generations. The adult males were isolated in cylindrical clear plastic containers (65mm diameter × 40mm depth) for 3 days prior to trials, with cardboard shelter and food and water available *ad libitum* as above. On the second day of isolation, to enable their differentiation during trials, each individual's dorsal-right wing was marked with one or two spots using a similar amount of white correction fluid (Tipp-Ex). Marking was performed on the day prior to males' use in trials to minimise the likelihood it would have an effect upon their behaviour.

Trials

Males of each wing morph were haphazardly assigned to one of three 'dyad' groups: normal-wing vs normal-wing (Nw.Nw), normal-wing vs flatwing (Nw.Fw), and flatwing vs flatwing (Fw.Fw). Trials and pre-trial treatments were conducted in an incubator at

24C, under red light. Immediately prior to use in trials, each male was introduced to a 210 x 230mm arena containing a female from the stock population of unknown age and mating status, and left to interact for 10 minutes. This pre-trial exposure to females has been found to increase the incidence of SSB in subsequent male-male trials due to mistaken identity (Bailey and French, 2012). SSB is an infrequent behaviour, so we performed the pre-trial exposure to females to facilitate comparisons between dyads by increasing the incidence of SSB across trials. Presence/absence of wing movement patterns of male courtship song (flatwing males still perform wing movement patterns associated with the production of song, despite obligate silence [Schneider, Rutz, Hedwig, & Bailey, 2018]) and female mounting was recorded over the course of the 10-minute treatment. In field crickets, females must mount the male for mating to occur (Rebar et al., 2009), and male courtship is characterised by the production of distinctive courtship song (Balakrishnan and Pollack, 1996). If the female mounted the male, the two were gently separated using a paintbrush to prevent copulation (Bailey and French, 2012). The same female was not used in multiple pre-trial treatments.

After the pre-trial treatment, the two males were removed from their respective arenas and gently placed at opposite ends of a third arena with the same dimensions. They were left to interact for 10 minutes, the duration of which was filmed using a Nikon D3300 digital camera, with no observers present. After trials, males were weighed to the nearest mg and their pronotum length recorded to the neared 0.01 mm. Equipment was cleaned with 80% ethanol between trials.

Scoring SSB and agonistic behaviours

Each of the films was studied by the same observer (JGR) and the presence of SSB and agonistic behaviours recorded. Videos were scored without audio to avoid biasing measurements between normal-wing and flatwing males. The strength of agonistic contests were scored between 0 and 3 using a weighting adapted from Dixon and Cade (1986), frequently used in studies of field cricket interactions (Bailey & French, 2012; Kuriwada, 2017): no aggressive contests=0; antennal fencing=1; mandible engagement=2; flipping=3. Presence of SSB was recorded when one or both males produced wing movement patterns characteristic of courtship song in the vicinity of the other. Courtship song could be distinguished by distinctive wing movement patterns; it includes a long, constant-intensity trill, distinct from the short chirps of calling song and intense repetitive aggressive song in which the lateral magnitude of wing movements is much greater and is visually distinctive (Balakrishnan and Pollack, 1996).

Statistical analyses

We first tested factors that might influence whether females mounted males in pre-trial treatments using a generalised linear model (GLM) with binomial error distribution. The response was whether females mounted the male. To examine whether the effect of male courtship upon female mounting differed between male wing morphs, we included in the full model “courted” (yes or no) and “morph” (flatwing or normal-wing) as categorical factors, their interaction, and “mass” and “pronotum length” as covariates. We also used a binomial GLM to test whether, given their inability to produce song, flatwing males were any less likely to produce wing movements associated with courtship song in the pre-trial exposure to females. Here the response was whether or not the focal male produced courtship song wing movements, with the same covariates and “morph” modelled as a categorical factor.

We next examined factors influencing the likelihood of SSB during the subsequent male-male behavioural trials. We treated the expression of SSB observed in each male-male dyad, irrespective of which cricket exhibited it, as a response in a binomial GLM. The unit of analysis in this initial test was therefore behaviour observed at the level of the dyad rather than the level of individual crickets (see below), which avoided pseudoreplication. Differences in mass and pronotum length for the two interacting males were included as covariates. Whether interacting males courted females in the pre-trial treatment (“courtship”) and whether they were mounted by females in the pre-trial treatment (“mounted”) were both modelled as categorical factors: because each male-male trial involved two males, these variables had three factor levels (i.e. neither male expressed or experienced the behaviour, only one did, or both males did).

We performed a post-hoc analysis to distinguish whether a given focal male’s tendency to express SSB was affected by his own prior experience with females, his interacting male partner’s prior experience, or both. To do this, we randomly selected one male from each of the dyads. Using this randomly selected focal male’s expression of SSB as a response, we ran a GLM with binomial distribution to examine the effects of pre-trial experiences (male courtship and female mounting) of the focal male and his interacting partner. The model also included predictor terms of focal and interacting male morph, mass, and pronotum length. The process of randomly selecting focal and interacting males for the above GLM was repeated 10,000 times to avoid random sampling bias, discarding results from models which produced convergence errors. Distributions of coefficients and significance of predictors describing pre-trial experiences of focal versus interacting males across all model runs were then compared, allowing us to evaluate whether SSB displayed by focal males was more strongly predicted by their own previous experience, or by the previous experience of their interacting partner.

All GLMs also included “generation” as a categorical predictor variable, specified as a fixed rather than random effect because it only had four levels, to account for any differences between cohorts. The strength of agonistic contests could not easily be transformed to approximate a normal distribution, so we used non-parametric Kruskal-Wallis and Wilcoxon rank sum tests to evaluate whether the strength of aggressive contests differed between trials in which SSB was or was not observed, or across dyads.

Analyses were performed in R v3.4.4 (R Core Team, 2018). Binomial GLMs were checked for overdispersion and significance-testing was performed using Chi-squared tests, with type II and III sum of squares for models with and without interaction terms, respectively.

Ethical note

We followed Animal Behaviour’s Guidelines for the treatment of animals in behavioural research and teaching. Individuals were marked using temporary correction fluid using a non-invasive procedure, and which gradually wore off over approximately 7 days, and arenas were large enough for males to escape aggressive rivals. After use in experiments, crickets were returned to the original stock population, with food and water available ad libitum.

Results

A total of 98 trials, involving 196 males, were recorded. Of these, 27 involved two normal-winged males (Nw.Nw), 30 two flatwing males (Fw.Fw), and 41 one of each male wing morph (Nw.Fw). Of trials in which males interacted (N=89), 60 (67.42%) exhibited aggressive interactions, 23 (25.74%) exhibited SSB, and 14 (15.73%) exhibited both aggressive interactions and SSB. (Fig. 1)

Behaviour in pre-trial treatment

Results for male courtship and female mounting behaviours during pre-trial treatments are shown in Table 1. In the presence of a female, flatwing males were no less likely to attempt courtship song than normal-wing males (χ^2_1=0.379, *P*=0.538), despite flatwing males' inability to generate an audible signal when making wing movements. Nevertheless, the effect of flatwing and normal-wing courtship efforts on female mounting differed significantly (χ^2_1=4.593, *P*=0.032), and in a predictable manner: flatwing males were less successful at eliciting female mounting behaviour if they tried to produce courtship song than were normal-wing males (Wilcox rank-sum test: *P*=0.013). In cases where males did not attempt courtship, there was a trend for flatwing males to receive more mountings but this was non-significant (Wilcox rank-sum test: *P*=0.074). It is worth noting that attempting to court did nevertheless increase the likelihood of flatwing males being mounted. (Fig. 2)

Rates of SSB

Results from the GLM for the incidence of SSB across trials are given in Table 2. The incidence of SSB was affected by the number of interacting males that had previously courted the female in the pre-trial exposure (χ^2_2=6.830, *P*=0.033): trials in which both males had courted females were on average 3.29 times more likely to exhibit SSB than those in which neither male had courted the female (Fig. 3). There was, however, little evidence for an effect of signalling ability or differences in size of males upon the expression of SSB, with no indication that expression of SSB differed between dyads with differing proportions of Nw and Fw males (χ^2_2=2.105, *P*=0.349), nor a strong indication of being affected by differences in mass or pronotum length (Table 2).

Follow-up analysis indicated that prior courtship by a focal male, rather than by their interacting male partner, increased the focal male's expression of SSB. Across 10,000 random subsets of single focal males selected from each dyad, prior courtship by the focal male was a significant positive predictor (P<0.05) of focal SSB in 5,932 subsets, while prior courtship by the interacting male was a significant positive predictor in only 84. There was also little evidence that the interacting male having been mounted by the female in the pre-trial treatment had an effect on SSB (a significant positive predictor of focal SSB in 594 iterations), making it unlikely that focal SSB was positively influenced by residual female olfactory cues on the interacting male. (Appendix: Fig. A1)

Rates of aggression

The strength of aggressive contests did not appear to differ between trials in which SSB was or was not observed (Wilcoxon rank sum test: W=785, *P*=0.803), nor between dyads (Kruskal-Wallis rank sum test: χ^2_2=1.383, *P*=0.501). Similarly, the likelihood of an aggressive contest occurring did not appear to be associated with whether or not SSB occurred (W=803, *P*=0.443), or dyad (χ^2_2=0.679, *P*=0.712).

Discussion

There is an intuitive hypothetical mechanism linking mistaken identity, frequently associated with SSB, with the evolutionary spread and persistence of alternative reproductive tactics. A common assumption in systems where males adopt alternative mating tactics is that males which are less readily distinguished from females will benefit from reduced levels of male-male competition (Peschke, 1985;

Norman, Finn, & Tregenza, 1999; Dukas, 2010), enabling access to receptive females. SSB has been considered likely to reduce the strength of aggressive interactions that occur during such competition (Kuriwada, 2017; Lane et al., 2016). The interaction of these two processes suggests a potential role for SSB in the evolutionary spread of less sexually dimorphic males which adopt alternative mating tactics. Despite these expectations, we found no evidence that a less sexually dimorphic, non-signalling male morph of field cricket, which benefits from satellite mating behaviours (Zuk, et al., 2006), is more likely to express or be the recipient of SSB compared with more sexually-dimorphic males. These results indicate that the rapid adaptive spread of silent, partially-feminised male crickets is unlikely to have been facilitated by flexible expression of SSB leading to a decrease in the fitness costs of aggressive contests. Instead, the best predictor of SSB was whether males courted females in pre-trial treatments, a result which emphasises the behaviour of the individual expressing SSB ('libido' *sensu* Logue, Mishra, McCaffrey, Ball, & Cade, 2009).

A male cricket's expression of SSB was predicted by his prior courtship behaviour, but was not strongly affected by the phenotype or prior experiences of the male with whom he interacted. Whether dyads were all-flatwing, all-normal-wing, or a mix had no apparent bearing on the likelihood that SSB would be expressed. These findings support the view that expression of SSB is influenced primarily by behaviour of the individual expressing it, rather than appearance or signalling of the male conspecific (Han, et al., 2016), and is consistent with interpretations of SSB as a spillover of ordinary courtship behaviour into a non-adaptive context (Bailey and Zuk, 2009, Logue et al., 2009), i.e. a behavioural syndrome (Sih, Bell, & Johnson, 2004; Boutin et al., 2016). Selection for male courtship behaviour is likely to be particularly strong in field crickets such as *T. oceanicus*, in which copulation can only occur if females mount males (Rebar et al., 2009), perhaps helping to explain the prevalence of SSB in this and related species (Bailey and French, 2012; Kuriwada 2017; Boutin et al., 2016) due to fitness benefits of increased courtship behaviour (Logue et al., 2009).

We introduced each of the males used in the experiment to a female prior to male-male behavioural trials, which has been shown to increase the rate of SSB owing to mistaken identity (Bailey and French, 2012). Flatwing males were no less likely to attempt courtship song during these pre-trial treatments, despite being unable to produce song at an appreciable amplitude (Schneider et al., 2018). However, patterns of wing movement associated with the production of courtship song (whether silent in the case of flatwing males or audible in the case of normal-wing males) were not equally effective in inducing female mounting behaviour – not surprisingly, courtship song by normal-winged crickets has a stronger effect in eliciting female mounting. This illustrates that flatwing males incur the substantial energetic costs associated with wing movement patterns that ordinarily generate song, despite their inability to sing (Hunt et al., 2004); courtship song is particularly costly, incurring twice the energetic expenditure of long-range advertisement song in the related field cricket *Acheta domesticus* (Hack, 1998). Although being silent clearly had a negative impact on male courtship ability, courtship by flatwing males nevertheless had a positive effect on the likelihood of female mounting. This could be due to low levels of noise produced during stridulation (Tinghitella, Broder, Gurule-Small, Hallagan, & Wilson, 2018; Rayner, Aldridge, Montealegre-Z, & Bailey, 2019), however a more plausible explanation is that this increase is due to the involvement of non-acoustic courtship cues, such as posturing and time spent near to the female, which were not recorded.

We did not find support for the prediction that less sexually dimorphic males of *T. oceanicus* receive, or benefit from, increased exposure to SSB, suggesting that SSB is unlikely to be a prominent mechanism of reducing male-male competition in this system. Nevertheless, observations from other species suggest this might elsewhere be the case (Mason and Crews, 1985; Norman, et al., 1999; Peschke, 1985; Dukas, 2010). Reduced sexual dimorphism, frequently referred to as 'female mimicry', is common among males of many species, and is thought to be an adaptive strategy which reduces the strength of intrasexual competition to which they are exposed, but whether a result of inconspicuousness, lack of perceived threat, or mistaken sex is often unclear. For example, in the ruff, *Philomachus pugnax*, less sexually dimorphic 'faeder' males sneak matings in the vicinity of territorial, ornamented males. Observations suggest these 'female-mimics' benefit from mistaken sex, and both express and receive SSB in interactions with aggressive territorial males (Jukema and Piersma, 2006). In red-sided garter snakes, *Thamnophis sirtalis parietali*, and marine isopods, *Paracerceis sculpta*, less sexually dimorphic males benefit from production of female-like pheromones in the former, and female-like appearance in the latter, by avoiding male-male competition and thereby gaining access to receptive females (Mason and Crews, 1985; Shuster, 1987).

In cases where less sexually dimorphic males which use alternative reproductive tactics benefit from reduced competition, they are often thought to do so by avoiding aggression from territorial males due to mistaken sex (e.g. Dominey, 1980; Mason and Crews, 1985). However, benefits of reduced investment in sexually dimorphic ornamentation could also derive from reduced conspicuousness to conspecific males and predators alike, and reallocation of nutritional and energetic resources (e.g. greater testes size in drab 'faeder' males of the ruff; Jukema and Piersma, 2006). Whether less sexually dimorphic males benefit from mistaken sex, providing a clear potential role for eliciting SSB as an adaptive strategy, or simply represent less conspicuous, unornamented males, is often unclear. Although we did not find evidence to support the hypothesis that SSB facilitated the spread of less sexually dimorphic male crickets, the potential for SSB to play a role in the spread of alternative reproductive tactics may be greater in cases where males actively 'mimic' female behaviours associated with courtship and reproduction (Arnold, 1976; Thornhill, 1979; Dominey, 1980).

Acknowledgements

We thank Audrey Grant, Megan McGunnigle and David Forbes for assistance with cricket rearing and maintenance. We also thank two anonymous reviewers and the editor for feedback which substantially improved our manuscript. NWB is grateful to the Natural Environmental Research Council for funding that supported this work (NE/L011255/1).

References

Arnold, S. J. (1976). Sexual Behavior, Sexual Interference and Sexual Defense in the Salamanders *Ambystoma maculatum*, *Ambystoma tigrinum* and *Plethodon jordani*. *Ethology, 42*(3), 247-300.

Bailey, N.W., & French, N. (2012). Same-sex sexual behaviour and mistaken identity in male field crickets, *Teleogryllus oceanicus*. *Animal Behaviour, 84*(4), 1031-1038.

Bailey, N.W., & Zuk, M. (2008). Acoustic experience shapes female mate choice in field crickets. *Proceedings of the Royal Society B: Biological Sciences, 275*, 2645-2650.

Bailey, N.W., & Zuk, M. (2009). Same-sex sexual behavior and evolution. *Trends in Ecology & Evolution, 24*(8), 439-446.

Balakrishnan, R., & Pollack, G.S. (1996). Recognition of courtship song in the field cricket, *Teleogryllus oceanicus*. *Animal Behaviour, 51*(2), 353-366.

Bierbach, D., Jung, C.T., Hornung, S., Streit, B., & Plath, M. (2013). Homosexual behaviour increases male attractiveness to females. *Biology Letters, 9*(1), 20121038.

Boutin, S.R.T., Harrison, S.J., Fitzsimmons, L.P., McAuley, E.M., & Bertram, S.M. (2016). Same-sex sexual behaviour in crickets: Understanding the paradox. *Animal Behaviour, 114*, 101-110.

Dixon, K.A., & Cade, W.H. (1986). Some factors influencing male-male aggression in the field cricket *Gryllus integer* (time of day, age, weight and sexual maturity). *Animal Behaviour, 34*(2), 340-346.

Dominey, W.J. (1980). Female mimicry in male bluegill sunfish—a genetic polymorphism? *Nature, 284*(5756), 546–548.

Dukas, R. (2010). Causes and consequences of male-male courtship in fruit flies. *Animal Behaviour, 80*(5), 913-919.

Gray, B., Bailey, N.W., Poon, M., & Zuk, M. (2014). Multimodal signal compensation: Do field crickets shift sexual signal modality after the loss of acoustic communication? *Animal Behaviour, 93*, 243–248.

Hack M.A. (1998). The energetics of male mating strategies in field crickets (Orthoptera: Gryllinae: Gryllidae). *Journal of Insect Behavior*, *11*, 853-867.

Han, C.S., & Brooks, R.C. (2015). Same-sex sexual behaviour as a by-product of reproductive strategy under male-male scramble competition. *Animal Behaviour, 108*, 193-197.

Han, C.S., Santostefano, F., & Dingemanse, N.J. (2016). Do social partners affect same-sex sexual behaviour in male water striders? *Animal Behaviour, 116*, 53-59.

Harari, A.R., Brockmann, H.J., & Landolt, P.J. (2000). Intrasexual mounting in the beetle *Diaprepes abbreviatus* (L.). *Proceedings of the Royal Society B: Biological Sciences, 26*(1457), 2071-2079.

Hoskins, J.L., Ritchie, M.G., & Bailey, N.W. (2015). A test of genetic models for the evolutionary maintenance of same-sex sexual behaviour. *Proceedings of the Royal Society B: Biological Sciences, 282*(1809), 20150429.

Hunt, J., Brooks, R., Jennions, M.D.,Smith, M.J., Bentsen, C.L., & Bussière L.F. (2004). High-quality male field crickets invest heavily in sexual display but die young. Nature, *432*(7020), 1024–1027.

Jukema, J., & Piersma, T. (2006). Permanent female mimics in a lekking shorebird. *Biology Letters, 2*(2), 161-164.

Kuriwada, T. (2017). Male–male courtship behaviour, not relatedness, affects the intensity of contest competition in the field cricket. *Animal Behaviour, 126*, 217-220.

Lane, S.M., Haughan, A.E., Evans, D., Tregenza, T. & House, C.M. (2016). Same-sex sexual behaviour as a dominance display. *Animal Behaviour, 114*, 113-118

Logue, D.M., Mishra, S., McCaffrey, D., Ball, D., & Cade, W.H. (2009) A behavioral syndrome linking courtship behavior toward males and females predicts reproductive success from a single mating in the hissing cockroach, *Gromphadorhina portentosa*. *Behavioral Ecology,* 20(4), 781-788.

Logue, D.M., Abiola, I.O., Rains, D., Bailey, N.W., Zuk, M., & Cade, W.H. (2010). Does signalling mitigate the cost of agonistic interactions? A test in a cricket that has lost its song. *Proceedings of the Royal Society B: Biological Sciences, 277*(1693), 2571–2575.

Macchiano, A., Razik, I., & Sagot, M. (2018). Same-sex courtship behaviors in male-biased populations: evidence for the mistaken identity hypothesis. *Acta Ethologica, 21*(3), 147-151.

MacFarlane, G.R., Blomberg, S.P., Kaplan, G., & Rogers, L.J. (2007). Same-sex sexual behavior in birds: Expression is related to social mating system and state of development at hatching. *Behavioral Ecology, 18*(1), 21-23.

Maklakov, A.A., & Bonduriansky, R. (2009). Sex differences in survival costs of homosexual and heterosexual interactions: evidence from a fly and a beetle. *Animal Behaviour, 77*(6), 1375-1379.

Mason, R.T., & Crews, D. (1985). Female mimicry in garter snakes. *Nature, 316*(6023), 59-60.

McRobert, S.P., & Tompkins, L. (1988). Two consequences of homosexual courtship performed by *Drosophila melanogaster* and *Drosophila affinis* males. *Evolution,* *42*(5), 1092-1097.

Norman, M.D., Finn, J. & Tregenza, T. (1999). Female impersonation as an alternative reproductive strategy in giant cuttlefish. *Proceedings of the Royal Society B: Biological Sciences, 266*(2426), 1347.

Pascoal, S., Liu, X., Fang, Y., Rockliffe, N., Paterson, S., Shirran, S.L., Botting, C.H., & Bailey, N.W. (2016). Rapid evolution and gene expression: a rapidly evolving Mendelian trait that silences field crickets has widespread effects on mRNA and protein expression. *Journal of Evolutionary Biology*, *29*(6), 1234–1246.

Pascoal, S., Risse, J.E., Zhang, X., Blaxter, M., Cezard, T., Challis, R.J., Gharbi, K., Hunt, J., Kumar, S., Langan, E., et al. (2018a). Silent crickets reveal the genomic footprint of recent adaptive trait loss. *bioRxiv* https://doi.org/10.1101/489526.

Pascoal, S., Liu, X., Fang, Y., Paterson, S., Ritchie, M.G. & Bailey, N.W. (2018b). Increased socially mediated plasticity in gene expression accompanies rapid adaptive evolution. *Ecology Letters*, *21*, 546-556.

Peschke, K. (1985). Immature males of *Aleochara curtula* avoid intrasexual aggressions by producing the female sex pheromone. *Naturwissenschaften, 72*(5), 274-275.

Preston-Mafham, K. (2006). Post-mounting courtship and the neutralizing of male competitors through "homosexual" mountings in the fly *Hydromyza livens* F. (Diptera: Scatophagidae). *Journal of Natural History,* 40*,* 101-105.

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rayner, J.G., Aldridge, S., Montealegre-Z, F., & Bailey, N.W. (2019). A silent orchestra: convergent song loss in Hawaiian crickets is repeated, morphologically varied, and widespread. *Ecology*. doi: 10.1002/ecy.2694

Rebar, D., Bailey, N.W., & Zuk, M. (2009). Courtship song's role during female mate choice in the field cricket *Teleogryllus oceanicus*. *Behavioral Ecology, 20*(6), 1307–1314.

Ruther, J. & Steiner, S. (2008). Costs of female odour in males of the parasitic wasp *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). *Naturwissenschaften, 95*(6), 547-552.

Sales, K., Trent, T., Gardner, J., Lumley, A.J., Ramakrishnan, V., Michalczyk, L., Martin, O.Y., & Gage, M.J.G. (2018). Experimental evolution with an insect model reveals that male homosexual behaviour occurs due to inaccurate mate choice. *Animal Behaviour, 139*, 51-59.

Scharf, I. & Martin, O.Y. (2013). Same-sex sexual behavior in insects and arachnids: Prevalence, causes, and consequences. *Behavioral Ecology and Sociobiology, 67*(11), 1719-1730.

Schneider, W.T., Rutz, C., Hedwig, B., & Bailey, N.W. (2018). Vestigial singing behaviour persists after the evolutionary loss of song in crickets. *Biology Letters, 14*(2), 20170654.

Shuster, S.M. (1987). Alternative Reproductive Behaviors: Three Discrete Male Morphs in *Paracerceis sculpta*, an Intertidal Isopod from the Northern Gulf of California. *Journal of Crustacean Biology, 7*(2), 318-127.

Sih, A., Bell, A., & Johnson, J.C. (2004). Behavioral syndromes: An ecological and evolutionary overview. *Trends in Ecology and Evolution*, *19*(7), 372–378.

Steiner, S., Steidle, J.L.M., & Ruther, J. (2005). Female sex pheromone in immature insect males - A case of pre-emergence chemical mimicry? *Behavioral Ecology* *and Sociobiology, 58*(2): 111-120.

Thornhill, R. (1979). Adaptive Female-Mimicking Behavior in a Scorpionfly. *Science, 205*(4404), 412-414.

Tinghitella, R.M., Broder, E.D., Gurule-Small, G.A., Hallagan, C.J., & Wilson, J.D. (2018). Purring Crickets: The Evolution of a Novel Sexual Signal. *The American Naturalist, 192*(6), 773-782.

Young, L.C., & VanderWerf, E.A. (2013). Adaptive value of same-sex pairing in Laysan albatross. *Proceedings of the Royal Society B: Biological Sciences, 281*(1775), 20132473.

Young, L.C., Zaun, B.J., & VanderWerf, E.A. (2008). Successful same-sex pairing in Laysan albatross. *Biology Letters, 4*(4), 323-325.

Zuk, M., Bailey, N.W., Gray, B., & Rotenberry, J.T. (2018). Sexual signal loss: The link between behaviour and rapid evolutionary dynamics in a field cricket. *Journal of Animal Ecology, 87*(3), 623-633.

Zuk, M., Rotenberry, J.T., & Tinghitella, R.M. (2006). Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. *Biology Letters, 2*(4), 521–524.

Figure 1. (a) Proportions of trials in which neither, one, or both interacting males expressed SSB. (b) proportions of trials involving aggressive contests of varying strength (see Methods for criteria used to score aggressive contests).

Figure 2. The likelihood of females mounting males of each wing morph that did and not perform courtship. Numbers in/above bars indicate sample sizes, asterisks indicate significance (* P<0.05, *** P<0.001) for "courtship" in the overall GLM (top comparison) and "morph" in post-hoc tests within each courtship category (comparisons between Nw and Fw males).

Figure 3. The relationship of male SSB to prior courtship of females across dyads with varying proportions of singing normal-wing and silent flatwing males. (a) Proportion of trials showing SSB, for each dyad group, in association with the number of males which previously courted a female. (b) Proportions of males from each dyad group which expressed SSB, separated on the X-axis by whether they had previously courted a female. Numbers above bars show sample sizes, and inside bars show the number of trials in which SSB was observed. Note differences in Y-axis limits between (a) and (b).

Figure A1. Histograms showing the frequency of P-values (a-d), and density plots showing the distribution of model coefficients ('estimates'; e-h) upon SSB by focal males of predictor terms describing courtship behaviour performed by, and female mounting elicited by, focal and opposite males in the pre-trial exposure to females. Dotted blue lines illustrate P=0.05, and dotted red lines illustrate an estimate of 0 (i.e. no effect upon expression of SSB in the focal individual). X-axes in plots of model coefficients have been truncated at ±30. Predictor terms were included in a GLM with a binomially distributed response variable of individual SSB, for randomly selected combinations of single males from 89 dyads. This process was repeated for 10,000 iterations.

Table 1. Results of binomial GLMs for male courting and female mounting behaviours in the pre-trial treatment.

Response	$\mathbf{R^2}$	**Predictor**	χ^2	**df**	**P-value**
Male courtship	0.052	Wing morph	0.379	1	0.538
		Mass	2.911	1	0.088
		Pronotum length	0.073	1	0.787
		Generation	3.755	3	0.289
Female mounting	0.394	Wing morph	4.593	1	**0.032**
		Courted	17.390	1	**<0.001**
		Mass	3.573	1	0.059
		Pronotum length	0.557	1	0.455
		Generation	0.960	3	0.811
		Morph:Courted	9.645	1	**0.002**

Significant ($P<0.05$) P-values are highlighted in bold. Data are from 196 observations.

Table 2. Results of a binomial GLM for the incidence of SSB across trials.

Predictor	χ^2	**df**	**P-value**
Dyad	2.105	2	0.349
Proportion courted female	6.830	2	**0.033**
Proportion mounted by female	2.072	2	0.355
Mass difference	1.752	1	0.186
Pronotum difference	3.080	1	0.079
Generation	3.003	3	0.391

Significant ($P<0.05$) P-values are highlighted in bold. Data are from 89 trials. The model had an R^2 of 0.236.