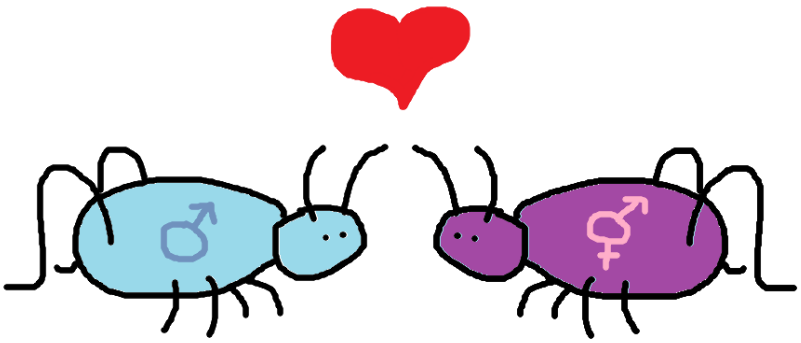
UNIVERSITY OF GOTHENBURG

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

INVESTIGATING THE CAUSE OF SAME-SEX SEXUAL BEHAVIOUR IN JAMAICAN FIELD CRICKETS USING MIXED SEX-PHEROMONES



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ABSTRACT

Same-sex sexual behaviours (SSB) are widespread in the animal kingdom, but their proximate and ultimate causes are still debated. A recent explanation, called the mating filter hypothesis, proposes that SSB results from having a broad mating filter, which means being permissive with what one regards as a potential mate. The hypothesis has been supported by a study showing SSB is condition-dependent male Pacific field crickets (*Teleogryllus oceanicus*). However, this hypothesis assumes that the proximate cause for SSB is inaccurate sex recognition, which is not yet known. Here I attempted to examine this assumption by investigating whether male Jamaican field crickets (*Gryllus assimilis*) in poor condition are less likely to court females when sex recognition cues are obscured. Male condition was manipulated through diet, and sex recognition cues were altered by extracting and reapplying male and female pheromones onto dead female crickets. Males were then exposed to live females and pheromone-manipulated females in behavioural trials. The sample size I achieved was limited, causing large uncertainties, and therefore estimates should be interpreted cautiously. In line with inaccurate sex recognition as a proximate cause for SSB, I found no evidence that mixed sex-pheromones affected either the likelihood or amount of male courtship. I also found no evidence for an interaction between diet and pheromone treatment, which does not support the prediction that males in poor condition become more selective when sex-recognition cues are unreliable. However, I found weak evidence that a low-quality diet made males less likely to court dead females despite not affecting courtship towards live females, which is in line with the mating filter hypothesis. Additionally, only males that courted a live female subsequently courted a mixed-pheromone female, suggesting that this behaviour is linked to normal mating motivation and providing some support for inaccurate sex-recognition as a proximate cause.

same-sex sexual behaviour | sex recognition | mating filter | mistaken identity | cuticular hydrocarbons | *Gryllus assimilis*

ABSTRACT (SWEDISH)

Sexuella beteenden riktade mot samkönade individer (SSB) förekommer i vitt skilda delar av djurriket, men deras proximata och ultimata orsaker är ännu omdebatterade. “Mating filter”-hypotesen är en nyligen presenterad förklaring som föreslår att SSB uppstår av ett brett mating filter, vilket innebär att vara öppen med vad man betraktar som en potentiell partner. Hypotesen stöds av en studie som visar att SSB påverkas av kroppsskick hos *Teleogryllus oceanicus*. Men denna hypotes innebär ett antagande om att den proximata orsaken till SSB är inexakt könsigenkänning vilket ännu inte är bekräftat. I denna studie ämnade jag att undersöka detta antagande genom att testa om *Gryllus assimilis*-hanar i dåligt skick är mindre benägna att uppvakta honor när signaler för könsigenkänning görs svårtolkade. Hanarnas skick manipulerades genom kost, och könsigenkänningssignaler förändrades genom att extrahera och återapplicera hanliga och honliga feromoner på döda honor. Hanar presenterades sedan för levande honor och feromonmanipulerade honor i beteendeexperiment. Provgruppstorleken som jag åstadkom var begränsad och ledde till stora konfidensintervall och därför bör estimeringarna tolkas försiktigt. Jag fann inga bevis för att blandade könsferomoner påverkade hanars benägenhet för uppvaktning. Jag fann heller inga bevis för en interaktionseffekt mellan kost och feromonbehandling vilket inte stödjer förutsägelsen att hanar i dåligt skick blir mer selektiva när könsigenkänningssignalerna är opålitliga. Däremot fann jag svaga bevis för att en lågkvalitativ kost gjorde hanar mindre benägna att uppvakta döda honor trots att kost inte påverkat benägenheten att uppvakta levande honor. Detta går i linje med mating filter-hypotesen. Dessutom var det bara hanar som hade uppvaktat levande honor som sedan uppvaktade feromonblandade honor, vilket antyder att beteendet är kopplat till vanlig parningsvilja och därmed ger stöd för inexakt könsigenkänning som proximat förklaring till SSB.

INTRODUCTION

Same-sex sexual behaviour in animals

Same-sex sexual behaviour (SSB) occurs in many taxa across the animal kingdom and is defined as any mating behaviour that is directed toward an individual of the same sex (Bailey & Zuk, 2009). Since SSB carries costs (e.g. energy expenditure or missed mating opportunities) while providing no obvious reproductive benefits it has been described as an evolutionary paradox (Bailey & Zuk, 2009; LeVan et al., 2009; Maklakov & Bonduriansky, 2009; Santtila et al., 2009). Many proximate and ultimate causes for SSB have been hypothesized, both as general explanations and species specific hypotheses (Bailey & Zuk, 2009; Monk et al., 2019).

One explanation that is commonly accredited for SSB in invertebrates is mistaken identity, which means that individuals engage in SSB when they fail to correctly recognize the sex of another individual (Bailey & French, 2012; Bailey & Zuk, 2009). Although in itself a proximate cause, SSB due to mistaken identity is sometimes categorized as a non-adaptive or maladaptive hypothesis despite being compatible with adaptive explanations (Bailey & French, 2012; Bailey & Zuk, 2009; Shine et al., 2003; Van Gossum et al., 2005).

Acceptance threshold theory and the mating filter hypothesis

By applying Reeve's acceptance threshold theory (Reeve, 1989), several researchers have proposed adaptive explanations for how SSB could be maintained over time in systems with unreliable sex recognition (Engel et al., 2015; Richardson et al., 2024; Richardson & Zuk, 2023). They have argued that if sex discrimination in a species is inherently imperfect, then adjusting the threshold for the sensory stimuli required to trigger mating behaviours will influence both opposite-sex and same-sex interactions. Specifically, lowering this threshold (or broadening the "mating filter"), would increase opportunities for mating with the desired different-sex individuals but simultaneously increase the likelihood of mating attempts directed toward same-sex individuals. This leads to a trade-off between the costs of SSB and the costs of missed mating opportunities, but since missed mating opportunities can be assumed to be more costly than occasional same-sex mating attempts, selection should favour a threshold low enough that some level of SSB persists as a by-product in an optimized mating strategy. To investigate this idea, research has looked at acceptance-threshold-based SSB as a flexible, condition-dependent phenomenon, examining how the frequency of SSB shifts as social and physiological factors are experimentally altered in order to alter the relative costs of sex-recognition errors (Engel et al., 2015; Richardson et al., 2024).

Sex recognition and courtship in crickets

In crickets, sex recognition is mediated by pheromones on the surface of the crickets' exoskeleton, commonly referred to as cuticular hydrocarbons (hereafter CHCs) (Hardy & Shaw, 1983; Tregenza & Wedell, 1997). Although still unknown for the Jamaican field cricket *Gryllus assimilis*, related cricket species do not have sex-specific pheromones; instead the same CHCs are present in both sexes but are sexually dimorphic in their relative abundance (Thomas & Simmons, 2008; Tregenza & Wedell, 1997). This could suggest that within-sex variation in CHC-profiles in these species might have a higher impact on rates of SSB than for species with sex-specific pheromones, since the most masculine females in terms of pheromone profile might resemble the most feminine males, and vice versa.

Male *G. assimilis*, like other cricket species, produce a distinct song when courting a female in order to persuade her to mount and mate with him (Vedenina & Pollack, 2012). This conspicuous courtship makes

crickets suitable for studying SSB. Producing courtship song is energetically costly (Hack, 1998; Mowles, 2014), and this led Richardson et al. (2024) to argue that the relative cost of courtship is higher for males with little metabolic energy available than for well-nourished males. Their mating filter hypothesis therefore predicts that males in poor condition will adopt a narrower mating filter, or a higher acceptance threshold for initiating courtship, because the relative cost of occasional same-sex courtship will then be greater. Consistent with this prediction, they showed that SSB was condition dependent in the Pacific field cricket (*Teleogryllus oceanicus*), as males in poor condition were less likely than males in good condition to court other males, while courtship directed toward females did not differ depending on condition. However, the mating filter hypothesis investigated in that study assumes that the proximate cause for the observed SSB is inaccurate sex recognition, which was not tested. A cricket may court another male because he thinks it is a female, or because he correctly recognizes the sex of the other male but still decides to court, and telling the two apart is difficult.

Interpretations of same-sex sexual behaviours in crickets

In an attempt to resolve this, Green et al. (2026) performed extensive male-male trials for 8 species of field crickets and analyzed the behaviours expressed by both males. They found that SSB in these species was better described as tension reduction than as mistaken identity, since SSB in one male was associated with reduced aggression in the other. They further argued that mistaken identity was unlikely, since SSB was frequently expressed even after the other male had sung, something only males do (Alexander, 1961).

However, alternative interpretations may also be consistent with their observations; aggression from one male was strongly associated with reduced SSB in the other, which in turn could be interpreted as support against tension reduction; the observation that SSB in one individual was associated with reduced aggression and increased SSB in the other could also be interpreted within a mating filter framework where courtship by another male may signal the presence of a female and thereby increase responsiveness and lead to a broadening of the mating filter.

The evidence presented against mistaken identity is the observation that males expressed SSB despite having heard song, which is only produced by males. This line of reasoning depends on the assumption that acoustic signals play a primary role in sex recognition. However, while male crickets are known to perceive and respond behaviourally to song from other males (Santori et al., 2020; Wilde et al., 2023), direct, individual-to-individual sex recognition has, to my knowledge, only been demonstrated through contact chemoreception and antennal movement (Hardy & Shaw, 1983; Tregenza & Wedell, 1997). It is therefore possible that acoustic cues are not the main basis for sex recognition during close interactions, which is conceivable since mating in most cricket species takes place in darkness where identifying the originator of a song might be highly unreliable (Alexander, 1968). A mistaken identity interpretation of SSB in a pheromone-biased sex recognition system is not incompatible with the occurrence of SSB despite the presence of male-specific song.

AIMS

In this study, I attempt to test the assumption that SSB in field crickets arises as a result of failed sex recognition. I investigate whether applying mixed sex-pheromones to dead female stimuli affects courtship in male Jamaican field crickets (*Gryllus assimilis*), and if males in poor condition are less likely to court a dead female when sex recognition cues are made less reliable.

To test this, I experimentally manipulated the chemical cues involved in sex recognition by extracting CHCs from dead male and female crickets and reapplying them to dead females, thereby creating females with mixed sex-pheromones. As a control, I used females subjected to the same procedure but using female-only pheromones, as well as pretrials with a live, female stimuli. Males were assigned to either a high- or low-quality diet and were then exposed to these CHC-manipulated females in behavioural trials. The courtship behaviour of the males was recorded to determine how condition and sex recognition reliability influence courtship. If my results mirror those of Richardson et al (2024), i.e. if a poor diet leads to a lower probability of courtship specifically in trials where sex recognition cues are obscured, they will be in line with their mating filter interpretation. If mixing sex-pheromones does not lower courtship, this would be in line with inaccurate sex-recognition as a proximate cause of SSB, since courtship directed towards females with mixed male and female pheromones could be considered a SSB-adjacent behaviour, but would not be attributable to accurate sex recognition since the CHC-profile of the female would not be typical to either sex.

METHODS

Cricket housing and rearing

The Jamaican field crickets used in this study were bred commercially and bought from a pet store. Juvenile crickets were separated immediately after purchase based on sex and were housed in 32-L plastic containers with egg cartons for shelter, mosquito mesh as substrate, cotton-plugged plastic vials for water and ad libitum access to dry cat food pellets (*Monster Cat Turkey & Chicken, Tree of Pets AB*) and rolled oats in Petri dishes. The containers were cleaned and water and food replaced once a week. The crickets were kept in climate controlled cabinets (*Aralab FitoClima 600 PLH*) set to 26°C, 50 % relative humidity under a photoreversed 12:12 h light:dark cycle. Containers were checked daily for dead crickets, and adult crickets were separated upon eclosion; adult females were placed in a separate 32-L container and adult males were weighed, isolated and put on their assigned diet.

Diet treatment

In order to manipulate the condition of the trial males, two experimental diets were made by mixing dry, ground cat food with microcrystalline cellulose (*Avicel PH-101, Sigma-Aldrich*) into a high-nutrient and a low-nutrient diet, containing 5% and 40% cellulose respectively. Diets of similar macronutrient-to-cellulose ratios have been shown to affect condition in related cricket species (Houslay et al., 2017; Richardson et al., 2024; Santori et al., 2020). Isolated males were randomly assigned a diet using a Fisher-Yates shuffle, and then placed in individual 324 ml (6x6x9 cm) plastic containers and provided with mosquito mesh, a cotton-plugged water vial and ad libitum access to the assigned diet (~5 ml) on a small paper tray. The containers and their contents were replaced once a week and all treatment males were kept in the same climate controlled cabinet. Males were kept on their respective diet a minimum of 8 days before being used in a behavioural trial.

Experimental manipulation of cuticular pheromones

Stimulus females with manipulated CHC's were prepared by first taking adult males and females from the stock population and killing them by freezing. These were then randomly paired with another cricket using a Fisher-Yates shuffle, and put in room temperature to thaw for 30 minutes. Pairs of two females were used to harvest female pheromones and create the control stimuli with female-only pheromones, and pairs of one male and one female were used to create the stimulus females with mixed sex-pheromones. Working in a fume cupboard, pairs were placed in glass containers and submerged fully in approx. 20 ml of hexane (*n-Hexane for analysis EMSURE® ACS, Sigma-Aldrich*) for 5 minutes before being removed and

left to dry for 15 minutes. The hexane extracts, now containing the CHC's from both individuals, were then painted back on both crickets with a fine brush until complete evaporation in order to reapply the CHC's. The crickets were set aside for 2 hours to ensure full evaporation of the hexane before finally being stored in -18 °C until used in a behavioural trial. This follows the procedure used by Tregenza & Wedell (1997) which was demonstrated to successfully strip and reapply pheromonal CHC's in *Gryllus bimaculatus*. In the mixed-pheromone pairs, only the female was used as stimulus in a trial.

Behavioural experiment

Diet-treated males were randomly assigned a stimulus female using a series of Fisher-Yates shuffles; assigning pheromone treatment within each diet group, followed by assigning stimulus ID:s within each stimulus group. The order of the trials were randomized, this too using a Fisher-Yates shuffle. The males were then placed in individual styrofoam boxes for acoustic isolation and were transported, along with females from the stock population, to a darkroom with constant temperature of 26 °C and left to readjust for 30 minutes. The assigned stimulus females were thawed for 30 minutes in ambient temperature before use in trial. All trials were performed under red light.

It has been shown in related species that preceding exposure to a female increases SSB (Bailey & French, 2012). Previous studies investigating SSB in field crickets have therefore included pretrial exposure to females (Richardson et al., 2024; Richardson & Zuk, 2023). The live female trial in this study serves both to increase probability of the SSB-adjacent behaviour investigated, to increase comparability between previous studies and as a baseline control to the dead female stimulus trial.

Live female trial

A diet treated male was placed in a 10x10 cm arena and left to acclimate for 3 minutes. A random female from the stock population was then placed in the arena. The trial started when the male made antennal contact with the female and behaviours and events were recorded for 5 minutes. A courtship chirp was defined as one of the high-frequency chirps that occur in singles, doubles or triplets (sometimes referred to as "ticks") between the pulsating segments of the courtship song (Vedenina & Pollack, 2012). This unit was used for the courtship counts due to being discrete and easy to count, as opposed to the song as a whole which is harder to quantify. If the female mounted, she was gently pushed off to prevent mating. When the trial ended the female was removed using a mesh cylinder with an opening diameter of ~5 cm.

Manipulated pheromone trial

Upon removal, the live female was immediately replaced by the assigned stimulus female. The trial started when the male made antennal contact with the stimulus female and behaviours and events were again recorded for 5 minutes. After first contact was broken, the female was nudged gently toward the male, mimicking live behaviour, until antennal contact was made again.

Statistical analysis

All statistical analyses were made in R (version 4.5.3). Survival was analyzed using a generalized linear model (GLM) with a binomial distribution. The body mass data lacked pre-diet mass and was only available for a small subset of individuals. Therefore, I decided to analyze this data visually rather than statistically.

I analyzed courtship probability in both the live female trials and the pheromone-manipulated female trials using generalized linear mixed models (GLMM) with binomial distributions. Here, courtship was analyzed as a binary response (courted vs did not court). The courtship chirp count-data was overdispersed, meaning variances were larger than the means, making poisson regressions inappropriate. Therefore, courtship chirp-data were analyzed using GLMMs with negative binomial distributions which are suitable for modeling overdispersed count-data.

For analysis of the courtship of pheromone-manipulated females, I initially built models with second order interactions. I tested whether the inclusion of these interactions produced models with better fit using likelihood ratio tests comparing full and reduced models. Since the interaction terms did not improve model fit they were removed from the final models in order to better estimate the main effects.

In several models the variance associated with male ID was estimated to be close to near zero. Despite this, male ID was retained in these models to account for non-independence between repeated trials on the same males.

RESULTS

Male mortality was high, as only ~35% of the males placed on diets survived until trial (≥ 8 days). Trials were performed on a total of 10 individual males tested between 1 and 10 times. The high mortality meant that the number of trials per diet x pheromone combination was low (Table 1). I conducted 42 sets of trials, each consisting of an initial trial with a live female and a subsequent trial with a dead, pheromone-manipulated female (Table 1). Only males who courted a live female in the preceding live trial ended up courting a dead female.

Most model estimates were associated with large confidence intervals relative to the estimated effect sizes, indicating considerable uncertainty. In all but one case, models including male ID as a random effect produced matrix singularities and near-zero variance estimates, indicating that the models were not able to attribute any variation to male identity. Despite this, male identity was retained as a random effect in order to appropriately account for non-independence in repeated measurements on the same individuals.

Table 1. Sample sizes for each diet x stimulus combination, and the number of biological replicates. Sample sizes are not adjusted for repeated measurements on the same individuals.

Number of trials performed				
	Control stimulus	**Mixed stimulus**	**Live stimulus**	**Number of males**
High quality diet	11	12	23	6
Low quality diet	10	9	19	4
Total	21	21	42	10

Survival

Male survival probability was modelled using a generalized linear model (GLM) with survival as a binomial response and diet treatment as a fixed effect. Survival was scored as surviving on the assigned diet until trial (≥ 8 days).

I found no evidence that survival differed between diet treatments (estimate ± SE = -0.511 ± 0.632, z = -0.808, p = 0.419), although individuals on the low nutrient diet showed a numerically lower survival than those on the high nutrient diet (survival rate H = 40% , L = 29%).

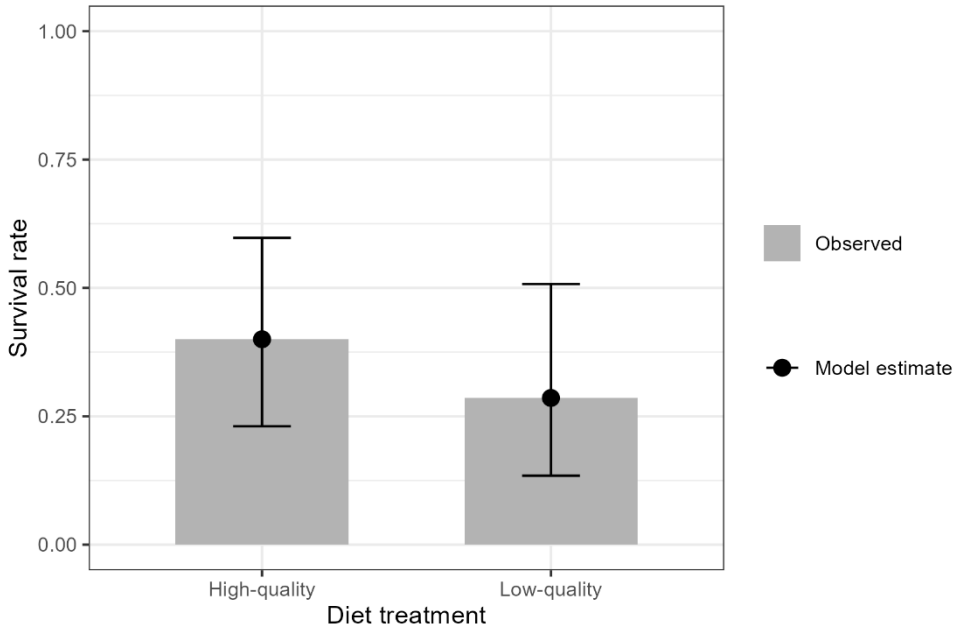

Figure 1. The effect of diet on survival ≥8 days after eclosion in Jamaican field crickets. Gray columns represent observed survival rates (high-quality diet: n = 25; low-quality diet: n = 21). Black points represent model predictions of survival probability from a generalized linear model with diet treatment as a predictor. Error bars represent 95% confidence intervals.

Body mass

Body mass was measured 2-8 times for a small subset of males. Among these males, there is no clear trend in mass change over time depending on diet treatment (Fig. 2).

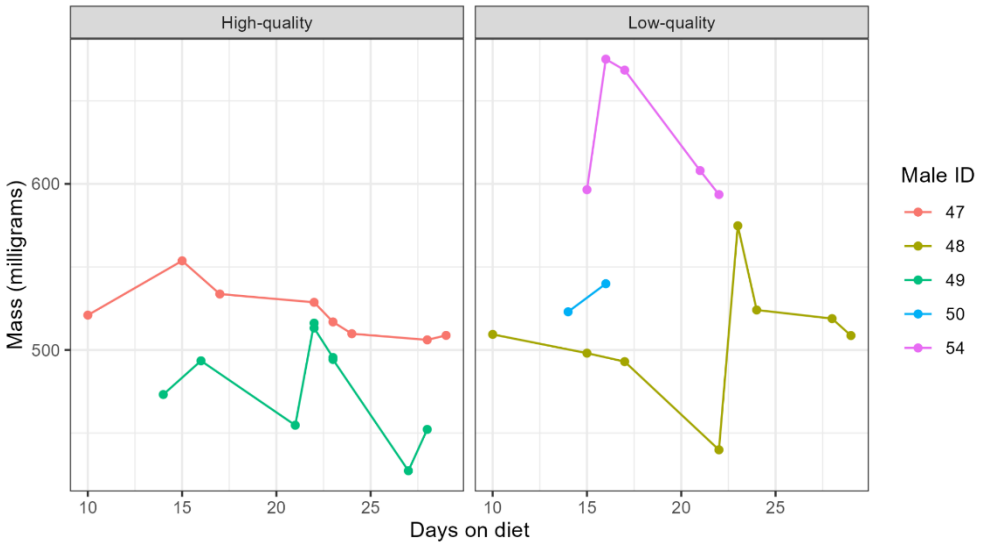

Figure 2. The effect of high- (H) and low quality diets (L) on body mass over time in male Jamaican field crickets. Y-axis represents pre-trial mass (mg), and x-axis represents number of days on the assigned diet at the time of the trial. The graph only includes individual males who were weighed multiple times.

Live female trials

Does diet affect the probability of a male courting?

To answer this I used a binomial generalized mixed model (GLMM) with diet treatment as a predictor, male ID as a random effect and courtship as a binary response variable (courted vs. did not court).

Males on the high- and low-quality diets both had an estimated 52% probability of courting a live female (Figure 3). As predicted, I found no evidence that diet treatment affected the probability of courting a live female (estimate ± SE = -0.021 ± 0.709, p = 0.977). The model could not detect any substantial between-male variation in the probability to court a live female (Table 2).

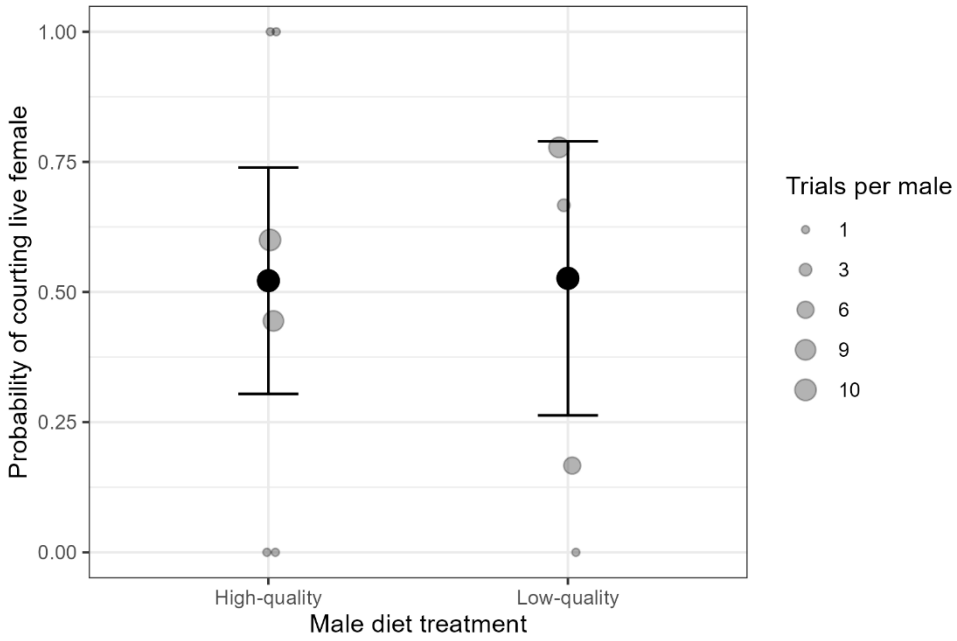

Figure 3. The effect of diet on the probability of courting a live female in male Jamaican field crickets. The grey points represent the observed probability of courting a live female for each individual male, and the size indicates the number of trials each male underwent. The black dots are model estimates, and the bars are 95% confidence intervals. Parametric bootstrapping was used to obtain CIs. Predictions were made at population level with no assigned male ID.

Table 2. Table of coefficients for the effect of diet on the probability of courting a live female (binomial GLMM).

Live female courtship binomial GLMM

Effect	Term	Estimate	Std. error	z-statistic	p-value
fixed	(Intercept)	0,082	0,474	0,174	0,862
fixed	diet_treatmentL	-0,021	0,709	-0,029	0,977
random	variance (male_ID)	0,133			

Does diet affect the number of courtship chirps produced by males?

A negative binomial GLMM with diet treatment as fixed effect, male ID as random effect and chirp count as response variable was used to analyze the effect of diet on the number of courtship chirps produced by males exposed to a living female.

As predicted, I found no evidence that diet affected the number of courtship chirps directed at live females (estimate ± SE = 0.126 ± 0.815, p = 0.877, Figure 4). Between-male variation in courtship chirps produced was estimated to be near-zero.

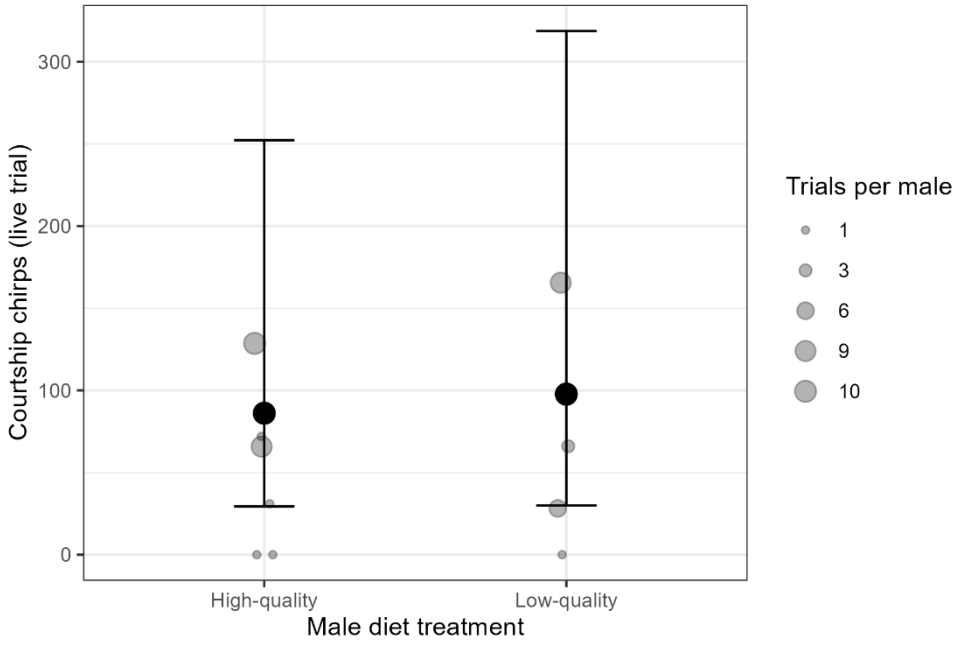

Figure 4. The effect of diet on the number of courtship chirps produced by male Jamaican field crickets when courting a live female. The grey points represent the observed number of live trial courtship chirps for each individual male, and the size indicates the number of trials each male underwent. The black dots are model estimates, and the bars are 95% confidence intervals. Predictions are made at population level with no assigned male ID.

Pheromone-manipulated female trials

Does diet or female pheromone treatment affect the probability of a male courting?

A binomial GLMM was used to determine probability of courting a pheromone-manipulated female, with diet treatment and pheromone treatment as fixed effects, male ID as random effect and courtship as a binary response variable. The initial model included an interaction between pheromone treatment and diet. However, I found no evidence to support an interaction, since including this term did not improve

model fit (likelihood ratio test, $\chi^2(1) = 1.076$, $p = 0.300$). Therefore, subsequent analysis was based on a reduced model without an interaction term to allow for more accurate estimations of main effects.

I predicted that diet would lower the probability of courtship in the low-quality-diet x mixed-pheromone group. However, I found weak evidence that males on a low nutrient diet were less likely to court a female regardless of pheromone treatment (estimate ± SE = -1.881 ± 1.137, p = 0.098) (Fig. 5). I found no evidence that the pheromone treatment affected the probability of courtship (estimate ± SE = -0.453 ± 0.872, p = 0.604). The model estimates approximately 0 between-male variance in courtship probability (Table 3).

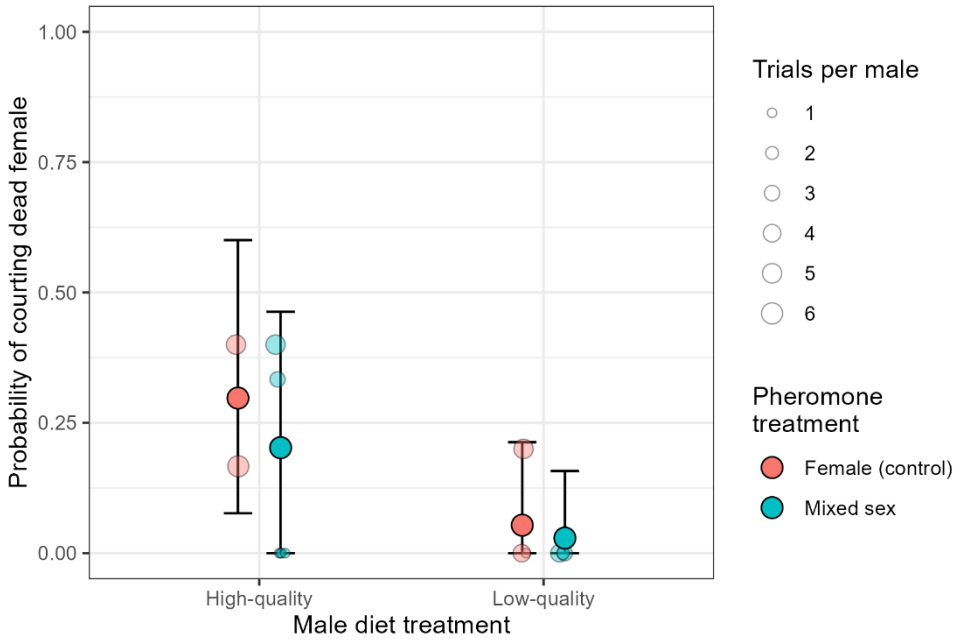

Figure 5. The effect of diet and female pheromone-treatment on the probability of courting a dead female in male Jamaican field crickets. Full-colored points represent models estimates of probability of courtship, with 95% confidence intervals. Semitransparent points represent observed probability of courtship for individual males, with the size of the point representing the number of trials performed on each male. Parametric bootstrapping was used to obtain 95% CIs. Predictions are made at population level with no assigned male ID.

Table 3. Table of coefficients for the effect of diet and female pheromone-treatment on the probability of courting a dead female (binomial GLMM).

Manip. pheromone courtship binomial GLMM

Effect	Term	Estimate	Std. error	z-statistic	p-value
fixed	(Intercept)	-0,817	0,628	-1,302	0,193
fixed	diet_treatmentL	-1,881	1,137	-1,655	0,098
fixed	stimulusmixed	-0,453	0,872	-0,519	0,604
random	variance (male_ID)	0			

Does diet or pheromone treatment affect the number of courtship chirps produced by males?

A negative binomial GLMM was used to analyze the number of courtship chirps produced by males presented with a pheromone-manipulated, dead female. It included diet and pheromone treatment type

as fixed effects, an interaction between these, male ID as random effect and chirp count as response variable. Including the interaction did not improve model fit so it was removed (likelihood ratio test, $\chi^2(1) = 2.162$, $p = 0.141$).

Contrary to my predictions, I found no evidence that the number of courtship chirps directed at females was affected by either diet (estimate ± SE = -4.082 ± 2.610, p = 0.118) or pheromone treatment of the stimulus female (estimate ± SE = -2.206 ± 2.595, p = 0.395). Model estimates approximately 0 between-male variance in courtship chirps performed (Table 4).

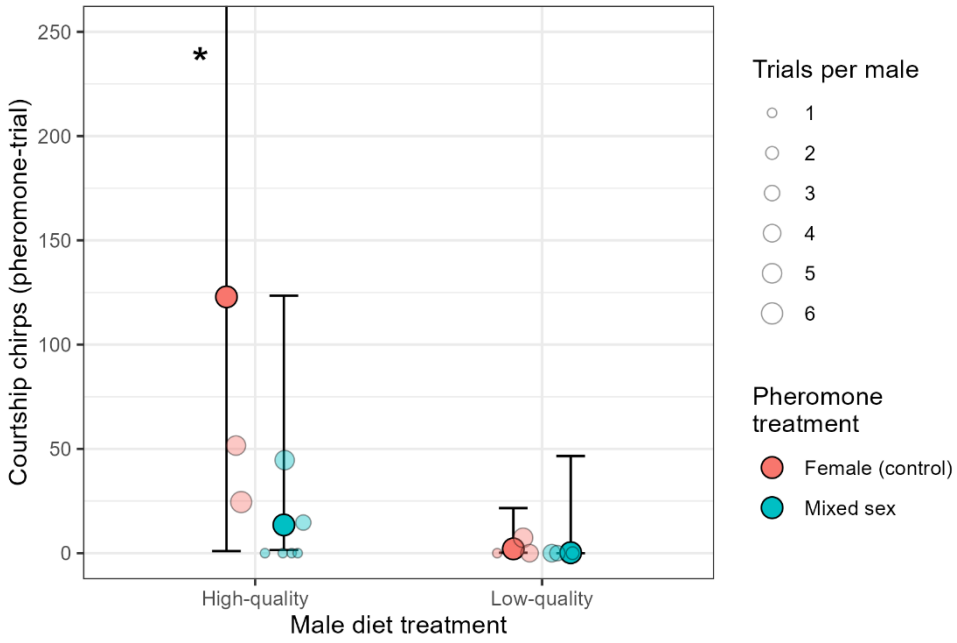

Figure 6. The effect of diet and female pheromone-treatment on the number of courtship chirps produced by male Jamaican field crickets when courting a dead female. Full-colored points represent models estimates of chirp count, with 95% confidence intervals. Semitransparent points represent observed chirp count for individual males, with the size of the point representing the number of trials performed on each male. Predictions are made at population level with no assigned male ID. *The upper confidence interval for high quality diet x control stimulus is 15377 chirps.

Table 4. Table of coefficients for the effect of diet and female pheromone-treatment on the number of courtship chirps produced by males courting a dead female (negative binomial GLMM).

Manip. pheromone courtship count (negative binomial) GLMM

Effect	Term	Estimate	Std. error	z-statistic	p-value
fixed	(Intercept)	4,811	2,464	1,953	0,051
fixed	diet_treatmentL	-4,082	2,61	-1,564	0,118
fixed	stimulusmixed	-2,206	2,595	-0,85	0,395
random	variance (male_ID)	0			

DISCUSSION

I tested how sex recognition cues and diet influence courtship of live and dead females in male Jamaican field crickets, *G. assimilis*. I found no evidence that diet affected body mass in males. As predicted, there was no evidence that male diet affected either likelihood or amount of courtship produced by males presented with a live, unmanipulated female. In the pheromone-manipulation trials, I found weak evidence that males on a poor diet were less likely to court regardless of the pheromone treatment of the stimulus female. I found no evidence that female pheromone treatment affected the likelihood of male courtship, nor that male diet and female pheromone treatment interacted to influence courtship probability. I also found no evidence that male diet, female pheromone treatment, or their interaction affected the number of courtship chirps produced by males in the pheromone-manipulation trials. Notably, while diet did not affect likelihood of a male courting a live female, only males that courted a live female in the preceding trial went on to court a dead female.

I found no direct support that the diet treatment succeeded in affecting body condition in the male crickets, as mortality did not differ significantly between diets and there are no obvious trends in weight change over time (Figure 1, Figure 2). The link between diet, body mass and mortality in post-eclosion Jamaican field crickets might not be simple; life-history strategies for resource allocation in male *Gryllodes sigillatus* for example have been found to have a complex effect on mass and mortality (Houslay et al., 2017). It could also be the case that the low sample size and lack of pre-diet weights hid the effect of diet on male mass. Previous studies of larger sample sizes, performing similar dietary manipulations have demonstrated an effect (Houslay et al., 2017; Richardson et al., 2024; Santori et al., 2020).

The experimental setup of the study drew inspiration from Richardson et al. (2024) to be able to compare my results to theirs. They found that males on a poor diet engaged in less SSB, meaning they found an interaction between diet and stimulus. If a similar effect can be found when manipulating pheromones, i.e. that males in poor condition are less likely to court when sex recognition cues are mixed, that would support both the mating filter-hypothesis and the assumption it relies on; that mistaken identity is the proximate cause for SSB. I did not find support for such an interaction, which provides some evidence against the mating filter hypothesis. However, the sample size was low, and the data were therefore sparsely distributed between the different treatment combinations which limits power, especially when trying to detect possible interactions (Table 1). We should therefore moderate the conclusions we draw from these results.

Some findings in this study provide tentative support for the mating filter-hypothesis and inaccurate sex recognition as a proximate cause for SSB. I did not find any evidence that mixing sex-pheromones affected either courtship probability or courtship effort. If SSB is preceded by accurate sex recognition, being presented with an individual with mixed sex-pheromones should render assessment the individual's sex difficult and result in reduced courtship. The absence of evidence for such an effect is in line with an inaccurate sex recognition interpretation of SSB. One could even argue that any courtship directed toward mixed-pheromone individuals in this study necessarily involved some degree of mistaken identity. Regardless of whether a male interpreted the stimulus as male or female before initiating courtship, that assessment would have been inaccurate since the pheromone profile contained pheromonal cues from both sexes. Additionally, I found a non-significant trend ($p = 0.098$, Table 3) that a low-quality diet decreased the likelihood of males courting a dead female, which was not anticipated. While not definitive, these findings could make sense within the mating filter hypothesis framework. The mating filter hypothesis suggests that SSB is no different from other misdirected sexual behaviours, such as those directed at juveniles or dead conspecifics (Richardson & Zuk, 2023). This could imply that the same mating-filter-narrowing effect that diet has on the male crickets is at play here, meaning males in poor condition

are both less likely to court other males and dead females. This effect does not, however, say anything about the proximate cause for SSB other than being in line with the mating filter hypothesis which presupposes inaccurate sex recognition.

The negative effect that a poor diet seemingly had on the probability of courting a dead female was not found on the amount of courtship chirps males produced. This could mean that diet only has an effect on the likelihood of courtship, and that when courtship is initiated the effort a male puts in is not affected by diet. However, Richardson et al. (2024) found that diet did have an effect on courtship count in *Teleogryllus oceanicus*, and looking at the observed chirp counts in my study does not immediately suggest that chirps are equally frequent between diet treatments (Figure 6). Count data are intrinsically more variable than binomial data, and the negative binomial models that were used require the estimation of an additional parameter which costs statistical power. It is likely that this, in combination with the small sample size and random effects, rendered the uncertainty in these models even higher than in the binomial courtship models, hiding any potential effects. I therefore find the estimates from the probability models to be more reliable than the courtship-count models.

An interesting pattern was that males only courted dead females if they had previously courted a live female. This was true for both stimulus types, i.e. control and mixed sex-pheromones. Analysing the data from Richardson et al. (2024) shows that only 1 out of the 58 males they presented with another male did not court a female during pre-trial female exposure, and this male did not court the male stimulus in the subsequent trial. I did 21 trials with mixed pheromone stimuli. In 7 of these trials the male did not court the living stimulus female, and in none of them did they court the mixed-pheromone female in the subsequent dead female trial. This pattern suggests that SSB is linked to normal mating motivation. This is consistent with a mistaken identity interpretation of SSB where same-sex courtship emerges as a byproduct of normal mating drive, but it would not be expected if SSB is induced by a separate drive, like tension reduction. Future research should investigate whether this pattern is consistent.

With limited sample size the power to estimate effects was also limited. This rendered model estimates uncertain which meant no strong conclusions could be drawn. However, if we ignore the non-independence of the trials performed repeatedly on the same males, the observed outcomes of the dead female trials suggests a trend worth investigating. Among males on a high-quality diet, the proportions courting control and mixed-pheromone females were essentially the same (27% and 25% respectively). In contrast, males on a low-quality diet showed lower courtship overall, particularly when exposed to mixed-pheromone females (10% for controls and 0% in the mixed pheromone group). Importantly, diet treatment had no detectable effect on male courtship of live females, suggesting that low-quality diet males were not too exhausted to court. If these patterns persist at larger sample sizes, they may suggest that poor condition lowers the probability of courting a dead female overall, and that mixed pheromone profiles reduce courtship primarily in males in poor condition. Such a pattern would be compatible with the mating filter hypothesis and with inaccurate sex recognition as a proximate explanation for SSB.

Conclusions and future studies

I investigated the proximate cause for SSB within a mating filter framework. The results provide both some evidence in line with the mating filter hypothesis and inaccurate sex recognition as a proximate cause for SSB, as well as evidence that does not provide further support for these ideas. Despite suffering from a low sample size, the study does provide insight into the inner workings of SSB, especially in the form of three compelling trends; that mixing sex-pheromones did not significantly lower males' likelihood to court dead females, that males in poor condition seemed to be less likely to court dead females, and that males only courted dead females if they had previously courted a live female.

The study also serves as a proof of concept, since male Jamaican field crickets were shown to court dead, CHC-manipulated individuals. Manipulation of pheromones therefore provides a promising new method for determining whether SSB arises following failed or successful sex recognition, since mixing pheromones renders correct interpretation of sex-pheromones difficult while still allowing for inaccurate interpretation. Possible next steps could include trials where males are exposed to a dead control male or a male with mixed sex-pheromones, perhaps in gradually increasing female-to-male CHC ratios. If SSB and regular courtship arises following successful sex recognition, one would then expect courtship to be lower in the mixed treatment than in the control. If, on the other hand, SSB occurs because of mistaken identity one would expect courtship to be higher in mixed than in control trials, because the mixed pheromone males simply resemble females more. Courtship would then also be expected to increase with increasing ratio of female pheromones. Performing pheromone mixing-experiments on species like *Teleogryllus oceanicus* or *Gryllus bimaculatus* where it is confirmed that pheromones are dimorphic in abundance rather than character would also be appropriate for future research (Thomas & Simmons, 2008; Tregenza & Wedell, 1997).

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