

ORIGINAL ARTICLE

House cricket males reared at different perceived acoustic population densities differ in adult behaviour but not physiology

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Abstract

1. If environments stay relatively constant over an individual's lifetime, a juvenile that accurately perceives environmental conditions, like population density, may adjust adult traits to better match their environment, thereby increasing success. While previous studies have explored how adult exposure to population density affects physiological and behavioural plasticity, the influence of juvenile density experience on adult traits is less studied.
2. Using the common house cricket, *Acheta domesticus*, we explored whether perceived acoustic population density during development affected adult physiology and behaviour. We simulated high- and low-densities using live ambient male song. Upon maturation, we measured metabolic (resting respiration) rate, reproductive investment (testes and accessory gland masses), calling song characteristics and aggressive behaviours from pairwise contests between males from different densities.
3. Male rearing density did not affect resting metabolic rates or reproductive organ masses. However, high-density males had significantly faster, longer chirps, with more pulses—known to be preferred by females—and higher dominant frequency. Low-density males won more aggressive contests and sang the aggressive song more. Initiation of aggressive behaviours or song and singing more aggressive song were the only other significant predictors of contest outcome.
4. These results suggest that males may plastically adjust calling song characteristics and aggressive behaviour, but not physiology, based on perceived density during development. We hypothesize that alternative mating tactics—that is, territory guarding versus attractive song production—may underlie these observed patterns.
5. Overall, our study highlights the significant influence of early-life biotic environments on adult behavioural decisions to enhance success in diverse environments.

KEYWORDS

aggressive behaviour, call characteristics, developmental plasticity, mating tactics, physiology, population density

INTRODUCTION

Reproductive behaviour, morphology and physiology can vary discontinuously among individuals of a species (i.e., alternative reproductive phenotypes; Brockmann, 2001; Gross, 1996; Oliveira et al., 2008; Shuster & Wade, 2003), which has long interested evolutionary biologists. This largely stems from the desire to explain the incredible diversity of such tactics found across animal species (Gross, 1996; Shuster, 2010). Alternative reproductive morphs can be influenced by environmental cues that are internal (e.g., body condition, size and age) or external—including abiotic (e.g., light, season and temperature) and biotic factors (e.g., social group, predation and population density; Brockmann, 2001; Gross, 1996). While the effects of internal environmental conditions (Brockmann, 2002; Forsyth & Montgomerie, 1987; Painting & Holwell, 2014) and abiotic external environmental conditions (Corlatti et al., 2020; Cusano et al., 2016; Denoël & Doellen, 2010) on tactics have been relatively well studied, biotic external environmental conditions have been much less studied (but see Formica & Tuttle, 2009; Ribeiro et al., 2010; Wilgers et al., 2014).

Population density—an external biotic environmental condition—has been shown to affect a variety of traits across taxa, such as reproductive success (Tveraa et al., 2013), early development (Albecker et al., 2020; Clutton-Brock et al., 1987), growth rate (Iba et al., 1995; Zewe & Booth, 2014), activity (Cade & Cade, 1992; de Rivera et al., 2003), acoustic signalling (Cade & Cade, 1992; Cade & Wyatt, 1984), female choosiness (Atwell & Wagner Jr., 2014; Holveck et al., 2015) and aggression (DiRienzo et al., 2012; Holveck et al., 2015; Knell, 2009). While many studies have found that adult environmental conditions can impact behaviours (Cade & Cade, 1992; Holveck et al., 2015), environmental conditions during development are known to have profound effects on adult behaviour in some systems (see Beach & Jaynes, 1954; Carlson, 2017).

Juvenile experience can influence behavioural and physiological responses upon emergence into adulthood due to delicate hormonal balances in the endocrine system associated with developmental moults (Dufty Jr. et al., 2002). Therefore, individuals may adaptively modify behaviour in response to environmental conditions experienced during development (Carlson, 2017). However, these behavioural changes are dependent on environmental conditions because a phenotype that may increase reproductive fitness in one environment could be maladaptive in another (Dufty Jr. et al., 2002).

Specifically, variation in population densities during development could entail physiological (e.g., metabolism or reproductive investment) or behavioural (e.g., aggression or mating behaviour) changes. Individuals with higher metabolic rates may be capable of performing longer or more frequent energy-demanding tasks, such as mate searching, organ development or predator/competitor interactions (Lorenz & Gäde, 2009). Both male weaponry and sperm production require energetic expenditure, leading to a trade-off between pre-copulatory and post-copulatory investments, respectively (Simmons & Emlen, 2006). When population densities are high, polyandrous species may experience heightened sperm competition (Buzatto et al., 2015), which may lead to increased investment in testes size

(Vahed et al., 2011). High population densities also increase the probability of interactions between conspecifics competing for mates and resources, which can escalate to aggressive contests, which may be energetically costly or increase the risk of injury (Knell, 2009).

Density may also affect investment in mate advertisement. For example, in species where males use song to attract females, longer call duration may be more advantageous in lower densities due to the possible infrequency of mate interactions, while shorter call duration helps reduce song overlapping at high densities (Eiriksson, 1992). However, call durations might increase at higher densities if females prefer longer calls (Wells & Taigen, 1986). While no study to our knowledge has investigated the role of population density in song quality variation (i.e., by changing characteristics), we might expect high-density males to invest more in producing an attractive song (a song preferred by females) to stand out from the crowd.

The effects of population density on these factors remain understudied or show mixed results for different species. Therefore, we chose to examine how perceived population density during juvenile development affects adult metabolism, reproductive investment, calling song characteristics and aggressive behaviours in individual males using a cricket model system. Measuring all the traits in the same individuals allowed us to look for relationships among traits within individuals; as this is difficult to do in many systems, we believe this is a strength of this experimental design.

Study system

We decided to test the effects of juvenile population density on the above adult traits using commercially bred *Acheta domesticus*, the common house cricket. In the field, cricket population densities vary due to variations in resource distributions and environmental conditions (Weidemann et al., 1990). Little is known about physiological changes in crickets that experience different population densities. One study using *Teleogryllus oceanicus* found that male crickets in higher population densities invest more in reproductive tissue due to heightened sperm competition (Bailey et al., 2010). No study to our knowledge has investigated population density effects on cricket metabolism.

Crickets produce a calling song that varies in duration and call characteristics between individuals. Previous studies show that adult *Gryllus* field cricket males called longer at low population densities (Cade & Cade, 1992; Cade & Wyatt, 1984; French & Cade, 1989; Hissmann, 1990), indicating that population density may influence behaviours when calling for a mate. While we know that call duration is negatively related to density, it is largely unknown how population density influences the calling characteristics of male crickets. Chirp intensity (loudness), chirp rate, chirp duration, the number of pulses per chirp, pulse duration, dominant frequency and pulse period have all been found to be important parameters for female preference in various cricket species (Stout et al., 1983; Stout & McGhee, 1988). We do know that females become less discriminatory of call characteristics of *T. oceanicus* when reared in isolation (Bailey & Zuk, 2008).

There is some conflict in the literature as to what role population density plays on aggression. Species differ in patterns of aggression associated with increasing population density: some increase in aggression and others decrease (Knell, 2009). Opposite to studies using non-cricket organisms (Erwin & Erwin, 1976; Moore, 1987; Parker & Nilon, 2008), field crickets are often most territorial at lower densities (Alexander, 1961). In particular, *Gryllus bimaculatus* (Simmons, 1986—density manipulated for adults; Iba et al., 1995—density manipulated for juveniles by changing the number of individuals in containers) and *Gryllus integer* (DiRienzo et al., 2012—perceived density manipulated for juveniles by the playing of recorded song) showed a reduction in both the number and intensity of aggressive contests at higher densities. Also, previous studies have shown mixed effects of population density on the number of aggressive song bouts (Adamo & Hoy, 1995; DiRienzo et al., 2012). No study, to our knowledge, has investigated the role of perceived population density via live ambient song on reproductive investment, metabolism, calling behaviour and aggression for a single species, nor with measuring these multiple traits in the same individuals.

A. domesticus males produce three distinct songs: the calling song to attract a female from afar, the courtship song performed once the female approaches the male and an aggressive song towards a competing conspecific male (Gray, 1997). Male *A. domesticus* will produce any of the three song types in laboratory settings, but the aggressive and courtship songs require contact (either physical or substrate chemical cues; Assis et al., 2016) to initiate. A range of aggressive behaviours performed by *A. domesticus* can be observed and easily identified (Hack, 1997a). Although it is unclear whether *A. domesticus* will defend burrows in the wild, they will defend burrows and use them as calling sites in the lab (BP personal observation; Hack, 1997b).

In this study, we investigated respiration rates, reproductive investment, calling song characteristics and male–male aggression for adult *A. domesticus* reared in perceived high or low population density. We simulated population density using live ambient cricket song, without direct contact or chemical cues with conspecifics—a novel method to the best of our knowledge. This method has several advantages: individuals in different conditions do not encounter differing competition for food and do not have differing prior experience of aggressive interactions. Lastly, using live song as opposed to recorded songs means that singing occurs at biologically relevant times (e.g., at night) and naturally varies in intensity.

Low population densities may lead to higher resting metabolic rates based on previous findings that low densities experience heightened aggression and longer calling durations. However, if males in high densities invest more in reproductive organs, producing attractive songs or searching for mates, they may require higher metabolic rates or suggest an energetic trade-off if no differences are found between densities. In addition, we expected that males perceiving high-density environments would have larger testes and accessory glands (that produce seminal fluid) due to increased sperm competition. Aggressively guarding a territory may be too costly for males in higher densities due to increased risk of injury and energy expenditure

associated with frequent male–male interactions. As a result, we predicted that males reared in a perceived higher density would invest more energy in mate attraction by producing a song with preferred characteristics and invest less in aggression to protect territory.

MATERIALS AND METHODS

General methods

For these experiments, we obtained commercially available common house crickets, *A. domesticus*, from Flukers Farms in June 2017. Until maturation, *A. domesticus* nymphs go through a series of eight instars and moults (Clifford et al., 1977). Previous studies show that nymphs are most susceptible to environmental influences on behaviour and physiology during the last two instars of their juvenile development (Friberg et al., 2011; Zera & Tiebel, 1989). Thus, we reared *A. domesticus* nymphs in the two (high or low) population density conditions from the start of their penultimate instar. We individually placed male crickets in their penultimate moult into small, clear plastic containers ($17 \times 10 \times 11$ cm). All containers had a paper towel substrate, cardboard egg crate shelter, ad libitum cat chow (Purina) and a water vial plugged with cotton. We placed all animals in Darwin Insect Rearing Chambers (IN055-AA-LT) maintained at 28°C, in a reversed light:dark cycle (LD 14:10 h). Nymphs went through multiple moults and several weeks under these reversed conditions. We monitored temperature and humidity in the chambers throughout the experiments using HOBO ProV2 U23-001 loggers, collecting measurements every 30 min.

We used live ambient male cricket song to simulate high- and low-density conditions. We placed nymphs in the high-density treatment in a rearing chamber with approximately 20–30 adult *A. domesticus* males, who call in the absence of female cues. These non-test males were housed in individual containers, as described above for the test crickets, and were placed on the shelf below our test animals (less than 30 cm between the test and non-test males). Adult *Gryllus campestris* males produce calls that are 70 dB SPL loud at a distance of 1 m (Bennet-Clark, 1989), and Staudacher (2009) found that the last instar nymphs of the closely related *G. bimaculatus* have a hearing threshold of 75–90 dB SPL at 5 kHz, suggesting that last instar crickets can hear calling males up to 1 m away. While it is unclear whether *A. domesticus* males produce and perceive similar amplitude calls, it seems likely that last instar nymphs could perceive male calls from 30 cm away. Therefore, our test juveniles could hear calling males during their development without physically interacting with them. We continually monitored calling in the environmental chamber over a 24-h period to note when and how much males sang. Dividing the 24 h into 30-min segments, we found that males sang throughout the dark period of the day (20 of 20 half-hour segments) and sang in 40% of the half-hour segments of the light period of the day (8 segments of 24). In addition, while it was not possible to tell from the recordings how many males were singing at any one time, more males were singing more loudly during the dark period (CM personal

observation). Using a Casella CEL-24 \times sound level meter, we measured the amplitude of male singing in the dark period in the range 60–70 dBA (impulse time weighting).

The lack of physical contact is important since prior physical interactions have been shown to affect aggressive behaviours (Iwasaki et al., 2006). We placed nymphs in the low-density treatment in a separate environmental chamber with no adult *A. domesticus* males. We use 'low-density' as compared to 'no density', as low-density conditions in the field would likely place conspecific males further than 1 m away, and outside of the likely hearing range of juvenile crickets. However, we have found little in the literature on field studies of *A. domesticus*, and so are basing this decision on field observations of other ground-dwelling crickets (*Gryllus* spp.—CM field observations; *Gryllodes supplicans*—Sakaluk, 1987). To investigate any potential confound of having separate incubators for the two acoustic treatment groups, we reared crickets in silence in the two incubators following these experiments and found no difference in adult calling song characteristics when measuring the same song traits in the same manner as described below ($N = 10, 16$; $p > 0.05$ for all measured characteristics; Table S5).

We monitored the individual containers daily and recorded the day when the crickets moulted into adults. Once high- and low-density crickets emerged as adults, we moved the adults from their associated chambers and placed them into a third chamber to ensure that nymphs in the low-density treatment remained isolated from male calls and nymphs in the high-density treatment did not experience an altered calling environment. Therefore, adults crickets from the two treatments were in the same calling environment where they could hear each other call. Chemical cues likely played no role as *A. domesticus* likely cannot sense airborne chemicals and require contact with a conspecific or chemical-laden substrate (Assis et al., 2016). As the calling male stimuli were less than 1 m from the developing nymphs during rearing (Bennet-Clark, 1989; Staudacher, 2009), the juveniles could likely perceive the auditory cues.

We used the same crickets for all analyses below to examine correlations between different traits within the same animal (e.g., resting metabolic rate and testes mass). The order of experiments a single male underwent was as follows—(1) respirometry, (2) song recordings, (3) aggression trials and (4) dissections. Due to reversing the cricket's circadian rhythms, we ran all tests and took all measurements during the dark cycle of the day (between 08:00 and 18:00) when crickets were most active. Before each type of trial, we measured male body mass to the nearest 0.1 mg using a Torbal Analytical Scale (AGZN120) and noted male age (defined as days since final moult).

Resting whole body respiration

To measure whole-body resting respiration rate, we used a closed-circuit respirometry system—Q-Box RP1LP Low range respiration package by Qubit. Before the start of the trials each day, we let the respirometer warm-up for 30 min and then recalibrated it using CO₂-free air, O₂-free N₂ gas and ambient outside air. Then, we further

scrubbed CO₂-free air (from a 30 L G122 gas bag) of CO₂ (using a column of soda lime) and water (using two columns of Drierite). We pumped this scrubbed air (Q-P103 gas pump, Q-G266 Flow Monitor 1 LPM) at a regulated flow rate of 50 mL/min into the 59.7 mL animal chamber, with an S132 temperature probe inserted in the chamber. We again scrubbed air flowing from the chamber of water (Drierite column), and we measured the O₂ and CO₂ levels using Q-S102 O₂ Analyzer 0%–100% enhanced, and a Q-S151 CO₂ Analyzer 0–2000 ppm. We used a C901 Logger Pro software, on a Windows desktop computer, for all analyses.

Before starting each trial, we allowed the chamber to stabilize at a CO₂ level of 0 ppm. We tested 93 animals: 46 high-density males and 47 low-density males between the ages of 4- and 5-day post-emergence. Males did not call during respirometry, and locomotion was minimal. Once the test individual was placed in the chamber, we once again waited for the CO₂ level to stabilize and then closed the respirometry system, so the air in the system was recycled repeatedly through the animal chamber. At this point, the measured CO₂ level started steadily increasing, and the O₂ level started steadily decreasing. When CO₂ reached 1000 ppm in the chamber (approximately 10 min), we stopped data collection. For analyses, we used a linear section of the CO₂ (increasing with time) and O₂ (decreasing with time) graphs to calculate the volume of carbon dioxide produced ($v\text{CO}_2$; $\mu\text{L}/\text{min}$) and the volume of oxygen consumed ($v\text{O}_2$; $\mu\text{L}/\text{min}$), using the equations specific to closed-flow systems in the Logger Pro Software and the volume of air in the closed system.

Reproductive investment: mass of testes and accessory glands

To examine the effect of population density during development on the masses of testes and accessory glands, we cold euthanized and dissected 73 males (36 high-density and 37 low-density) between the ages of 6- and 16-day post-maturation. After dissecting a male, we removed and weighed the testes and accessory glands to the nearest 0.1 mg.

Male calling song characteristics

To record male calling song, we used wooden anechoic chambers (inside dimensions—30 \times 55 \times 55 cm) lined with 5 cm wide acoustic wedge foam (Auralex Studiofoam). We recorded males individually, using a Sennheiser ME66 shotgun microphone attached to a K6 power module, which fit through the chamber lid. We placed the male cricket, housed in its container, into the anechoic chamber for recording. We amplified male calling song from the microphones using Pre-Sonus BlueTube V2 preamps and recorded using Cambridge Electronic Design Limited's Micro-1401-3 data acquisition unit and Spike2 (software for multi-channel continuous data acquisition and analysis) on a Windows desktop computer. Songs were digitized at 22 kHz and analysed using a custom script specific for cricket song

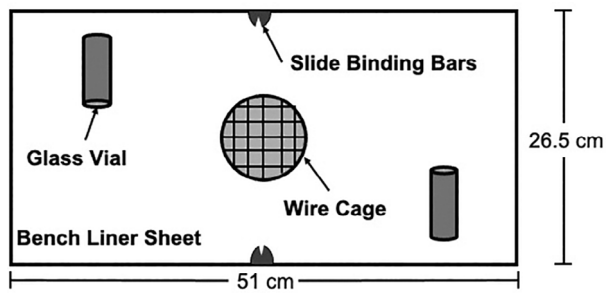


FIGURE 1 Experimental setup of the aggression trial. Graphic is the view from the top of a 32 cm tall clear glass aquarium. We slid a fitted opaque plastic sheet into the slide binding bars during the 10-min acclimation.

analysis (recording and analyses methods similar to Tolle & Wagner Jr., 2011). We visually examined the recorded period (ranging between 30 min if a male called right away, and 3 h) and selected a continuous bout of song that was at least 5 min long and analysed the chirp rate, average chirp duration, average number of pulses per chirp, average pulse duration and average dominant frequency for each male. If a male did not call, we tested them the following day. Males within the correct age range (4–11 days) were chosen randomly for recording, and recordings occurred from the start of the dark period (08:00) to the end of the dark period (18:00)—there was no significant difference in start time of recording for males in the two treatments ($F_{1,83} = 0.03$; $p = 0.866$).

We recorded males before and after aggression trials. As we found no differences in call characteristics between recordings (paired t-tests comparing before to after), we used five continuous minutes of calling song from either the first (if possible) or second recordings. We analysed the recordings of a total of 85 animals: 40 high-density males (36 recorded before aggression trials, 4 recorded after) and 45 low-density males (39 recorded before aggression trials, 6 recorded after), and all tested between ages 4- and 11-day post-emergence. Dropping the post-aggression males from the analyses yielded the same results as including them, and therefore, we only present the results for the combined dataset.

Male–Male aggressive contests

To investigate the effect of rearing density on male aggression, we staged 31 pairwise aggressive interactions using a low-density male and a high-density male for a total of 62 males tested, each tested only once. Males were between the ages of 5- and 12-day post-emergence. To conduct male–male aggression trials during the cricket's perceived night-time, we transferred the males to a room lit only by red lights—which crickets cannot perceive (Zufall et al., 1989)—to simulate night-time. We carried out trials in an aquarium (26.5 × 51 × 32 cm) bottom-lined with a white bench liner sheet to make the crickets more visible in video recording (Figure 1). We crafted two burrows from glass vials (15 mL, 21 × 70 mm) coated in duct tape to make them opaque and secured them to the bottom of

the aquarium. We fashioned a round metal cage from window wire and secured it to the bottom-middle of the aquarium. The metal cage housed a mature female in 12 of the 31 trials to test the effects of female presence on male aggression. We included female presence as a covariate in models of aggressive behaviour but it had no effects (Table S1). We glued slide binding bars to the sides of the aquarium, and we slid a plastic sheet—fitted to the width of the aquarium and made to fit over the wire cage—into them to create a divider. We set a Panasonic 4 K video camera (HC-WXF991) to a night scene infrared setting in 4 K mode to record all trials.

Immediately before the trial, we marked crickets (randomly) with either a white or black mark on the pronotum with a sharpie paint pen for identification during video analysis. We placed the crickets in the aquarium on either side of the divider for a 10-min acclimation. After this, we removed the divider and recorded interactions for 15 min following the first contact of the dyad. For video analysis, we used a behavioural-logging software—Behavioural Observation Research Interactive Software (BORIS; Friard & Gamba, 2016). All 15-min trials consisted of multiple contests (when a pair were in physical contact). We defined a contest as aggressive when there was a clear winner, which occurred when an opponent withdrew from the contest. We collected the number of aggressive contests that took place, noted the winner and respective contest lengths defined from the initiation of physical contact to the loser withdrawal.

The behaviours of one opponent depend on the behaviours of the other, especially after the first couple of seconds of a contest (Alexander, 1961; Iwasaki et al., 2006). As such, we recorded the rearing density of the male that completed the first unique aggressive behaviour [from the ethogram by Adamo and Hoy (1995)] in the first aggressive contest. In addition, we recorded the rearing density of the first male to sing the aggressive song in each dyad and the time spent singing the first bout (the end defined as at least a 5-s pause) of the aggressive song for both males. We also summed the total length of time individuals sang the aggressive song over the entire 15-min trial and divided it by the sum of the contest lengths for that particular dyad (hereafter, 'aggressive song length per total contest length') to correct for the proportion of time spent in the contest.

Statistical analysis

We conducted all statistical analyses using IBM SPSS Statistics 25. We visualized distributions using histograms and tested dependent variables for normal distributions using the Shapiro–Wilk normality test. We used R studio (R v. 4.2.2, R studio v. 2023.09.0 + 463) and the tidyverse package (Wickham et al., 2019) to produce the graphs. If the data were not normally distributed, we transformed the data by taking the log or the square root—depending on the skew of the data—to meet normality assumptions for parametric analyses. When normality assumptions were not met, we completed parametric and non-parametric analyses and reported the parametric statistics if both gave similar results. To determine the effects of perceived population density during development on continuous, normally distributed dependent variables traits, we performed backwards stepwise

TABLE 1 Variation in respiration rate and reproductive tissue investment for males reared in high- and low-density perceived during development.

	Mean \pm standard error		ANCOVA
	High density	Low density	
vCO ₂ (μ L/min) produced	3.38 \pm 0.18	3.49 \pm 0.16	$F_{1,90} = 0.79, p = 0.377$
vO ₂ (μ L/min) consumed	5.60 \pm 0.25	5.55 \pm 0.23	$F_{1,90} = 1.43, p = 0.235$
Body mass (mg)	311 \pm 8.29	314 \pm 7.81	$F_{1,71} = 0.06, p = 0.814$
Accessory gland mass (mg)	20.14 \pm 0.90	21.92 \pm 0.70	$F_{1,69} = 0.62, p = 0.435$
Testes mass (mg)	31.80 \pm 0.83	30.61 \pm 0.76	$F_{1,69} = 0.10, p = 0.753$

Note: For the volume of carbon dioxide (vCO₂) produced and volume of oxygen (vO₂) consumed data were log transformed before an ANCOVA was performed. Covariate statistics are in Table S1. The descriptive statistics are the calculated mean and standard error of the untransformed data.

ANCOVA analyses where we dropped non-significant covariates stepwise. For continuous, non-parametric dependent variables, we applied a Mann–Whitney *U*-test (in which case we were unable to account for covariates). Additionally, we used ANOVA tests to investigate differences in the covariates between rearing densities.

To test for differences between rearing population density, winner/loser and categorical aggression variables, we performed forward stepwise likelihood ratio logistic regressions. Additionally, we used ANOVA tests to see if body mass, age, respiration rates, testes mass, accessory gland mass, calling song characteristics and the aggressive song length (first bout length and total time per total contest time) predicted the overall winner. Finally, we used Pearson correlations to test for relationships between respirometry, reproductive investment, calling song and aggressive traits.

RESULTS

Resting whole body respiration

Perceived rearing population density had no effect on resting whole-body respiration—vCO₂ and vO₂ were not significantly different between males reared in high or low densities (Table 1). As body mass increased, so did vCO₂ produced and vO₂ consumed (Table S1). Age and ambient temperature were initially run as covariates but were dropped from the analysis as they did not affect vCO₂ or vO₂ (Table S1). There was no difference in either age or ambient temperature during respirometry between males in the two treatments (Table S2). Males in the low-density treatment, however, were slightly heavier than males in the high-density treatment (Table S2). We also measured the length of the hind leg, hind wing, and forewing and hind leg mass and found no differences between rearing environments (Table S2).

Reproductive investment: mass of testes and accessory glands

Perceived population density during development had no effect on reproductive investment. Body mass, accessory gland and testes mass

did not differ significantly between males reared in high or low densities (Table 1). Not surprisingly, both accessory gland mass and testes mass increased with body mass (Table S1). While age did not significantly affect the body mass of dissected males and was dropped as a covariate (Table S1), as the age of the animal increased, testes mass decreased and accessory gland mass increased (Table S1). We note that males in the low-density were dissected when they were on average about 1 day older than males in the high-density treatment (Table S2).

Male calling song characteristics

Rearing density had significant effects on several male calling song characteristics. We found that males reared in high-density environments performed calling songs with significantly higher chirp rate (square root transformed) than males reared in low-density environments ($F_{1,83} = 35.08, p < 0.001$; Figure 2a). Similarly, males reared in high-density environments had calling songs with significantly longer chirp duration than males reared in low-density environments ($F_{1,83} = 7.90, p = 0.006$; Figure 2b). In addition, high-density males had calling songs with significantly more pulses per chirp (log transformed) than males reared in low-density environments ($F_{1,83} = 11.54, p = 0.001$; Figure 2c). Age and ambient temperature were initially run as covariates but were dropped from the analysis as they did not affect chirp rate, chirp duration or the number of pulses per chirp (Table S1). Body mass was retained in the final model for chirp rate as it had marginal non-significant effects but was dropped from the models of chirp duration and the number of pulses per chirp (Table S1). Similarly, relative humidity had marginal non-significant effects on chirp duration but was dropped from models of chirp rate and the number of pulses per chirp (Table S1).

There was no effect of rearing density on the mean pulse duration of male calling songs ($F_{1,81} = 0.02, p = 0.892$; Figure 2d). Examining the effects of the covariates, mean pulse duration increased with increasing body mass and increasing ambient temperature but was not affected by age or relative humidity (Table S1). Further, we found that males reared in high-density environments had calling songs with significantly higher dominant frequency than males reared in low-

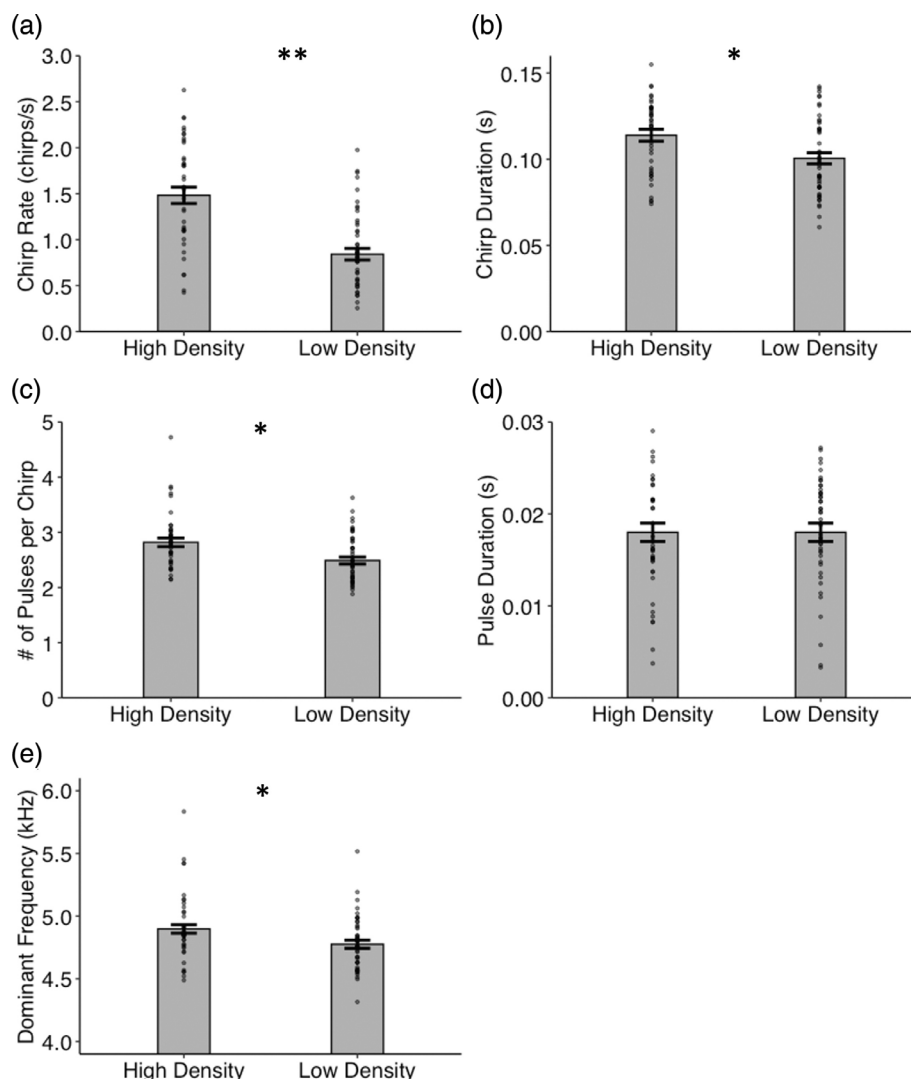


FIGURE 2 Variation in calling song characteristics of males reared in high versus low density. The number of pulses per chirp and dominant frequency was log transformed and chirp rate square root transformed prior to model testing. The bars and error bars represent the back-transformed model estimates of the mean and standard error, while the points show the raw data. Asterisks above bars denote statistically significant differences (* $p < 0.05$, ** $p < 0.001$). (a) Chirp rate (chirps/s); (b) chirp duration (s); (c) number of pulses per chirp; (d) mean pulse duration (s); (e) dominant frequency (kHz).

density environments ($F_{1,82} = 5.56$, $p = 0.021$; Figure 2e). As ambient temperature increased, so did the dominant frequency (Table S1). Age, body mass and relative humidity were initially run as covariates but were dropped from the analysis as they did not affect dominant frequency (Table S1). Lastly, there was no difference in age, body mass, ambient temperature or relative humidity during recordings among males in the two treatments (Table S2).

Male–Male aggression

There was a non-significant trend that more low-density males won the first aggressive contest than high-density males ($\chi^2_{1,62} = 3.16$, $p = 0.075$; Figure 3). In addition, significantly more low-density males won greater than 50% of the aggressive contests during the entire

15-min trial ($\chi^2_{1,62} = 5.23$, $p = 0.022$; Figure 3). The number of aggressive contests in a trial ranged from 2 to 21 (mean \pm SE: 8.68 ± 0.87 contests). Of the 31 aggression trials, there were only six trials where one of the males in the pair did not win 100% of the contests. In those six trials, the number of contests won by each male in the dyad was split as follows: 10-1, 10-1, 8-3, 6-3, 3-1, 2-1.

In the first contest, rearing density did not affect which individual completed the first unique aggressive behaviour ($\chi^2_{1,62} = 0.58$, $p = 0.446$) or sang the first aggressive song ($\chi^2_{1,62} = 1.64$, $p = 0.200$). Generally, we observed that the winner often began singing the aggressive song near the end of the contest or, in contests where aggression escalated, both started the aggressive song, but only the winner would continue singing at the end of the contest. Rearing density also did not affect the length of the first aggressive song bout ($F_{1,44} = 0.13$, $p = 0.720$; Figure 4a). However, low-density males sang

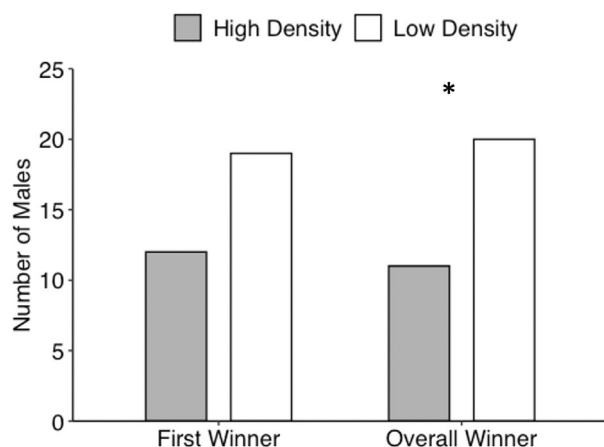


FIGURE 3 Number of males by rearing density that won the first aggressive contest (First Winner) and number of males that won greater than 50% of the aggressive contests within a 15-min trial (Overall Winner). Count data are shown. Asterisks above bars denote statistically significant differences (* $p < 0.05$, ** $p < 0.001$).

significantly more aggressive songs per total contest time over the entire 15-min trial ($F_{1,43} = 5.07$, $p = 0.029$; Figure 4c). Ambient temperature, relative humidity, female presence and marker colour at the time of the aggression trial were initially run as covariates, and all were dropped from the analysis of the first aggressive song bout and the total aggressive song per total contest time because they did not significantly affect the measured behaviours (Table S1). Body mass was retained in the final model for the first aggressive song length, and age was retained in the final model of the total aggressive song length per total contest time as they were marginally non-significant (Table S1).

Body mass at the time of the aggression trial did not significantly differ by rearing environment (Table S2). However, low-density males were slightly older (<1 day) at the time of the aggression trial than high-density males (Table S2).

To examine whether any factor other than rearing environment affected which males won their aggression trials, we looked at whether winners differed from losers in any other of our measured traits. While there was no difference in the first aggressive song length between males that won or lost ($F_{1,44} < 0.01$, $p = 0.998$; Figure 4b), males that won more aggressive encounters (overall winners) sang the aggressive song for more time per total contest time during the 15-min trial ($F_{1,44} = 79.71$, $p < 0.001$; Figure 4d). Overall winners were most likely to perform the first aggressive behaviour ($\chi^2_{1,62} = 10.90$, $p = 0.001$) and to first sing an aggressive song ($\chi^2_{1,62} = 18.96$, $p < 0.001$). However, no other relevant factor—body mass or age at the time of the aggression trial, vCO_2 , vO_2 , testes mass, accessory gland mass or any of the measured calling song characteristics—predicted the outcome of aggressive contests (Table 2).

Lastly, we tested for correlations between respiration rate, reproductive investment, calling song characteristics and aggressive behaviours (Tables S3, S4) and found that resting metabolism rate (vCO_2

produced) is positively correlated with testes mass ($r = 0.37$; a similar relationship was seen in vO_2 consumed). This finding is still robust after a Bonferroni correction for multiple comparisons.

DISCUSSION

To determine the physiological and behavioural responses that result from varying population densities, we exposed juvenile *A. domesticus* males to perceived high- or low-density conditions using live ambient male song. We tested for differences in resting respiration, male investment in reproductive tissues, male calling song characteristics and male-male aggression in adults. In terms of physiological responses, rearing density did not affect resting respiration or male reproductive investment. We did find behavioural differences in both song structure and aggression. Males reared in higher densities sang the calling song with higher chirp rates, longer chirp durations, more pulses per chirp and higher dominant frequencies. Low-density males, on the other hand, were more likely to win more than 50% of the aggressive contests in the trial and sang the aggressive song more per total contest time. Rearing density and aggressive behaviours—but not any other measured trait—were significant predictors of the outcome of aggressive contests. Lastly, higher volumes of CO_2 production were associated with heavier testes.

Past studies examining the effects of density on body mass have yielded mixed results. Some found that isolation results in larger males (Heifetz & Applebaum, 1995; Iba et al., 1995), while others found males reared in high densities are larger (McFarlane, 1962; Watler, 1982). Still others have shown no differences in body mass due to rearing density (Cade & Cade, 1992; French & Cade, 1989). Interestingly, we found that low-density males were significantly heavier than high-density males at the time of respirometry (average age = 4 days), yet there were no density-related differences in male body mass at the time of dissection (average age = 9 days). This could suggest that the high-density males reached the final moult more rapidly than the low-density males, as there may be advantages to early maturity in environments with more competition for mates or resources (Heifetz & Applebaum, 1995; Peters & Barbosa, 1977; Yang et al., 2015). Slower development time would allow low-density males more time for growth. Once our test animals underwent their final moult, we placed them together in the same adult acoustic environment. The loss of body mass difference at age 9 days may be caused by the animals from the two conditions differentially adjusting to their new adult density environment, as social environment has been shown to affect food consumption and weight gain (Gutiérrez et al., 2020; Watler, 1982). Additionally, low-density males were older (by ~1 day), on average, at the time of dissection, which might also explain the loss of body mass differences.

Many previous studies have found that higher sperm competition caused by higher population densities leads to larger investment in testes mass (Gage, 1995; Klemme et al., 2014; Stockley & Seal, 2001). However, we found no differences in either testes or accessory gland mass between our two density treatments by the time males were

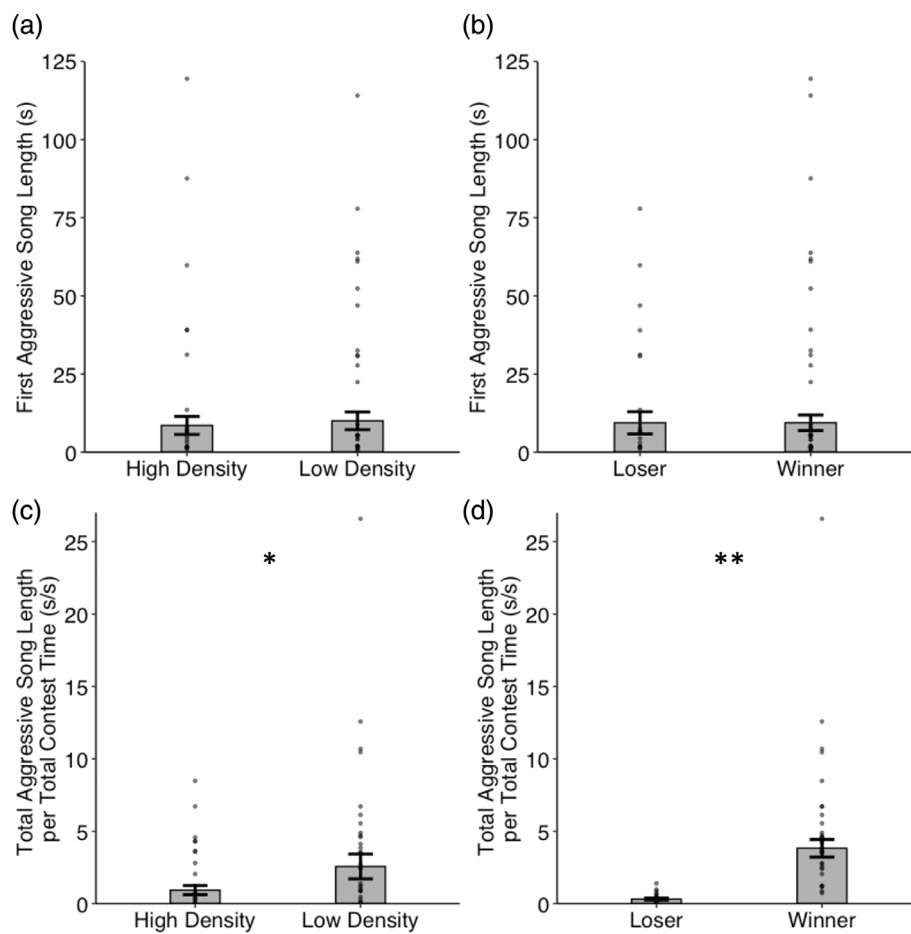


FIGURE 4 Variation in aggressive song length of males reared in high versus low density (a and c) and males that won versus lost (b and d). Data were log transformed prior to model testing. The bars and error bars represent the back-transformed model estimates of the mean and standard error, while the points show the raw data. Asterisks above bars denote statistically significant differences (* $p < 0.05$, ** $p < 0.001$). (a and b) First aggressive song length (s); (c and d) total aggressive song length per total contest time (s/s).

TABLE 2 Variation in respiration rate of, reproductive tissue investment by, and calling song characteristics of males that won or lost.

	Mean \pm standard error		ANOVA
	Winner	Loser	
Body mass (mg)	311.6 \pm 7.4	309.5 \pm 8.5	$F_{1,60} = 0.01, p = 0.925$
Age (days)	7.00 \pm 0.28	6.90 \pm 0.31	$F_{1,60} = 0.06, p = 0.816$
vCO ₂ (μ L/min) produced	3.34 \pm 0.17	3.41 \pm 0.17	$F_{1,60} = 0.02, p = 0.882$
vO ₂ (μ L/min) consumed	5.41 \pm 0.31	5.62 \pm 0.31	$F_{1,60} = 0.39, p = 0.534$
Testes mass (mg)	31.46 \pm 0.89	30.58 \pm 0.91	$F_{1,60} = 0.48, p = 0.489$
Accessory gland mass (mg)	21.20 \pm 0.86	20.95 \pm 0.99	$F_{1,61} = 0.03, p = 0.857$
Chirp rate (chirps/s)	1.17 \pm 0.10	1.33 \pm 0.12	$F_{1,59} = 0.92, p = 0.342$
Chirp duration (s)	0.106 \pm 0.004	0.103 \pm 0.006	$F_{1,59} = 0.01, p = 0.913$
Number of pulses per chirp	2.65 \pm 0.08	2.72 \pm 0.11	$F_{1,59} = 0.24, p = 0.628$
Pulse duration (s)	0.017 \pm 0.001	0.017 \pm 0.001	$F_{1,59} = 0.05, p = 0.824$
Dominant frequency (Hz)	4812 \pm 38.3	4741 \pm 166.1	$F_{1,59} = 1.759, p = 0.190$

Note: For the volume of carbon dioxide (vCO₂) produced, volume of oxygen (vO₂) consumed and number of pulses per chirp, data were log transformed before an ANOVA was performed. Similarly, chirp rate was square root transformed. The descriptive statistics are the calculated mean and standard error of the untransformed data.

dissected (average age = 9 days). Since we placed low-density and high-density males into the same incubator once they emerged as adults, increased perceived mate competition as adults may have driven males to increase energetic investment into reproductive organs as adults. Also, age was a significant covariate of reproductive tissue mass—older males had smaller testes and larger accessory glands. These results raise the question: how plastic is reproductive organ investment? In order to determine the degree of plasticity of reproductive organ and body mass, future studies should compare testes and accessory gland masses to body mass at different ages post-maturation or as population density conditions change. On the other hand, the sperm precedence pattern, to our knowledge, is unknown for *A. domesticus*, so it remains possible that sperm competition is generally not strong in this species.

We found no differences in resting metabolic rates between males reared in the two density environments. This suggests that the behaviours of males in different population densities may require approximately equivalent energy expenditures. For example, we know that acoustic signalling (Hoback & Wagner Jr., 1997) and aggressive behaviours (Hack, 1997a) are both energetically costly. Future experiments should investigate variation in maximum metabolic rates between males in different perceived population densities.

The most profound density effects were in the behavioural responses: calling song and aggression. We looked for differences in density-dependent calling characteristics, including chirp rate, chirp duration, pulses per chirp, pulse duration and dominant frequency. We found that high-density males had significantly faster chirp rates (HD— 1.52 ± 0.089 vs. LD— 0.90 ± 0.062 chirps per second), longer chirp durations (HD— 0.11 ± 0.003 vs. LD— 0.10 ± 0.003 second), more pulses per chirp (HD— 2.86 ± 0.082 vs. 2.53 ± 0.064) and higher dominant frequencies (HD— 4.91 ± 0.032 vs. 4.78 ± 0.043 kHz). We believe our study to be the first to find variation in cricket calling song characteristics due to perceived population density. Previous studies have found that females prefer males with faster chirp rates (1.5–2 chirps/second preferred over 0.5–1 chirps/second in *A. domesticus*; Stout et al., 1983; in *Gryllus lineaticeps*; Wagner Jr. & Reiser, 2000), longer chirp durations (in *Allonemobius socius*; Olvido & Wagner Jr., 2004; in *G. lineaticeps*; Wagner Jr. & Reiser, 2000) and more pulses per chirp (three pulses per chirp preferred over two pulses per chirp in *A. domesticus*; Gray, 1997; Stout et al., 1983). Conversely, females have been found to either prefer lower dominant frequencies (in *G. campestris*; Simmons & Ritchie, 1996) or to not have a preference within a normal range (positive phonotaxis between 3 and 5 kHz in *A. domesticus*; Stout et al., 1983). Therefore, as females in this species prefer more pulses per chirp (Gray, 1997; Stout et al., 1983) and higher chirp rates (Stout et al., 1983), our results suggest that males reared in high perceived densities may produce more attractive songs and therefore could be more likely to attract mates. Future studies should directly test female preferences for male song characteristics associated with different population densities. In addition, we did not measure call amplitude in this experiment; as that is known to be important in female choice in some species (Nandi &

Balakrishnan, 2013), whether call amplitude changes as a result of perceived rearing density is an open question.

Interestingly, pulse duration, which was not significantly affected by rearing density, increased with increasing body mass and ambient temperature. Typically, as temperature increases, pulse duration decreases as wings are capable of closing faster at higher temperatures (Martin et al., 2000). It is possible, however, that rising temperatures had a greater impact on the interpulse duration or how fast the wings opened. Though, we caution conclusions made from this result as ambient temperature varied less during this study (21–24°C) than in previous studies designed specifically to test the effect of temperature on pulse duration (18–33°C; Martin et al., 2000). The relationship between body mass and pulse duration has been less studied. In addition, the dominant frequency was higher when ambient temperature increased, a result that has been previously shown in multiple studies and is thought to be associated with faster chirp rates (Beckers, 2020; Martin et al., 2000).

After staging pairwise contests between low- and high-density males, we found that males reared in low-density conditions were more likely to win greater than 50% of the aggressive contests than high-density males. There was a trend that low-density males won the first aggressive contest over high-density males. This result has been commonly found in many cricket species, including *G. bimaculatus* (Iba et al., 1995) and *G. integer* (DiRienzo et al., 2012), which have both additionally found a reduction in both the amount and intensity of aggressive contests as densities increased. The first aggressive contest is likely the most biologically relevant because repeated interactions in the field occur across a longer time span than the 15-min trials in this study. However, a male would likely encounter neighbouring males multiple times, as males in many cricket species tend to sing from the same burrow, often through one night and sometimes across multiple nights (Alexander & Meral, 1967 in *Gryllus veletis* and *Gryllus pennsylvanicus*; Rodríguez-Muñoz et al., 2019 in *G. campestris*; Sakaluk, 1987 in *G. supplicans*; Walker & Whitesell, 1982 in *Anurogryllus muticus*; CM field observations of *Gryllus lineaticeps* and *Gryllodes sigillatus*). Therefore, we believe that examining repeated interactions in these species is important. Males experiencing low field population densities are less likely to repeatedly encounter neighbours, so heightened aggression may occur less frequently at low population densities in the field, making this a less costly tactic.

Also, low-density males sang the aggressive song more per total contest time. DiRienzo et al. (2012) found similar results, as they showed that males in a low-density treatment performed more aggressive song bouts. Aggressive songs are typically performed by the winner of an aggressive contest to decrease further aggression from the losing male (Phillips II & Konishi, 1973). Since low-density males were more likely to win repeated contests, singing the aggressive song more in total may serve to deter competitor males from re-initiating contests. In addition, initiating aggression through behaviour and song were significant predictors of winning aggressive contests, as was singing the aggressive song for longer total time per total contest time.

In examining correlations between our measured traits, we found that males with larger testes masses had higher vCO_2 production. Larger testes have been found to be correlated with higher sperm production from heightened sperm competition (Ramm et al., 2005), which is inevitably energetically costly. However, this may be an indirect effect of body size as both vCO_2 and testes size are positively correlated with body size. Interestingly, we found no other significant correlations between our measured traits. It is somewhat surprising that metabolic measurements did not significantly affect call structure or aggression. Hoback and Wagner Jr. (1997) found that oxygen consumption was positively correlated with chirp rate and pulse duration, and that chirp rate drove the energetic cost of calling. Our lack of difference between metabolic rate and calling characteristics may suggest that males with faster chirps are decreasing the amount of time spent calling, which is supported by numerous studies finding lower call durations in high densities (Cade & Cade, 1992; Cade & Wyatt, 1984; French & Cade, 1989; Hissmann, 1990), although experiments directly testing a trade-off between call duration and chirp rate are needed. Similarly, we might expect that more aggressive individuals have higher metabolic rates, but aggressive males may be less active or spend less time singing the calling song outside of our 15-min trial.

We present these novel and interesting findings with a caveat. The males used in these experiments were produced commercially, where there has likely been inadvertent selection for traits conducive to being maintained in high population densities. As such, future studies should include field observations and offspring reared from field-caught specimens.

The results of this study lead us to hypothesize that male *A. domesticus* may invest in more aggressively guarding a territory or in producing more attractive songs as alternative mating tactics depending on the perceived density that they were reared in. We have shown that juvenile *A. domesticus* can take in information about their external biotic environment via acoustic cues and change their behaviours in adulthood. These changes in behaviour may allow individuals to achieve higher fitness in either low-density or high-density environments. Since aggressive contests entail higher risks of injury and energy loss, the tactic may be more advantageous in low-density conditions when the probability of coming across a potential competitor is much lower than in a high-density environment. Conversely, producing an attractive song may help a male stand out to a female when there are many other potential suitors present. This last hypothesis needs further testing to see whether females show preference for high-density male calling song.

AUTHOR CONTRIBUTIONS

Brandi J. Pessman: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; software; validation; visualization; writing – original draft; writing – review and editing. **Rosaria J. Rae:** Conceptualization; investigation; writing – review and editing. **Veronica Wagner:** Conceptualization; investigation; writing – review and editing. **Chandreyee Mitra:** Conceptualization; data curation; formal analysis; funding acquisition; investigation;

methodology; project administration; resources; software; supervision; validation; visualization; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest to declare and no disputes over ownership of the data.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GitHub at https://github.com/brandipessman/Acheta_density.

ETHICS STATEMENT

All participants completed responsible conduct of research (RCR) training through the Collaborative Institutional Training Initiative [CITI] program through North Central College prior to conducting this research. We obtained *Acheta domesticus* crickets commercially from Fluker's Farms and separated the crickets into individual containers upon arrival. We tested a total of 93 male crickets during these lab experiments. We housed crickets individually in $17 \times 10 \times 11$ cm containers with a paper towel substrate, cardboard egg crate shelter, ad libitum food (Purina cat chow) and water. We maintained an ambient temperature of 28°C and a reversed light: dark cycle (LD 14:10 h). We monitored ambient temperature and relative humidity throughout the duration of the experiments to ensure they stayed within an acceptable range. We monitored aggression trials to ensure males were not injured, and no male was injured during aggressive interactions. We provided two burrows for duelling dyads to give competing males refuges from aggression. After we completed trials, each male was cold euthanized to reduce pain and dissected to determine testes and accessory gland masses.

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REFERENCES

Adamo, S.A. & Hoy, R.R. (1995) Agonistic behaviour in male and female field crickets, *Gryllus bimaculatus*, and how behavioural context

- influences its expression. *Animal Behaviour*, 49(6), 1491–1501. Available from: [https://doi.org/10.1016/0003-3472\(95\)90070-5](https://doi.org/10.1016/0003-3472(95)90070-5)
- Albecker, M.A., Pahl, M., Smith, M., Wilson, J.G. & McCoy, M.W. (2020) Influence of density and salinity on larval development of salt-adapted and salt naïve frog populations. *Ecology and Evolution*, 10(5), 2436–2445. Available from: <https://doi.org/10.1002/ece3.6069>
- Alexander, R.D. (1961) Aggressiveness, territoriality, and sexual behaviour in field crickets (orthoptera: Gryllidae). *Behaviour*, 17, 130–223. Available from: <https://doi.org/10.1163/156853961X00042>
- Alexander, R.D. & Meral, G.H. (1967) Seasonal and daily chirping cycles in the northern spring and fall field crickets, *Gryllus veletis* and *G. pennsylvanicus*. *The Ohio Journal of Science*, 67, 200–209.
- Assis, B.A., Trietsch, C. & Foellmer, M.W. (2016) Male mate choice based on chemical cues in the cricket *Acheta domesticus* (orthoptera: Gryllidae). *Ecological Entomology*, 42, 11–17. Available from: <https://doi.org/10.1111/een.12353>
- Atwell, A. & Wagner, W.E., Jr. (2014) Female mate choice plasticity is affected by the interaction between male density and female age in a field cricket. *Animal Behaviour*, 98, 177–183. Available from: <https://doi.org/10.1016/j.anbehav.2014.10.007>
- Bailey, N.W., Gray, B. & Zuk, M. (2010) Acoustic experience shapes alternative mating tactics and reproductive investment in male field crickets. *Current Biology*, 20(9), 845–849. Available from: <https://doi.org/10.1016/j.cub.2010.02.063>
- Bailey, N.W. & Zuk, M. (2008) Acoustic experience shapes female mate choice in field crickets. *Proceedings of the Royal Society B*, 275, 2645–2650. Available from: <https://doi.org/10.1098/rspb.2008.0859>
- Beach, F.A. & Jaynes, J. (1954) Effects of early experiences upon the behavior of animals. *Psychological Bulletin*, 51(3), 239–263. Available from: <https://doi.org/10.1037/h0061176>
- Beckers, O.M. (2020) Phenotypic plasticity related to temperature induces song variation in the field cricket *Gryllus rubens*. *Ethology*, 126(8), 781–790. Available from: <https://doi.org/10.1111/eth.13035>
- Bennet-Clark, H.C. (1989) Songs and the physics of song production. In: Huber, F., Moore, T.E. & Loher, W. (Eds.) *Cricket behavior and neurobiology*. Ithaca, New York: Comstock Publication Associates, pp. 227–261.
- Brockmann, H.J. (2001) The evolution of alternative strategies and tactics. *Advances in the Study of Behaviour*, 30, 1–51. Available from: [https://doi.org/10.1016/S0065-3454\(01\)80004-8](https://doi.org/10.1016/S0065-3454(01)80004-8)
- Brockmann, H.J. (2002) An experimental approach to altering mating tactics in male horseshoe crabs (*Limulus polyphemus*). *Behavioral Ecology*, 13(2), 232–238. Available from: <https://doi.org/10.1093/beheco/13.2.232>
- Buzatto, B.A., Roberts, J.D. & Simmons, L.W. (2015) Sperm competition and the evolution of pre-copulatory weapons: increasing male density promotes sperm competition and reduces selection on arm strength in a chorusing frog. *Evolution*, 69(10), 2613–2624. Available from: <https://doi.org/10.1111/evo.12766>
- Cade, W.H. & Cade, E.S. (1992) Male mating success, calling and searching behaviour at high and low densities in the field cricket, *Gryllus integer*. *Animal Behaviour*, 43, 49–56. Available from: [https://doi.org/10.1016/S0003-3472\(05\)80070-3](https://doi.org/10.1016/S0003-3472(05)80070-3)
- Cade, W.H. & Wyatt, D.R. (1984) Factors affecting calling behaviour in field crickets, *Teleogryllus* and *Gryllus* (age, weight, density, and parasites). *Behaviour*, 88, 61–75. Available from: <https://doi.org/10.1163/156853984X00489>
- Carlson, B.A. (2017) Early life experiences have complex and long-lasting effects on behavior. *Proceedings of the National Academy of Sciences of the United States of America*, 114(44), 11571–11573. Available from: <https://doi.org/10.1073/pnas.1716037114>
- Clifford, C.W., Roe, R.M. & Woodring, J.P. (1977) Rearing methods for obtaining house crickets, *Acheta domesticus*, of known age, sex, and instar. *Annals of the Entomological Society of America*, 70, 69–74. Available from: <https://doi.org/10.1093/aesa/70.1.69>
- Clutton-Brock, T.H., Major, M., Albon, S.D. & Guinness, F.E. (1987) Early development and population dynamics in red deer. I. Density-dependence effects on juvenile survival. *Journal of Animal Ecology*, 56, 53–67. <https://www.jstor.org/stable/4799>
- Corlatti, L., Bassano, B. & Lovari, S. (2020) Weather stochasticity and alternative reproductive tactics in northern chamois, *Rupicapra rupicapra*. *Biological Journal of the Linnean Society*, 130(2), 359–364. Available from: <https://doi.org/10.1093/biolinnean/blaa030>
- Cusano, D.A., Matthews, L.P., Grapstein, E. & Parks, S.E. (2016) Effects of increasing temperature on acoustic advertisement in the Tettigoniidae. *Journal of Orthoptera Research*, 25, 39–47. Available from: <https://doi.org/10.1665/034.025.0101>
- de Rivera, C.E., Backwell, P.R., Christy, J.H. & Vehrencamp, S.L. (2003) Density affects female and male mate searching in the fiddler crab, *Uca beebei*. *Behavioral Ecology and Sociobiology*, 53, 72–83. Available from: <https://doi.org/10.1007/s00265-002-0555-2>
- Denoël, M. & Doellen, J. (2010) Displaying in the dark: light-dependent alternative mating tactics in the alpine newt. *Behavioral Ecology and Sociobiology*, 64, 1171–1177. Available from: <https://doi.org/10.1007/s00265-010-0933-0>
- DiRienzo, N., Pruitt, J.N. & Hedrick, A.V. (2012) Juvenile exposure to acoustic sexual signals from conspecifics alters growth trajectory and an adult personality trait. *Animal Behaviour*, 84(4), 861–868. Available from: <https://doi.org/10.1016/j.anbehav.2012.07.007>
- Duffy, A.M., Jr., Clobert, J. & Moller, A.P. (2002) Hormones, developmental plasticity and adaptation. *Trends in Ecology and Evolution*, 17(4), 190–196. Available from: [https://doi.org/10.1016/S0169-5347\(02\)02498-9](https://doi.org/10.1016/S0169-5347(02)02498-9)
- Eiriksson, T. (1992) Density dependent song duration in the grasshopper *Omocestus viridulus*. *Behaviour*, 122, 121–132. Available from: <https://doi.org/10.1163/156853992X00345>
- Erwin, N. & Erwin, J. (1976) Social density and aggression in captive groups of pigtail monkeys (*Macaca nemestrina*). *Applied Animal Ethology*, 2(3), 265–269. Available from: [https://doi.org/10.1016/0304-3762\(76\)90059-6](https://doi.org/10.1016/0304-3762(76)90059-6)
- Formica, V.A. & Tuttle, E.M. (2009) Examining the social landscapes of alternative reproductive strategies. *Journal of Evolutionary Biology*, 22(12), 2395–2408. Available from: <https://doi.org/10.1111/j.1420-9101.2009.01855.x>
- Forsyth, A. & Montgomerie, R.D. (1987) Alternative reproductive tactics in the territorial damselfly *Calopteryx maculata*: sneaking by older males. *Behavioral Ecology and Sociobiology*, 21(2), 73–81. Available from: <https://doi.org/10.1007/BF02395434>
- French, B.W. & Cade, W.H. (1989) Sexual selection at varying population densities in male field crickets, *Gryllus veletis* and *G. pennsylvanicus*. *Journal of Insect Behavior*, 2, 105–121. Available from: <https://doi.org/10.1007/BF01053621>
- Friard, O. & Gamba, M. (2016) BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, 7(11), 1325–1330. Available from: <https://doi.org/10.1111/2041-210X.12584>
- Friberg, M., Haugen, I.M.A., Dahlerus, J., Gotthard, K. & Wiklund, C. (2011) Asymmetric life-history decision-making in butterfly larvae. *Oecologia*, 165, 301–310. Available from: <https://doi.org/10.1007/s00442-010-1804-0>
- Gage, M.J.G. (1995) Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proceedings of the Royal Society B*, 261, 25–30. Available from: <https://doi.org/10.1098/rspb.1995.0112>
- Gray, D.A. (1997) Female house crickets, *Acheta domesticus*, prefer the chirps of large males. *Animal Behaviour*, 54(6), 1553–1562. Available from: <https://doi.org/10.1006/anbe.1997.0584>
- Gross, M.R. (1996) Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology and Evolution*, 11(2), 92–98. Available from: [https://doi.org/10.1016/0169-5347\(96\)81050-0](https://doi.org/10.1016/0169-5347(96)81050-0)

- Gutiérrez, Y., Fresch, M., Ott, D., Brockmeyer, J. & Scherber, C. (2020) Diet composition and social environment determine food consumption, phenotype and fecundity in an omnivorous insect. *Royal Society Open Science*, 7(4), 200100. Available from: <https://doi.org/10.1098/rsos.200100>
- Hack, M.A. (1997a) The energetic costs of fighting in the house cricket, *Acheta domesticus*. *Behavioral Ecology*, 8, 28–36. Available from: <https://doi.org/10.1093/beheco/8.1.28>
- Hack, M.A. (1997b) Assessment strategies in the contests of male crickets, *Acheta domesticus* (L.). *Animal Behaviour*, 53, 733–747. Available from: <https://doi.org/10.1006/anbe.1996.0310>
- Heifetz, Y. & Applebaum, S.W. (1995) Density-dependent physiological phase in a non-migratory grasshopper *Aiolopus thalassinus*. *Entomologia Experimentalis et Applicata*, 77(3), 251–262. Available from: <https://doi.org/10.1111/j.1570-7458.1995.tb02322.x>
- Hissmann, K. (1990) Strategies of mate finding in the European field cricket (*Gryllus campestris*) at different population densities: a field study. *Ecological Entomology*, 15, 281–291. Available from: <https://doi.org/10.1111/j.1365-2311.1990.tb00810.x>
- Hoback, W.W. & Wagner, W.E., Jr. (1997) The energetic cost of calling in the variable field cricket, *Gryllus lineaticeps*. *Physiological Entomology*, 22, 286–290. Available from: <https://doi.org/10.1111/j.1365-3032.1997.tb01170.x>
- Holveck, M., Gauthier, A. & Nieberding, C.M. (2015) Dense, small and male-biased cages exacerbate male-male competition and reduce female choosiness in *Bicyclus anynana*. *Animal Behaviour*, 104, 229–245. Available from: <https://doi.org/10.1016/j.anbehav.2015.03.025>
- Iba, M., Nagao, T. & Urano, A. (1995) Effects of population density on growth, behaviour and levels of biogenic amines in the cricket, *Gryllus bimaculatus*. *Zoological Science*, 12(6), 695–702. Available from: <https://doi.org/10.2108/zsj.12.695>
- Iwasaki, M., Delago, A., Nishino, H. & Aonuma, H. (2006) Effects of previous experience on the agonistic behaviour of male crickets, *Gryllus bimaculatus*. *Zoological Science*, 23(10), 863–872. Available from: <https://doi.org/10.2108/zsj.23.863>
- Klemme, I., Soulsbury, C.D. & Henttonen, H. (2014) Contrasting effects of large density changes on relative testes size in fluctuating populations of sympatric vole species. *Proceedings of the Royal Society B*, 281, 20141291. Available from: <https://doi.org/10.1098/rspb.2014.1291>
- Knell, R.J. (2009) Population density and the evolution of male aggression. *Journal of Zoology*, 278(2), 83–90. Available from: <https://doi.org/10.1111/j.1469-7998.2009.00566.x>
- Lorenz, M.W. & Gäde, G. (2009) Hormonal regulation of energy metabolism in insects as a driving force for performance. *Integrative and Comparative Biology*, 49(4), 380–392. Available from: <https://doi.org/10.1093/icb/icp019>
- Martin, S.D., Gray, D.A. & Cade, W.H. (2000) Fine-scale temperature effects on cricket calling song. *Canadian Journal of Zoology*, 78(5), 706–712. Available from: <https://doi.org/10.1139/z99-262>
- McFarlane, J.E. (1962) A comparison of the growth of the house cricket (orthoptera: Gryllidae) reared singly and in groups. *Canadian Journal of Zoology*, 40(4), 559–560. Available from: <https://doi.org/10.1139/z62-048>
- Moore, A.J. (1987) The behavioral ecology of *Libellula luctuosa* (Burmeister) (Anisoptera: Libellulidae): I. Temporal changes in population density and the effects on male territorial behavior. *Ethology*, 75(3), 246–254. Available from: <https://doi.org/10.1111/j.1439-0310.1987.tb00657.x>
- Nandi, D. & Balakrishnan, R. (2013) Call intensity is a repeatable dominant acoustic feature determining male call attractiveness in a field cricket. *Animal Behaviour*, 85(5), 1003–1012. Available from: <https://doi.org/10.1016/j.anbehav.2013.09.003>
- Oliveira, R.F., Taborsky, M. & Brockmann, H.J. (2008) *Alternative reproductive tactics: an integrative approach*. Cambridge, UK: Cambridge University Press.
- Olvido, A.E. & Wagner, W.E., Jr. (2004) Signal components, acoustic preference functions and sexual selection in a cricket. *Biological Journal of the Linnean Society*, 83(4), 461–472. Available from: <https://doi.org/10.1111/j.1095-8312.2004.00404.x>
- Painting, C.J. & Holwell, G.I. (2014) Flexible alternative mating tactics by New Zealand giraffe weevils. *Behavioral Ecology*, 25(6), 1409–1416. Available from: <https://doi.org/10.1093/beheco/aru140>
- Parker, T.S. & Nilon, C.H. (2008) Gray squirrel density, habitat suitability, and behaviour in urban parks. *Urban Ecosystems*, 11, 243–255. Available from: <https://doi.org/10.1007/s11252-008-0060-0>
- Peters, T.M. & Barbosa, P. (1977) Influence of population density on size, fecundity, and developmental rate of insects in culture. *Annual Review of Entomology*, 22, 431–450. Available from: <https://doi.org/10.1146/annurev.en.22.010177.002243>
- Phillips, L.H., II & Konishi, M. (1973) Control of aggression by singing crickets. *Nature*, 241, 64–65. Available from: <https://doi.org/10.1038/241064a0>
- Ramm, S.A., Parker, G.A. & Stockley, P. (2005) Sperm competition and the evolution of male reproductive anatomy in rodents. *Proceedings of the Royal Society B*, 272, 949–955. Available from: <https://doi.org/10.1098/rspb.2004.3048>
- Ribeiro, P.D., Daleo, P. & Iribarne, O.O. (2010) Density affects mating mode and large male mating advantage in a fiddler crab. *Oecologia*, 164, 931–941. Available from: <https://doi.org/10.1007/s00442-010-1801-3>
- Rodríguez-Muñoz, R., Hopwood, P., Fisher, D., Skicko, I., Tucker, R., Woodcock, K. et al. (2019) Older males attract more females but get fewer matings in a wild field cricket. *Animal Behaviour*, 153, 1–14. Available from: <https://doi.org/10.1016/j.anbehav.2019.04.011>
- Sakaluk, S.K. (1987) Reproductive behaviour of the decorated cricket, *Grylodes supplicans* (orthoptera: Gryllidae): calling schedules, spatial distribution, and mating. *Behaviour*, 100(1/4), 202–225. <https://www.jstor.org/stable/4534582>
- Shuster, S.M. (2010) Alternative mating strategies. In: Fox, C. & Westneat, D.F. (Eds.) *Evolutionary behavioural ecology*. Cambridge, UK: Cambridge University Press, pp. 434–450.
- Shuster, S.M. & Wade, M.J. (2003) *Mating systems and strategies*. Princeton, NJ: Princeton University Press.
- Simmons, L.W. (1986) Inter-male competition and mating success in the field cricket, *Gryllus bimaculatus* (de Geer). *Animal Behaviour*, 34(2), 567–579. Available from: [https://doi.org/10.1016/S0003-3472\(86\)80126-9](https://doi.org/10.1016/S0003-3472(86)80126-9)
- Simmons, L.W. & Emlen, D.J. (2006) Evolutionary trade-off between weapons and testes. *Proceedings of the National Academy of Sciences of the United States of America*, 103(44), 16346–16351. Available from: <https://doi.org/10.1073/pnas.0603474103>
- Simmons, L.W. & Ritchie, M.G. (1996) Symmetry in the songs of crickets. *Proceedings of the Royal Society B*, 263, 305–311. Available from: <https://doi.org/10.1098/rspb.1996.0191>
- Staudacher, E.M. (2009) The auditory system of last instars in *Gryllus bimaculatus* DeGeer. *Physiological Entomology*, 34, 18–29. Available from: <https://doi.org/10.1111/j.1365-3032.2008.00647.x>
- Stockley, P. & Seal, N.J. (2001) Plasticity in reproductive effort of male dung flies (*Scatophaga stercoraria*) as a response to larval density. *Functional Ecology*, 15, 96–102. Available from: <https://doi.org/10.1046/j.1365-2435.2001.00496.x>
- Stout, J.F., DeHann, C.H. & McGhee, R.W. (1983) Attractiveness of the male *Acheta domesticus* calling song to females. *Journal of Comparative Physiology*, 153, 509–521. Available from: <https://doi.org/10.1007/BF00612605>

- Stout, J.F. & McGhee, R. (1988) Attractiveness of the male *Acheta domestica* calling song to females. *Journal of Comparative Physiology A*, 164, 277–287. Available from: <https://doi.org/10.1007/BF00603958>
- Tolle, A.E. & Wagner, W.E., Jr. (2011) Costly signals in a field cricket can indicate high- or low-quality direct benefits depending upon the environment. *Evolution*, 65(1), 283–294. Available from: <https://doi.org/10.1111/j.1558-5646.2010.01123.x>
- Tveraa, T., Stien, A., Bårdsen, B.J. & Fauchald, P. (2013) Population densities, vegetation green-up, and plant productivity: impacts on reproductive success and juvenile body mass in reindeer. *PLoS One*, 8(2), e56450. Available from: <https://doi.org/10.1371/journal.pone.0056450>
- Vahed, K., Parker, D.J. & Gilbert, J.D.J. (2011) Larger testes are associated with a higher level of polyandry, but a smaller ejaculate volume, across bushcricket species (Tettigoniidae). *Biology Letters*, 7, 261–264. Available from: <https://doi.org/10.1098/rsbl.2010.0840>
- Wagner, W.E., Jr. & Reiser, M.G. (2000) The importance of calling song and courtship in female mate choice in the variable field cricket. *Animal Behaviour*, 59(6), 1219–1226. Available from: <https://doi.org/10.1006/anbe.1999.1428>
- Walker, T.J. & Whitesell, J.J. (1982) Singing schedules and sites for a tropical burrowing cricket (*Anurogryllus muticus*). *Biotropica*, 14(3), 220–227. Available from: <https://doi.org/10.2307/2388028>
- Watler, D. (1982) Influence of social situation on food consumption and growth in nymphs of the house cricket, *Acheta domestica*. *Physiological Entomology*, 7(3), 343–350. Available from: <https://doi.org/10.1111/j.1365-3032.1982.tb00307.x>
- Weidemann, S., Stiedl, O. & Kalmring, K. (1990) Distribution and population density of the bushcricket *Decticus verrucivorus* in a damp-meadow biotope. *Oecologia*, 82, 369–373. Available from: <https://doi.org/10.1007/BF00317485>
- Wells, K.D. & Taigen, T.L. (1986) The effect of social interactions on calling energetics in the gray treefrog (*Hyla versicolor*). *Behavioral Ecology and Sociobiology*, 19, 9–18. Available from: <https://doi.org/10.1007/BF00303837>
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R. et al. (2019) Welcome to the {tidyverse}. *Journal of Open Source Software*, 4(43), 1686. Available from: <https://doi.org/10.21105/joss.01686>
- Wilgers, D.J., Wickwire, D. & Hebets, E.A. (2014) Detection of predator cues alters mating tactics in male wolf spiders. *Behaviour*, 151(5), 573–590. Available from: <https://doi.org/10.1163/1568539X-00003149>
- Yang, F., Hu, G., Shi, J.J. & Zhai, B.P. (2015) Effects of larval density and food stress on life-history traits of *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). *Journal of Applied Entomology*, 139(5), 370–380. Available from: <https://doi.org/10.1111/jen.12179>
- Zera, A.J. & Tiebel, K.C. (1989) Difference in juvenile hormone esterase activity between presumptive macropterous and brachypterous *Gryllus rubens*: implications for the hormonal control of wing polymorphism. *Journal of Insect Physiology*, 35, 7–17. Available from: [https://doi.org/10.1016/0022-1910\(89\)90031-0](https://doi.org/10.1016/0022-1910(89)90031-0)
- Zewe, F.L. & Booth, D.T. (2014) A preliminary study on the effect of isolation on frog larval growth and metamorphosis. *Australian Zoologist*, 37(2), 173–177. Available from: <https://doi.org/10.7882/AZ.2014.003>
- Zufall, F., Schmitt, M. & Menzel, R. (1989) Spectral and polarized light sensitivity of photoreceptors in the compound eye of the cricket (*Gryllus bimaculatus*). *Journal of Comparative Physiology A*, 164, 597–608. Available from: <https://doi.org/10.1007/BF00614502>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting information.

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