**Linking Mating Preferences to Sexually Selected Traits and Offspring Viability:
Good- vs Complementary-Genes Hypotheses**

Running Title: Good-Genes Versus Complementary-Genes

Susan M. Bertram^{1,4}, Michelle J. Loranger¹, Ian R. Thomson¹,
Sarah J. Harrison¹, Genevieve L. Ferguson¹, Mykell L. Reifer¹,
Deborah H. Corlett¹, and Patricia Adair Gowaty^{2,3}

¹Department of Biology, Carleton University, Ottawa, Ontario, Canada K1S 5B6

²Department of Ecology and Evolutionary Biology and Institute of Environment and
Sustainability, University of California Los Angeles
Los Angeles, California, United States 90095

³Smithsonian Tropical Research Institute, APO, USA

⁴ Corresponding Author: Susan M. Bertram
Department of Biology
Carleton University
1125 Colonel By Drive
Ottawa, Ontario
Canada K1S 5B6
Email: Sue_Bertram@carleton.ca
Phone: 613-520-2600 x1585

KEY WORDS

Good genes hypothesis, Indirect fitness benefits, Compatible genes, Viability, Productivity, Fitness, Female mating preferences, Immune-gene-complementarity hypothesis

ABSTRACT

Indirect-fitness benefits hypotheses suggest that offspring of preferred mates should exhibit greater survival or reproductive success. For example, good-genes hypotheses propose that female mating preferences are mediated by secondary sexual traits because they honestly reflect the ability to pass on genes that will enhance offspring survival or reproduction. Conversely, complementary-genes hypotheses propose that mating preferences are mediated by complementary-genes because they enhance offspring viability. While these two research traditions are not strict alternatives and both may operate simultaneously, they have never been tested together. Here we explore the multiple potential underlying factors influencing mating preference evolution in Jamaican field crickets, *Gryllus assimilis*. After evaluating female preferences for randomly selected males, we tested if preferred males differed from non-preferred males in their body size, relative mass, or mate attraction signals. We then mated females to their preferred or non-preferred partners and tested offspring viability. Results revealed: (1) females preferred larger males, (2) larger females oviposited more eggs, (3) neither morphology nor mate attraction signaling explained variation in offspring viability, and (4) mating with a preferred partner did not enhance offspring viability. Overall, in our current study population, cricket mate preferences were inconsistent with complementary-genes and good-genes hypotheses for indirect-fitness-benefits. Our current research explores whether male secondary sexual traits honestly reflect the ability to pass on genes that enhance offspring reproduction.

INTRODUCTION

Search for a mate can be costly as it exposes individuals to predators and takes time away from other tasks (Hubbell and Johnson 1987; Pomiankowski 1988; Hedrick and Dill 1993; Andersson 1994; Johnstone 1997; Houle and Kondrashov 2002; Hutchinson and Halupka 2004; Wiegmann et al. 2013). Why then have mate preferences evolved? Direct-fitness benefits, such as females receiving greater material contributions that enhance their fertility or fecundity, provide easy explanations for the evolution of mate preferences (reviewed by Andersson 1994; Hoelzer 1989; Hamilton 1990; Wagner 2011). Mate preference evolution may also be explained by indirect-fitness benefits such as enhanced offspring viability, parasite resistance of offspring, or the attractiveness of sons in the subsequent generation (Fisher 1930; Zahavi 1975; Heywood 1989; Andersson 1994). For example, Fisher (1930) and later Zahavi (1975) posited that secondary sexual traits reflect their bearers' health and viability (good-genes). When both secondary sexual traits and female preferences are heritable, females that mate with more elaborate males (males with brighter, louder, or more complex secondary sexual traits) will produce offspring that may inherit their father's good-genes for health and viability, and their mothers' good-genes for preference, resulting in greater survival and/or reproductive success. A correlation may result between preference and viability [demonstrated by Lande (1981)]. The difficulty with these hypotheses is that continuous directional selection for elaborate traits may remove heritable variation, resulting in the elimination of the indirect benefits associated with mate preference (e.g. Falconer and Mackay 1996; Borgia 1979; Andersson 1994; but see Heywood 1989; Pomiankowski 1988).

The main rationale for Hamilton and Zuk's (1982) insightful paper on mate choice was to

provide a way around the conundrum of erosion of variation. Hamilton and Zuk (1982) reasoned that by associating with males with more elaborate traits, females would avoid exposure to pathogens and parasites and, assuming that appropriate heritable variation exists, their offspring would exhibit greater health and viability. Because pathogens and parasites have much shorter generation times, they evolve rapidly relative to their hosts. Given the evolutionary red queen dynamics between hosts and pathogens/parasites, the cycles of co-adaptation should ensure a continual source of genetic variation in elaborate traits, provided that elaborate traits are associated with genes for disease resistance.

There is substantial support for Hamilton and Zuk's (1982) hypothesis as males with more elaborate traits are healthier and carry fewer pathogens and parasites than males with less elaborate traits (Zuk 1992; Hamilton and Poulin 1997; Buchanan et al. 1999; Figuerola et al. 1999; Merila et al. 1999; McGraw and Hill 2000, Balenger and Zuk 2014), and females often avoid exposure to pathogens and parasites when they associate with males with more elaborate traits (because these males are healthier), thus decreasing the risks to their own health and survival (Borgia and Collis 1989; Pruett-Jones et al. 1990; Zuk et al. 1990; Petrie et al. 1991; Zuk 1992; Sheldon and Verhulst 1996; McGraw and Hill 2000; Roberts et al. 2004; Lawniczak et al. 2007; Balenger and Zuk 2014). However, few studies show consistency with Hamilton and Zuk's prediction that more elaborate sexual traits predict offspring health and viability (reviewed in Clayton 1991; Siva-Jothy and Skarstein 1998; Barber et al. 2001). Given weak support for Hamilton and Zuk's offspring viability prediction, more consideration might be given to other indirect-fitness benefits hypotheses.

Several indirect-fitness benefits hypotheses do not require mating preferences to be proximately dependent on elaborate male sexual traits. Instead, these "complementary-genes"

hypotheses assume that mating preferences are mediated by heterozygosity or complementary (i.e. dissimilar) alleles (Downhower et al. 1983; Wedekind et al. 1995; Brown 1997; Wedekind and Furi 1997; Penn and Potts 1999; Wedekind 1999; Bluhm and Gowaty 2004a,b; Gowaty et al. 2007; Gowaty 2008, 2010). Individuals should benefit from mating with genetically dissimilar partners because their offspring should have a greater number of heterozygous loci and a reduced probability of expressing deleterious recessive alleles (Tregenza and Wedell 2000). For example, the immune-gene-complementarity hypothesis stems from the fact that offspring are often more viable, vigorous, etc. when their parent's immunity genes are complementary (Bluhm and Gowaty 2004a,b; Gowaty et al. 2007; Gowaty 2008, 2010). The immune-gene-complementarity hypothesis echoes the long-standing logic of "heterozygote vigor", where alleles at immunity-related loci act as a defense against pathogens and parasites, and individuals with the highest likelihood of survival often carry the greatest heterozygosity (Zeh and Zeh 1997; Penn and Potts 1999; Tregenza and Wedell 2000; Colegrave et al. 2002; Neff and Pitcher 2005; Kempenaers 2007). Given that the immune-gene-complementarity hypothesis assumes that variation in immune coding alleles mediate individual mating preferences, it predicts that individuals selecting mates (discriminators can be female or male) "predict" which of their potential mates should enable them to produce more viable offspring (Gowaty et al. 2007). Therefore, complementary-genes hypotheses do not assume that all discriminators will prefer a single best phenotype in a mate. Instead, these hypotheses predict mating preferences will be self-referential, as the best mate for one discriminator is unlikely to be the best mate for other discriminators (Downhower et al. 1983; Bluhm and Gowaty 2004 a,b; Gowaty et al. 2007). Complementary-genes hypotheses thus provide a way for genetic variation to be maintained in the population even in the face of strong mating preferences.

Past studies designed to test the immune-gene-complementarity hypothesis tested preferences of male and female discriminators, then randomly paired half the discriminators with their behaviorally preferred partners and the other half with their non-preferred partners, and then quantified offspring viability (reviewed in Gowaty 2008). The offspring of discriminators mated with their preferred partners had significantly higher viability than the offspring of discriminators mated with their non-preferred partners in ducks, flies, mice, cockroaches, and fish (Bluhm and Gowaty 2004 a,b; Anderson et al. 2007; Drickamer et al. 2000, 2003; Gowaty et al. 2003; Moore et al. 2003; Sandvik et al. 2000; Gowaty et al. 2007). These studies support the crucial indirect-fitness benefits prediction that offspring of individuals experimentally mated with their preferred partners were healthier, and survived longer than the offspring of individuals experimentally mated with their non-preferred partners.

In all aforementioned experiments performed to date to test the immune-gene-complementarity hypothesis, the investigators were blinded to variation in elaborate sexual traits or morphology of subjects, as they intentionally picked individuals (discriminators and discriminatees) for inclusion in each experiment at random with respect to their phenotypic characteristics. Further, the investigators did not conduct pre- or post-experimental evaluations of elaborate trait variation among potential mates (but they argued that such tests should be the next step). The investigators utilized this approach because they were motivated by a desire to ascertain whether individuals constrained to breed with their non-preferred partners produced offspring with lower viability, not by a desire to explain whether morphological or elaborate trait variation influenced offspring survival or reproduction. The investigators were therefore unable to determine whether the result of higher offspring viability might stem from females associating with males with more elaborate traits and/or from females associating with males with more

complementary-genes (but they argued that combining these ideas should be the next step).

Here we use field crickets to simultaneously test the two indirect-fitness benefits hypotheses that have been introduced to explain the evolution of female mating preferences. The “good-genes” hypothesis predicts that (a) males with more elaborate traits (e.g., biggest males and/or the loudest, brightest, most complex, or highest effort signalers) are healthier and carry fewer pathogens and parasites than males with less elaborate traits; (b) that females prefer to mate with males that have the best health-predictive elaborate traits; and (c) female preference positively affects offspring fitness. Conversely, the “complementary-genes” hypothesis predicts that (a) mate preferences are self-referential and therefore not necessarily correlated with traditionally measured phenotypic traits (e.g., size and/or elaborate traits), and (b) when females mate with their preferred partners their broods have higher egg to adult survival (viability). These two hypotheses primarily differ in whether elaborate male traits predict indirect fitness benefits: with good-genes, they do; with complementary-genes they do not.

Field crickets are appropriate organisms to test these two hypotheses because (1) females use male acoustic mate attraction signals to distinguish between potential mates and (2) females assess relatedness between potential mates. Field cricket males raise their forewings and rub them together, with each closing stroke producing a pulse of sound. Males concatenate these pulses into chirps or trills and they exhibit extensive and repeatable inter-individual variation in their acoustic signaling behavior (Alexander 1961; Bertram et al. 2014). Females often use male morphology and acoustic signals to distinguish between potential mates, preferring to mate with larger males (*G. bimaculatus*: Simmons 1986; Bateman et al. 2001; Saleh et al. 2014; *G. assimilis*: Loranger and Bertram 2016), males that signal louder or with higher effort (Cade 1979; *G. texensis*: Cade and Cade 1992; *G. firmus*: Crnokrak and Roff 1995; *G. campestris*:

Holzer et al. 2003; *Teleogryllus commodus*: Hunt et al. 2004; *G. pennsylvanicus*: Judge et al. 2008, 2014; *G. assimilis*: Pacheco and Bertram 2014), at higher chirp rates (*G. lineaticeps*: Wagner 1996, Wagner and Reiser 2000), with long chirp durations (*G. lineaticeps*: Wagner 1996), and intermediate to higher pulses per chirp/trill (more: *G. bimaculatus*: Popov and Shuvalov 1997; *G. texensis*: Wagner et al. 1995; average: *G. campestris*: Popov and Shuvalov 1997). Females also appear to be able to assess relatedness between potential mates, as they bias sperm storage and paternity towards unrelated males (Tuni et al. 2013; Tregenza and Weddell 2002; Bretman et al. 2004; Simmons et al. 2006; Tuni et al. 2013).

Given both good- and complementary-genes hypotheses provide plausible explanations for the evolution of female mating preferences, we used Jamaican field crickets (*Gryllus assimilis*) to test the good-genes predictions that (1) preferred males are larger, relatively heavier, and/or use more elaborate sexual signals, and (2) preferred males possessing these traits produce offspring with higher viability resulting in more adult offspring. Additionally, we also tested the complementary-genes prediction that (3) offspring viability (egg hatching success and juvenile to adult molting success) is significantly higher when females breed with their preferred partners compared to when they breed with the partners that they do not prefer. While it is possible for both predictions 2 and 3 to be supported, good-genes validation requires support for predictions 1 and 2, while complementary-genes validation requires support for only prediction 3. The design of our experiments allows us to begin to explore the multiple potential hypotheses put forth to explain the evolution of female mating preferences.

METHODS

Establishing and Rearing the Lab Population

We conducted our study in accordance with the guidelines of the Canadian Council on Animal Care. We used 17th generation laboratory-reared *G. assimilis* field crickets descended from wild crickets captured in Smithville, Texas, from 15 to 24 September, 2008. We captured several hundred wild crickets and allowed them to mate freely for several days, and then imported the eggs and adults to establish our laboratory population (Canadian Food Inspection Agency permit # 2007-03130). Given *G. assimilis* had not been observed in this region before, we used morphological keys and acoustic song to identify it to species and then sent specimens to D. B. Weissman, a taxonomic authority on *Gryllus*, who confirmed our *G. assimilis* identification (detailed in Montroy et al. 2016).

We reared *G. assimilis* from egg to final juvenile instar in communal plastic containers (L x W x H = 64 x 40 x 42 cm) and maintained an illumination regime of 14:10 h light:dark at a temperature of $\bar{X} \pm SD = 28 \pm 2^{\circ}\text{C}$ in our greenhouse at Carleton University, Ottawa, Canada. Crickets had ample shelter in the form of several stacked egg cartons and *ad libitum* water and standard food (Harlan's Teklad Rodent diet 8604M; 24.3% protein, 40.2% carbohydrate, 4.7% lipid, 16.4% fiber, 7.4% ash).

We checked communal bins daily for any individuals that had undergone final (imaginal) molt (day of eclosion = day 1 of adulthood or post-imaginal molt). We housed adult crickets individually in 520 mL clear plastic containers with lids that contained a 4 x 4 cm opening covered with wire mesh to allow airflow. Individually housed crickets experienced the same "habitat" conditions as those in communal containers, except that individually housed subjects had a crumpled unbleached paper towel for shelter.

Experiment

To examine the multiple potential factors underlying mating preference evolution we quantified male acoustic signaling behavior from 7-13 days post-imaginal molt (detailed in *Acoustic Signals* section). At 14 days post-imaginal molt we allowed females choose between two randomly selected males (detailed in *Preference Trials* section). We then placed females to breed with either their preferred or non-preferred partners (detailed in *Mating and Egg Ovipositing* section). We quantified variation in number of eggs oviposited (fecundity), variation in egg hatching success (egg viability), juvenile to adult molting success (juvenile viability), and estimated the number of adult offspring produced (productivity; detailed in *Direct and Indirect Fitness Measures* section). We united the data on morphology, elaborate sexual signals, mate preference, and offspring hatching and molting success to test for treatment effects and correlations (detailed in *Data Analysis* section).

Acoustic Signals

We monitored male acoustic sexual signaling continuously from 7-13 days post imaginal-molt (6 days total) using our Electronic Acoustic Recording System (EARS-II designed and built by Cambridge Electronic Design Ltd., Cambridge, UK). The EARS-II is comprised of 96 Styrofoam boxes, each lined with acoustically-isolating foam and containing a suspended microphone and LED light bulb set to the same illumination regime of 14:10 h light:dark as the rest of the greenhouse. We placed each male into his own Styrofoam box and then continuously monitored his acoustic signaling behavior using CricketSong software (Cambridge Electronic Design Ltd., Cambridge, UK). CricketSong filters out background noise, dynamically adjusts its amplitude threshold to ensure all sound pulses are recorded, and continuously quantifies time

spent signaling (a broad-scale measure of acoustic signaling effort) and seven fine-scale characteristics of acoustic signaling behavior: pulse duration (ms), interpulse duration (ms), number of pulses per chirp, chirp duration (ms), interchirp duration (ms), carrier frequency (Hz), and amplitude [Pa; converted to decibels using $20 \times \log_{10}(\text{Mean Amp (Pa)} / 0.00002)$]. Because *G. assimilis* signaling characteristics exhibit high repeatability across the days we monitored them (ranging from $r=0.80-0.97$; Bertram et al 2012), we averaged fine-scale characteristics using a weighted protocol that allowed each pulse or chirp to contribute equally (detailed in Bertram et al 2012). Daily time spent signaling was averaged across days without weighting.

Preference Trials

We moved crickets to a quiet behavior room (temperature $\bar{X} \pm \text{SD} = 25 \pm 2^\circ\text{C}$) when they were 13 days post-imaginal molt. We assessed female mating preference for two randomly selected males (blind to morphological and behavioral trait variation) at 14 days post-imaginal molt (after a 1 day acclimation period in the behavior room). The mate preference arena consisted of two male zones abutted to one side of the female discriminator's zone (Figure S1). An opaque barrier that included 5 cm of empty space separated the two male's zones, ensuring males had no physical or visual contact. The barrier separating the female's zone from the males' zones was clear and perforated, allowing the potential for physical (antennal), chemical, visual, and acoustic contact between female and males. Within the female's zone we demarcated two choice (preference) zones, one directly in front of each male's zone. Each choice zone comprised 1/6 of the total area of the female's zone. As a result, 2/3 of the female's zone was demarcated as a no-choice zone. The bottom of the arena contained gravel, which was thoroughly mixed after

each preference trial. After mixing the gravel we wiped the walls with ethanol to reduce potential transmission of contact sex pheromones (cuticular hydrocarbons).

Figure S1. Schematic diagram of the mate preference arena. Two males (A and B) were placed in separate zones (white), divided by an opaque 5 cm thick barrier. The female was placed in the center of the no-choice zone. After an acclimatization period, the female moved freely and time spent in each choice (preference) zone and the no-choice zone was quantified.

We placed each male into their respective randomly assigned zones. We placed a female (N=128) in the center of the no-choice zone under an inverted 30 ml plastic opaque cup. We allowed 2 min for acclimatization, after which we carefully raised the cup, enabling the female to move freely within her zone. We quantified the time it took for the female to initiate exploratory behavior, and females had to initiate exploratory behavior within 20 minutes to be included in the experiment. We also quantified time spent in the no-choice zone and the time spent in each male's choice zone. If the female showed a clear preference (spent ≥ 60 sec in a male's choice zone, and spent at least 15 sec more in the preferred male's choice zone than in the non-preferred male's choice zone), we immediately swapped the males' zones and repeated the trial with the

same individuals. To be considered to have made a mating preference the female had to exhibit repeatable behavior by choosing the same male in both preference trials using the aforementioned criteria (N=60). If any of these preference criteria were not met, the mating preference of the female was considered to have been ambiguous. All mate preference trials were performed between 06:00 h and 10:00 h.

Mating and Egg Ovipositing

We randomly assigned females that exhibited a clear preference to a mating partner [either their preferred (N=30) or non-preferred (N=30) male; mating treatment] at 14 days post-imaginal molt. We then measured each individual's mass (detailed below) and then placed each assigned mating pair into a clean 520 mL plastic container that had been provisioned with a sand-filled egg oviposition cup, an egg-carton for shelter, and *ad libitum* food and water. Assigned pairs were kept in the container for 24 hours and allowed to freely mate. Males were then removed and housed individually in a clean 520 mL plastic container that had been provisioned with a shelter and *ad libitum* food and water. We cleaned individual containers, moistened sand in the egg oviposition cups, replenished cricket food and water, and monitored adults for survival on a thrice-weekly schedule (every Monday, Wednesday and Friday). We euthanized all surviving adults at 28 days post-imaginal molt as adult life span of several wild field cricket species is around 28 days (Murray and Cade 1995).

Direct and Indirect Fitness Measures

Two weeks after we first provided the egg oviposition cup to the mated pair we transferred each cup to its own clear plastic 'family container' (L x W x H = 16.5 x 10.5 x 16

cm). The sand in the oviposition cups kept moist to enable egg development, and the eggs were given up to 8 weeks to hatch. We provided all hatched offspring with paper egg cartons for shelter and *ad libitum* water and food. We maintained offspring together in the family container, in the same abiotic conditions that their parents experienced during development. When siblings reached ~1 cm in length, we randomly assigned 20 to each of two family containers, resulting in 40 siblings per family remaining in the observation exercise. If families had fewer than 40 siblings, we kept them all. Thrice weekly we monitored offspring to determine when they completed their imaginal molt and euthanized (froze) all adult offspring except for a small subset of males that we utilized in a separate behavioral experiment (subject of a future paper). We removed egg oviposition cups from the family containers eight-weeks after they were introduced and continued to monitor them for three or more weeks to see if there were any subsequent hatches (none observed). We dried the sand in each egg oviposition cup and then carefully separated the eggs by sifting the sand through a 500 μ m sieve. We counted all egg casings (hatched) and whole eggs (un-hatched) produced by each mated pair. We quantified number of eggs oviposited, egg-hatching success ($\# \text{ eggs hatched} / \# \text{ eggs oviposited}$), juvenile-to-adult-molting success ($\# \text{ offspring achieving imaginal molt} / \# \text{ offspring kept per family}$), and per couple productivity ($\# \text{ eggs oviposited} * \text{egg-hatching success} * \text{juvenile-to-adult-molting success}$). Number of eggs oviposited is a measure of direct fitness, egg-hatching success and juvenile-to-adult-molting success both represent offspring viability and are therefore measures of indirect fitness. Conversely, per couple productivity combines measures of both direct and indirect fitness.

Morphological Traits

We used a Denver Instruments analytical balance (Pinnacle Series model PI-314) to obtain an overall wet mass measure. To obtain an overall measure of body size we photographed each euthanized individual (dorsal side up) using a Mycro USB Microscope (EmCal Scientific Inc, San Diego, California) with a standard scale visible in each image. We used ImageJ (version 1.48, National Institutes of Health, <http://imagej.nih.gov/ij/>) to quantify three size measures from each photograph: pronotum length (maximal distance down the sagittal length of the pronotum), pronotum width (maximal measure transversely across the pronotum) and head width (maximal distance between the outer edges of the eyes).

Data Analysis

We used JMP 11.0.0 (SAS Institute Inc., 100 SAS Campus Drive, Cary, North Carolina, USA) to analyze all data. We used coefficient of variation (CV = ratio of the standard deviation to the mean) to provide standardized measures of the amount of variation expressed by each signaling trait. We performed a Principal Components Analysis (PCA) to extract orthogonal vectors from all seven fine-scale acoustic signaling characteristics. To facilitate interpretation of this signaling PCA we performed varimax rotations on the first three principal components; we selected three principal components (signal factors 1-3) because all three met the Kaiser (1960) criterion (eigenvalues above 1), and the conditions of the Scree Test (Cattell 1966) for factor rotation. To obtain an overall measure of body size we performed PCA's (by sex) to extract orthogonal vectors from pronotum length, pronotum width, and head width. We used the first principal component (PC1 size) in all statistical models that required a body size measure. We standardized mean residual mass to zero by saving the residuals from standard least squares linear regressions of body mass on PC1 size, using separate regressions for males and females.

We used general linear models (GLMs) to explore how elaborate signaling traits were influenced by male morphology. Our dependent variables were signal factors 1, 2, and 3 and our independent variables were male size and residual mass. We also used a GLM to explore whether the overall effort males spent on mate attraction could be predicted by fine-scale elaborate signaling components. Our dependent variable was time spent signaling and our independent variables were signal factors 1, 2, and 3. We tested each of these models for violations of normality by examining the distribution of residuals using the Shapiro-Wilks Goodness of Fit tests; we also tested each model for violations of homoscedasticity by looking for dependency between the residuals and the fitted values. Normality and homoscedasticity assumptions were only violated for time spent signalling, and a box-cox transformation allowed these data to meet both assumptions.

We used paired t-tests to test the good-genes prediction that preferred males are larger, relatively heavier, and/or use more elaborate sexual signals (Prediction 1). We used a GLM and two-part logit-linear models to test the good-genes prediction that parents' ability to produce adult offspring is influenced by parent size and/or elaborate sexual signals (Prediction 2) and the immune-gene-complementarity prediction that offspring viability is significantly higher when individuals breed with their preferred partners compared to non-preferred partners (Prediction 3). The GLM allowed us to explore how number of eggs laid (dependent variable) was influenced by male and female body size, male elaborate traits, and preferred / non-preferred mating treatment (independent variables). The two-part logit-linear models allowed us to explore how offspring viability (egg hatching success and juvenile to adult molting success; dependent variables) and parents' ability to produce adult offspring (estimated number of adult offspring; dependent variable) were influenced by body size, elaborate sexual traits, and preferred/non

preferred mating treatment (independent variables). For example, in the two-part logit-linear model for egg hatching success, the first part of the model incorporated a logistic regression to test how female PC1 size, male PC1 size, signal factor 1, residuals of signal factor 2 on male PC1 size, signal factor 3, and preferred / non-preferred mating treatment affected the probability of females producing viable eggs or not. For females that produced viable eggs, the second part of the model used a GLM to investigate how the same independent variables influenced the proportion of eggs that hatched. Similarly, we used two-part logit-linear models to explore how the same independent variables influenced the juvenile to adult molting success and the estimated number of adult offspring produced. Correlations between several variables of interest (male signaling factors, time spent signalling, body size, and residual mass) resulted in problems with multicollinearity. To ameliorate these problems we used the residuals from a regression of signal factor 2 on male PC1 size and excluded residual mass and time spent signalling. We tested each model for violations of normality by examining the distribution of residuals using the Shapiro-Wilks Goodness of Fit tests; we also tested each model for violations of homoscedasticity by looking for dependency between the residuals and the fitted values. Normality and homoscedasticity assumptions were only violated for estimated number of adult offspring produced model, and a cubed-root transformation allowed these data to meet both assumptions. Note: (1) we initially included interaction terms in our models but they were all non-significant so we dropped them from our final models, (2) we ran these models using rotated factors from a PCA that incorporated all signalling traits and all morphological traits together, but the output did not differ from what is presented, and (3) we ran these models again after including different measures of female preference (time to approach the preferred mate or

proportion of time spent in the preferred female's zone), but the output did not differ from what is presented.

RESULTS

Do Females Exhibit Consistent Mating Preferences?

Forty-two percent of the females we tested (54/128) were 'non-performers', as they either did not initiate exploratory behavior within 20 min or they moved randomly around the arena spending <60 sec total time in a male's choice zone. Eleven percent of the females we tested (14/128) were 'indiscriminate', as they changed the male they preferred across the two mating trials. Forty-seven percent of the females we tested (60/128) expressed 'consistent' preferences for one male over the other across both mating trials. Our findings clearly reveal non-random female discriminatory behavior: of the females that completed the mating preference task ('indiscriminate' and 'discriminate' females), >81% (60/74) preferred the same male across both trials while <19% (14/74) switched the male they preferred across trials (chi-square: 78.15, $P<0.0001$). This finding lies in contrast to random female discriminatory behavior, which would have the null expectation of 50% switchers and 50% clear choosers.

We combined the non-performers and the indiscriminate females together into one group (deemed the females that exhibited ambiguous preferences) and asked whether morphology explained whether a female would exhibit ambiguous or clear mating preferences. Female body size, female residual mass, and the difference between males in their body size did not explain significant variation in whether females expressed a clear or ambiguous mating preference (unpaired t-tests: female size PC1: $t=0.033$, $P=0.974$; residual female mass: $t=-1.1251$, $P=0.263$;

male size difference: $t=-1.123$, $P=0.264$). We note that only the discriminate females (females that repeatedly chose the same male across both mate preference trials) are included in all subsequent results.

Morphological and Song Variation

Both male and female crickets varied extensively in their body morphology (CVs range from 5-16). For example, females exhibited a 2-fold difference (497-1042 mg) and males exhibited a 3-fold difference (312-916 mg) in body mass. Head width, pronotum width and pronotum length all loaded positively (eigenvectors: 0.57-0.59) onto the first principal component (PC1 size). PC1 size explained 84% of the variation in female size (Eigenvalue = 2.52) and 86% of variation in male size (Eigenvalue = 2.59). Male crickets also exhibited extensive variation in their elaborate acoustic signaling traits. For example, some males never signaled while others signaled for an average of ~7 hours a night (CV=91). Males also varied in the fine-scale acoustic properties of their signals, including number of pulses per chirp, chirp duration, interchirp duration, carrier frequency, and amplitude (CVs range from 8-28). The signaling PCA included three significant factors (Table 1): Signal Factor 1 was indicative of chirp duration as high scoring males signal with more pulses per chirp and longer chirp durations; Signal Factor 2 was indicative of carrier frequency and amplitude, as high scoring males signal loudly at lower carrier frequencies; Signal Factor 3 was indicative of pulse rate, as high scoring males signal with longer interpulse duration and lower pulse rates.

Table 1. Results from principal component analyses with varimax rotation on acoustic mate attraction signaling traits. Signaling traits with eigenvectors >0.60 are bolded.

	Factor 1	Factor 2	Factor 3
Eigenvalue	2.38	1.68	1.11
Percent	34.05	23.95	15.93
Cumulative Percent	34.05	58.00	73.92
Pulse Duration	0.12	0.16	0.11
Interpulse Duration	-0.08	-0.08	0.93
Pulses/Chirp	0.95	0.26	-0.19
Chirp Duration	0.82	0.25	0.51
Interchirp Duration	0.07	-0.06	0.38
Carrier Frequency	-0.19	-0.60	0.08
Amplitude	0.12	0.97	-0.22

Variation in elaborate acoustic signaling traits was partially explained by variation in morphology, as larger males signaled louder and with lower carrier frequencies than smaller males (Signal factor 2; Table 2). Additionally, males that were relatively light for their size spent more time signaling throughout the course of the night (time spent signaling) and signaled with more pulses per chirp, resulting in longer chirp durations, than relatively heavy males. Variation in fine-scale signaling components explained 34% of the variation in time spent signaling: males that signaled with more pulses per chirp and longer chirp durations (Signal Factor 1), higher amplitude and lower carrier frequencies (Signal Factor 2), and shorter interpulse durations (Signal Factor 3) also signaled more often (higher signaling effort).

Table 2. Factors influencing elaborate sexual signaling behavior. Male body size and residual mass influenced most signaling traits.

Fine scale signaling factors predicted time spent signaling. While all males were included in the analyses, using only the mated males yielded the same qualitative results. Significant model effects are bolded.

Signaling Trait	Term	Estimate $\pm$ SE	F/t	DF	R²_{adj}	P
Signal Factor 1	Model		4.71	2,234	0.031	0.0098
	Size (PC1)	-0.041 $\pm$ 0.04	-1.05			0.2942
	Residual Mass	-0.003 $\pm$ 0.00	-2.90			0.0041
Signal Factor 2	Model		7.31	2,234	0.051	0.0008
	Size (PC1)	0.150 $\pm$ 0.04	3.77			0.0002
	Residual Mass	-0.001 $\pm$ 0.00	-0.57			0.5676
Signal Factor 3	Model		0.30	2,234	-0.006	0.7389
	Size (PC1)	0.029 $\pm$ 0.04	0.70			0.4848
	Residual Mass	0.000 $\pm$ 0.00	-0.33			0.7412
Time Spent Signaling	Model		2.98	2,244	0.016	0.0528
	Size (PC1)	1.840 $\pm$ 2.60	0.71			0.4807
	Residual Mass	-0.155 $\pm$ 0.07	-2.34			0.0203
Time Spent Signaling	Model		64.71	3,319	0.372	<0.0001
	Signal Factor 1	28.072 $\pm$ 2.75	10.21			<0.0001
	Signal Factor 2	23.906 $\pm$ 2.75	8.69			<0.0001
	Signal Factor 3	-10.435 $\pm$ 2.75	-3.79			0.0002

Good- Versus Complementary-Genes Hypotheses:

There was partial support for good-genes Prediction 1: that preferred males have more elaborate sexual signals, are larger, and/or are relatively heavier than non-preferred males. On average females preferred larger males to smaller males in the dichotomous mate choice trials (Figure 1; Table 3). However, females exhibited extensive variation in their mating preferences, with several females preferring the smaller of the two males (Figure 1). This variation suggests that not all females prefer a single best phenotype in a mate and aligns with the idea that female mate preferences might be partially self-referential. Females did not associate more with males that had higher residual mass. Further, females did not prefer males that signaled with higher effort (time spent signaling), more pulses per chirp (Signal Factor 1), higher amplitude (Signal Factor 2), or with lower interpulse duration (Signal Factor 3).

Figure 1. Plot showing the body size of preferred versus non-preferred males for female discriminators that made a clear mating preference. Each line represents the choice of a female and indicates the difference in size between the preferred and not-preferred male. Overall, females preferred larger males to smaller males (paired t-tests, $P=0.0204$, Table 3), but also exhibited extensive variation in their mating preferences.

Table 3. Morphological and elaborate sexual trait differences between preferred and non-preferred males (paired T-tests). Significant model effects are bolded.

Male Trait	Preferred	Not-Preferred	t	DF	P
PC1 Size	0.33 ± 0.21	-0.28 ± 0.22	-2.38	1,57	0.0204
Residual Mass	-5.33 ± 7.34	-2.15 ± 9.14	0.85	1,55	0.3975
Signal Factor 1	-0.02 ± 0.15	0.04 ± 0.10	0.14	1,53	0.8861
Signal Factor 2	-0.03 ± 0.14	0.09 ± 0.12	0.37	1,53	0.7106
Signal Factor 3	-0.01 ± 0.15	-0.05 ± 0.15	-0.81	1,53	0.4190
Time Spent Signaling	112 ± 13	101 ± 13	-0.99	1,59	0.3277

Even though females mated to their preferred partner oviposited 30% more eggs on average than females mated to their non-preferred partner (421 ± 45 vs. 325 ± 33 , respectively), variation in number of eggs oviposited was so high that mating treatment was not significant ($P=0.2809$; effect size $r=0.2169$; Table 4). Similarly, variation in number of eggs oviposited was not explained by differences in male body size or male elaborate sexual signals (Table 4). Female body size, did, however significantly explain variation in the number of eggs oviposited (larger females oviposited more eggs than smaller females; $P=0.0339$).

There was no support for good-genes Prediction 2: parents' ability to produce adult offspring is influenced by parent size and/or elaborate sexual signals. Variation in the estimated number of offspring to reach adulthood was not significantly explained by female body size, male body size, nor male elaborate sexual signals (Table 4). Similarly, even though females

mated with their preferred partner were estimated to have had 23% more offspring reach adulthood compared to females mated to their non-preferred partner (319 ± 40 vs. 259 ± 36 , respectively), variation in the estimated number of adult offspring was so high that mating treatment was not significant ($P=0.6835$; effect size $r=0.1430$; Table 4).

Table 4. Results of general linear models examining factors influencing the number of eggs laid ($F=1.918$; $R^2_{adj}=0.091$; $DF=6,49$; $P=0.0965$) and the estimated number of adult offspring produced ($F=0.6865$; $R^2_{adj}=-0.043$; $DF=6,40$; $P=0.6616$). Significant model effects are bolded.

Dependent Parameter	Model Parameter	Estimate $\pm$ SD	t	P
# Eggs Laid	Intercept	377.65 $\pm$ 28.53	13.24	<0.0001
	Mating Treatment[Non Preferred]	-32.69 $\pm$ 29.99	-1.09	0.2809
	Female PC1 Size	38.16 $\pm$ 17.48	2.18	0.0339
	Male PC1 Size	3.05 $\pm$ 19.01	0.16	0.8733
	Male Signal Factor1	-26.72 $\pm$ 32.62	-0.82	0.4166
	Residuals Male Signal Factor2	-34.61 $\pm$ 33.18	-1.04	0.3020
	Male Signal Factor3	-34.45 $\pm$ 28.59	-1.20	0.2340
Estimated # Adult Offspring	Intercept	5.58 $\pm$ 0.22	25.75	<0.0001
	Mating Treatment[Non Preferred]	-0.23 $\pm$ 0.23	-1.01	0.3204
	Female PC1 Size	0.2 $\pm$ 0.13	1.53	0.1351
	Male PC1 Size	-0.1 $\pm$ 0.14	-0.67	0.5059
	Male Signal Factor1	-0.01 $\pm$ 0.25	-0.04	0.9653
	Residuals Male Signal Factor2	-0.14 $\pm$ 0.27	-0.52	0.6030
	Male Signal Factor3	-0.07 $\pm$ 0.22	-0.30	0.7651

There was no support for complementary-genes Prediction 3: that offspring viability is significantly higher when individuals breed with preferred partners compared to non-preferred partners. The probability of a female producing some eggs that successfully hatched (egg viability) was not explained by whether the female was mated to their preferred partner or not (mating treatment; $P=0.3205$), nor was it explained by any of our other predictor variables (male or female body size, male elaborate sexual signals; Table 5). For females that produced some viable eggs, the proportion of successfully hatching eggs was also not explained by mating treatment. Females mated to their preferred partners produced eggs with an average hatching success rate of $49\pm 6\%$ compared to an only slightly lower hatching success rate of $46\pm 5\%$ for females mated to their non-preferred partners, variation in hatching rate was high enough that mating treatment was not remotely significant ($P=0.4825$; Table 5). Similarly, female body size, male body size, and male elaborate sexual signals did not explain the proportion of eggs that successfully hatched (Table 5).

The probability of a female having some juveniles that successfully reached adulthood (juvenile viability) was also not explained by whether the female was mated to their preferred partner compared to their non-preferred partner (mating treatment; $P=0.5184$), nor was it explained by any of our other predictor variables (male or female body size, male elaborate sexual signals; Table 6). Similarly, for females that produced some viable juveniles, the proportion of juveniles that successfully reached adulthood was also not explained by mating treatment. While the rate for juveniles molting to adulthood was $67\pm 6\%$ for females mated to their preferred partners; it was only slightly lower ($61\pm 6\%$) for females mated to their non-preferred partners; this mating treatment difference was not significant ($P=0.6835$; Table 6).

Similarly, female body size, male body size, and male acoustic mate attraction signals did not explain the proportion of juveniles successfully molting to adulthood (Table 6).

Table 5. Results of a two-part logit-linear model examining factors influencing the probability of producing some eggs that successfully hatch (nominal logistic regression; $\chi^2=8.64$; DF=6,56; P=0.1947) and, for females with egg hatching success, the proportion of eggs that hatched (general linear model; F=0.61; DF=6,46; $R^2_{adj}=-0.05$; P=0.7199).

Model	Model Parameter	Estimate $\pm$ SE	χ^2	t	P
Logistic Regression	Intercept	-5.19 $\pm$ 2.02	6.59		0.0103
	Mating Treatment[Non Preferred]	-1.28 $\pm$ 1.29	0.99		0.3205
	Female PC1 Size	0.17 $\pm$ 0.43	0.17		0.6841
	Male PC1 Size	-1.06 $\pm$ 0.87	1.49		0.2221
	Male Signal Factor1	2.58 $\pm$ 1.75	2.17		0.1404
	Residuals Male Signal Factor2	1.9 $\pm$ 1.26	2.28		0.1314
	Male Signal Factor3	0.18 $\pm$ 0.94	0.04		0.8483
General Linear Model	Intercept	0.53 $\pm$ 0.04		13.76	<0.0001
	Mating Treatment[Non Preferred]	-0.03 $\pm$ 0.04		-0.71	0.4825
	Female PC1 Size	-0.01 $\pm$ 0.02		-0.50	0.6217
	Male PC1 Size	-0.03 $\pm$ 0.03		-1.05	0.2999
	Male Signal Factor1	-0.02 $\pm$ 0.04		-0.51	0.6129
	Residuals Male Signal Factor2	0.05 $\pm$ 0.04		1.22	0.2276
	Male Signal Factor3	-0.02 $\pm$ 0.04		-0.46	0.6474

Table 6. Results of a two-part logit-linear model examining factors influencing the probability of producing some juveniles that successfully reach adulthood (nominal logistic regression; $\chi^2=5.21$; DF=6,52; P=0.5175) and, for females with offspring that reach adulthood, the proportion of juveniles that successfully molted to adulthood (general linear model; F=1.22; DF=6,40; $R^2_{adj}=0.028$; P=0.3172).

Model	Model Parameter	Estimate $\pm$ SE	χ^2	t	P
Logistic Regression	Intercept	-2.68 $\pm$ 0.68	15.50		<0.0001
	Mating Treatment[Non Preferred]	0.32 $\pm$ 0.49	0.42		0.5184
	Female PC1 Size	0.3 $\pm$ 0.31	0.93		0.3351
	Male PC1 Size	0.17 $\pm$ 0.34	0.27		0.6063
	Signal Factor1	0.04 $\pm$ 0.52	0.00		0.9456
	Residuals Signal Factor2	-0.2 $\pm$ 0.55	0.13		0.7174
	Signal Factor3	0.78 $\pm$ 0.54	2.07		0.1498
General Linear Model	Intercept	0.8 $\pm$ 0.02		41.14	<0.0001
	Mating Treatment[Non Preferred]	-0.01 $\pm$ 0.02		-0.41	0.6835
	Female PC1 Size	-0.02 $\pm$ 0.01		-1.36	0.1824
	Male PC1 Size	-0.01 $\pm$ 0.01		-0.52	0.6066
	Signal Factor1	-0.02 $\pm$ 0.02		-0.99	0.3273
	Residuals Signal Factor2	0.02 $\pm$ 0.02		0.94	0.3533
	Signal Factor3	-0.03 $\pm$ 0.02		-1.49	0.1445

DISCUSSION

Our study's significance lies in bringing together two disparate research traditions regarding the evolution of mating preferences via indirect-fitness benefits. Good-genes hypotheses propose that elaborate sexual traits mediate mating preferences because elaborate traits honestly reflect an individual's ability to pass on genes that will enhance their offspring's survival and reproduction. Complementary-genes hypotheses propose mating preferences for complementary mates evolved to enhance offspring viability. Our findings lend only minimal support to good-genes hypotheses and are largely inconsistent with complementary-genes hypotheses.

Good-Genes Hypotheses

Good-genes models assume elaborate sexual traits are honest indicators of a male's health and vigor (i.e. condition) because of the energetic costs involved in elaborate sexual trait development, maintenance, and expression (Zahavi 1975; Berglund et al. 1996; Rowe and Houle 1996; Maynard Smith and Harper 2003). There are several indications that *G. assimilis*' elaborate sexual signals honestly reflect male condition: (1) Males in our study were highly variable in the amount of time they spent signaling for a mate; males that signaled most often also signaled with more energetically expensive chirps (more pulses per chirp, shorter interpulse durations, longer chirp durations, and higher amplitudes); and males that signaled most often also had less body reserves (lower residual mass) following signaling than males that signaled less often. Given signaling costs increase with increased signaling time (Prestwich 1994; Prestwich and Walker, 1981; Hoback and Wagner 1997; Hack 1998), our results suggest that

high effort signalers may be more able to afford the energetic costs of signaling than low effort signalers. (2) Thomson et al. (2014) revealed *G. assimilis*' signaling effort and fine-scale signal characteristics are positively correlated with muscular enzyme activity and fuel stores, suggesting that signaling variation is partially influenced by the ability to mobilize and/or metabolize glycogen stores. (3) Male *G. assimilis*' elaborate sexual traits also appear to be indicative of past condition. Whattam and Bertram (2011) fed juvenile *G. assimilis* high (unlimited) or low (limited) amounts of food. Juveniles fed unlimited food were larger at adulthood, had higher residual mass, and signaled with longer and louder chirps at lower carrier frequencies than males fed low amounts of food (Whattam and Bertram 2011). This manipulative study suggests that body size reliably reflects the ability to accumulate sufficient dietary resources during development. Thus, *G. assimilis*' elaborate sexual signals seem to be somewhat indicative of male condition.

The good-genes corollary to the assumption that elaborate traits reflect health and vigor is that females preferentially mate with males possessing more elaborate traits (bigger males and/or males that signal louder or more often), and in doing so produce offspring that inherit their fathers' health and viability and their mothers' preferences. We tested whether females prefer to mate with males with more elaborate traits using a very carefully controlled environment that minimized the potential for males to see or physically interact with each other. This ensured individuals were largely free of the normal social competitions associated with female mate preference and male-male competition (e.g., courtship interference). We were therefore able to examine the effects of 'freely expressed' mating preferences on fitness. Our mate preference findings revealed that female *G. assimilis* prefer larger males significantly more often than smaller males, adding to the growing list of mate preference experiments revealing consistent

mating preference for larger mates (Simmons 1986, 1995; Zuk 1988; Bateman et al. 2001; Deb et al. 2012; Loranger and Bertram 2016). However, we note that females exhibited inter-individual variation in their preference for larger males, with some females preferring smaller males over larger males (Figure 1).

We found no female preference for elaborate male sexual signals. Females may not have had the opportunity to hear differences in male acoustic signals because we did not broadcast male calls during mate preference trials. Instead, we let each male signal at will, an approach that may have weakened the opportunity to detect preference for elaborate songs. There are, however, strong indications that females prefer the chirps of larger males, as female *G. assimilis* preferentially orient to louder chirps over quieter chirps (Pacheco and Bertram 2014) and larger males signal louder (at higher amplitudes) than smaller males (present study and Bertram et al. 2014). Further, we may have underestimated female mating preferences because we used virgins. Virgin *G. bimaculatus* seem to bet-hedge by mating with the first male they encounter, and then exhibiting stronger mating preferences in their successive male encounters (Bateman et al. 2001). Lastly, we examined female preference for elaborate traits by presenting females with randomly selected males, resulting in males being quite different in morphology and/or elaborate traits in some trials and quite similar in others. While our approach is more akin to the trait distribution females would naturally encounter (Brown 1999; Gerhardt and Huber 2002), it decreases the likelihood of observing female preference relative to a design that uses stimuli from opposite ends of the continuum (Wagner 1998; Deb et al. 2012; Pacheco and Bertram 2014). While good-genes effects are notoriously weak (Møller and Alatalo 1999), they were likely weakened even further for the aforementioned reasons.

We also found no evidence for the prediction that female *G. assimilis* who mate with elaborate males produce offspring that inherit their father's good-genes for health and viability, as elaborate traits were not correlated with offspring viability measures. Here, as in most studies, we are guessing about the traits that supposedly indicate that an individual has good-genes and which mediate females' preferences. One may find correlates, as we have, of females' preference and male morphological or elaborate traits, but it is hard to know if these traits signify the good-genes that are at work against the specific pathogens operative in the population. This lack of knowing might explain why, even though females preferred significantly larger males, we found no association between male body size and offspring viability.

Immune-Gene-Complementarity Hypothesis

Females mated to their preferred partner produced eggs that had a 3% higher hatching success and their juveniles had a 6% higher adult molting success than females mated to their non-preferred partner; these differences did not approach statistical significance. Given that mating preferences did not "predict" offspring hatching or juvenile to adult molting success (viability measures), our findings are inconsistent with the immune-gene-complementarity hypothesis that mating with preferred mates enhances offspring viability. Previous experimental studies evaluating this hypothesis include female mallards (Bluhm and Gowaty 2004 a,b), female and male feral house mice (Drickamer et al. 2000, 2003; Gowaty et al. 2003; Raveh et al. 2014), female Tanzanian cockroaches (Moore et al. 2003), and female and male fruit-flies (Anderson et al. 2007), as well as several species of fish (Gowaty et al. 2007). Similar to our study, the investigators ensured that the discriminators and potential partners (discriminatees) were virgins, and individuals were picked for inclusion in each experiment at random with respect to their

obvious phenotypic characteristics. In these aforementioned experiments, offspring viability (% egg to adult or % pup to adult) was always significantly higher when discriminators bred with their preferred compared to their non-preferred mate. Thus, our study is perhaps the first published to not find support for immune-gene-complementarity hypothesis.

The lack of an offspring viability benefit of cricket mate preferences remains strange in the context of these other aforementioned studies and might be explained by the fact that our experimental crickets had been in a lab-adapted population for ~17 generations. The lab-adapted population was likely more devoid of pathogens and parasites than in nature, and females may not need to select mates based on immune gene complementarity when the environment has few pathogens and the crickets are raised in a pristine, food-rich environment. Further, it is not clear how inbred our population is. If our population has experienced inbreeding then this would have reduced our ability to detect indirect-fitness effects. In contrast, in the other aforementioned studies that evaluated complementary-genes hypotheses (reviewed in Gowaty et al. 2007), duck subjects were collected as eggs from the wild, mice subjects were also collected from the wild as pups, as were fish subjects. The cockroach cultures had been in captivity for over 20 years and showed the weakest of the offspring viability effects. While the flies had been in culture for 7 or 8 years, the isofemale lines were mixed to bring lines back to genetic diversity similar to wild caught populations.

Concerns and Future Directions

It is possible that under more natural conditions we might have been able to detect the effects of good-genes or complementary-genes, given fitness is highly dependent on the environment (Johnston et al. 2001). Further, the environmental conditions in the laboratory were

not particularly stressful and evidence from a number of studies suggests that the benefits of mating with preferred males (and maybe the benefits of mating with complementary males) can be strongly environment dependent, not only because fitness is dependent on the environment, but also because of genotype by environment effects on male signals (Kokko and Heubel 2008; Higginson and Reader 2009; Ingelby et al 2010). Because of these genotype by environment effects, male signals can be more strongly correlated with female mating benefits under stressful conditions. Future research should therefore re-examine these three predictions under more stressful conditions (e.g., restricted diets and pathogen exposure).

Future research should also explore whether the offspring of females mated to larger or more elaborate signaling males receive indirect fitness benefits such as growing larger or signaling more attractively. Larger sons should be preferred as mates, and larger daughters should be more fecund. In support of this good-genes notion, Wedell and Tregenza (1999) revealed that the sons of preferred male *G. bimaculatus* obtained more copulations than the sons of non-preferred males.

Future research should also explore the idea that direct fitness benefits influence the evolution of mating preferences. Direct fitness benefits are commonly dismissed when males provide no obvious resources to females, such as in the field cricket mating system (Wagner 2011). However, direct benefits hypotheses need to be explicitly tested (Reynolds and Gross 1990; Wagner 2011). For example, our study reveals that females mated to preferred partners oviposited 30% more eggs on average than females mated to non-preferred partners. While females were so variable in the number of eggs they oviposited that these mating treatment differences did not approach statistical significance, the findings suggest that preferred males might provide some direct-fitness benefits that enhance female fecundity. Carefully controlling

the number of matings and quantifying egg oviposition throughout natural life would help to ascertain whether mating treatments influence direct-fitness benefits. While female *G. assimilis* only receive sperm and seminal fluid from the males, the seminal fluid products may benefit female fecundity or longevity. Direct fitness effects have been found in *G. lineaticeps*, as multiply mated females lived 32% longer and produced 98% more eggs than females that were mated singly. These intriguing results suggest that the direct-fitness benefits hypothesis needs to be explicitly tested in the *G. assimilis* system.

CONCLUSIONS

Indirect-fitness benefits hypotheses for the evolution of mating preferences fall into two disparate classes: hypotheses about good-genes and hypotheses about complementary-genes. Good-genes hypotheses suggest mating preferences are mediated by elaborate traits, with elaborate males producing more fit offspring. Complementary-genes hypotheses suggest mating preferences are mediated by dissimilar immune alleles, with dissimilar mates producing offspring with enhanced viability. Our study's significance lies in bringing together these two disparate research traditions. Our intention was to experimentally separate the questions about whether female mate preferences resulted in fitness payouts, while being blind to potential mediating traits. Thus, our "non traditional" approach took out any possibility of unconscious bias associated with investigators' knowledge of variation in male phenotypes. Our findings are only partially consistent with good-genes hypotheses: while elaborate sexual signals appear to be honest indicators of male morphology and while females discriminate between mates based on male morphology, females mated to larger males (or more elaborate signalers) did not exhibit

greater indirect fitness benefits. Our findings are inconsistent with the immune-gene-complementarity hypothesis, as mating treatment did predict offspring viability. Our study appears to be the first published research to not find support for immune-gene-complementarity hypothesis. In context this might not be strange, as there are only a few published studies (but on a wide range of taxa from flies to cockroaches, fish, birds, and mammals) that evaluate the fitness outcomes for females mating with their preferred and non-preferred partners, when investigators were blind to discriminatee phenotypes. Our null result on the offspring viability prediction leaves us with an open question about whether offspring viability is a factor in mate preferences in crickets.

REFERENCES

- Alexander, R. D. 1961. Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera: Gryllidae). *Behaviour* 17:130-223.
- Anderson, W. W., Y. K. Kim, and P. A. Gowaty. 2007. Experimental constraints on mate preferences in *Drosophila pseudoobscura* decrease offspring viability and fitness of mated pairs. *P. Natl. Acad. Sci. USA* 104:4484-4488.
- Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton.
- Balenger, S. L., and M. Zuk. 2014. Testing the Hamilton–Zuk hypothesis: past, present, and future. *Integr. Comp. Biol.* 54:601-613.
- Barber, I., S. A. Arnott, V. A. Braithwaite, J. Andrew, and F. A. Huntingford. 2001. Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections. *Proc. R. Soc. Lond. B.* 268:71-76.

- Bateman, P. W., L. N. Gilson, and J. W. H. Ferguson. 2001. Male size and sequential mate preference in the cricket *Gryllus bimaculatus*. *Anim. Behav.* 61:631-637.
- Berglund, A., A. Bisazza, and A. Pilastro. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biol. J. Linn. Soc.* 58:385-399.
- Bertram, S. M., L. P. Fitzsimmons, E. M. McAuley, H. D. Rundle, and R. Gorelick. 2012. Phenotypic covariance structure and its divergence for acoustic mate attraction signals among four cricket species. *Ecol. Evol.* 2:181-195
- Bluhm, C. K., and P. A. Gowaty. 2004a. Reproductive compensation for offspring viability deficits by female mallards, *Anas platyrhynchos*. *Anim. Behav.* 68:985-992.
- Bluhm, C. K., and P. A. Gowaty. 2004b. Social constraints on female mate preferences in mallards *Anas platyrhynchos* decrease offspring viability and mother productivity. *Anim. Behav.* 68:977-983.
- Borgia, G. 1979. Sexual selection and the evolution of mating systems. In: M. S. Blum and N. A. Blum, editors. *Sexual Selection and Reproductive Competition in Insects*. New York: Academic Press. p. 19-80.
- Borgia, G. and K. Collis. 1989. Female choice for parasite-free male satin bowerbirds and the evolution of bright male plumage. *Behav. Ecol. Sociobiol.* 25:445-453.
- Bretman, A., N. Wedell, and T. Tregenza. 2004. Molecular evidence of post-copulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. *Proc. R. Soc. Lond. B.* 271:159-164.
- Bretman, A., R. Rodriguez-Munoz, and T. Tregenza. 2006. Male dominance determines female egg laying rate in crickets. *Biol. Lett.* 2:409-411.
- Brown, J. L. 1997. A theory of mate choice based on heterozygosity. *Behav. Ecol.* 8:60-65.

- Brown, W. D. 1999. Mate choice in tree crickets and their kin. *Annu. Rev. Entomol.* 44:371-396.
- Buchanan, K. L., C. K. Catchpole, J. W. Lewis, and A. Lodge, A. 1999. Song as an indicator of male parental effort in the sedge warbler. *Proc. R. Soc. Lond. B.* 267:321-326.
- Cade, W. H. 1979. The evolution of alternative male reproductive strategies in field crickets. In: *Sexual Selection and Reproductive Competition in Insects* (Ed. by M. S. Blum and N. A. Blum), pp. 343-379. New York: Academic Press.
- Cade, W. H. and E. S. Cade. 1992. Male mating success, calling and searching behavior at high and low densities in the field cricket, *Gryllus integer*. *Anim. Behav.* 43:49-56.
- Cattell, R. B. 1966 The scree test for the number of factors. *Multivar. Behav. Res.* 1:245-276.
- Clayton, D. H. 1991. The influence of parasites on host sexual selection. *Parasitol. Today.* 7:329-334.
- Colegrave, N., J. S. Kotiaho, and J. L. Tomkins. 2002. Mate choice or polyandry: reconciling genetic compatibility and good genes sexual selection. *Evol. Ecol. Res.* 4:911-917.
- Crnokrak, P. and D. A. Roff. 1995. Fitness differences associated with calling behaviour in the two wing morphs of male sand crickets, *Gryllus firmus*. *Anim. Behav.* 50:1475-1481.
- Deb, R., M. Bhattacharya, and R. Balakrishnan. 2012. Females of a tree cricket prefer larger males but not the lower frequency male calls that indicate large body size. *Anim. Behav.* 84:137-149.
- Downhower, J. F., L. Brown, R. Pederson, and G. Staples. 1983. Sexual selection and sexual dimorphism in mottled sculpins. *Evolution* 37:96-103
- Drickamer, L. C., P. A. Gowaty, and C. M. Holmes. 2000. Free female mate choice in house mice affects reproductive success and offspring viability and performance. *Anim. Behav.* 59:371-378.

- Drickamer, L. C., P. A. Gowaty, and D. M. Wagner. 2003. Free mutual mate preferences in house mice affect reproductive success and offspring performance. *Anim. Behav.* 65:105-114.
- Falconer, D. S. and T. F. C. Mackay. 1996. *Introduction to Quantitative Genetics*. 4th edn. Menlo Park, California: Benjamin Cummings.
- Figuerola, J., E. Muñoz, R. Gutiérrez, and D. Ferrer. 1999. Blood parasites, leucocytes and plumage brightness in the Cirl Bunting, *Emberiztia cirlus*. *Funct. Ecol.* 13:594-601.
- Fisher, R. A. 1930. *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.
- Gerhardt, H. C. and F. Huber. 2002. *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. Chicago: University of Chicago Press.
- Gowaty, P. A. 2008. Reproductive compensation. *J. Evolution. Biol.* 21:1189-1200.
- Gowaty, P. A. 2010. Compensation in reproduction. *Encyclopedia of Animal Behavior*, 1:345-349.
- Gowaty, P. A., L. C. Drickamer, and S. Schmid-Holmes. 2003. Male house mice produce fewer offspring with lower viability and poorer performance when mated with females they do not prefer. *Anim. Behav.* 65:95-103.
- Gowaty, P. A., W. W. Anderson, C. K. Bluhm, L. C. Drickamer, Y. K. Kim, and A. Moore. 2007. The hypothesis of reproductive compensation and its assumptions about mate preferences and offspring viability. *Proc. Natl. Acad. Sci. USA.* 104:15023-15027.
- Hack, M. A. 1998. The energetics of male mating strategies in field crickets (Orthoptera: Gryllinae: Gryllidae). *J. Insect Behav.* 11:853-867.
- Hamilton, W. D. 1990. Mate choice near and far. *Am. Zool.* 30:341-352
- Hamilton, W. D. and R. Poulin. 1997. The Hamilton and Zuk hypothesis revisited: a meta-

- analytical approach. *Behaviour* 134:299-320.
- Hedrick, A. V. and L. M. Dill. 1993 Mate choice by female crickets is influenced by predation risk. *Anim. Behav.* 46:193-196.
- Heywood, J. 1989. Sexual selection by the handicap mechanism. *Evolution* 43:1387-1397.
- Higginson, A. D. and T. Reader. 2009. Environmental heterogeneity, genotype-by-environment interactions and the reliability of sexual traits as indicators of mate quality. *Proc. Roy. Soc. B.* 276:1153-1159.
- Hoback, W. W. and W. E. Wagner, 1997. The energetic cost of calling in the variable field cricket, *Gryllus lineaticeps*. *Physiol. Entomol.* 22:286-290.
- Hoelzer, G. A. 1989. The good parent process of sexual selection. *Anim. Behav.* 38:1067-1078.
- Holzer, B., A. Jacot, and M. W. Brinkhof. 2003. Condition-dependent signaling affects male sexual attractiveness in field crickets, *Gryllus campestris*. *Behav. Ecol.* 14:353-359.
- Houle, D. and A. S. Kondrashov. 2002. Coevolution of costly mate choice and condition-dependent display of good genes. *Proc. R. Soc. Lond. B.* 269:97-104.
- Hubbell, S. P. and L. K. Johnson. 1987. Environmental variance in lifetime mating success, mate choice and sexual selection. *Am. Nat.* 130:91-112.
- Hunt, J., R. Brooks, M. D. Jennions, M. J. Smith, C. L. Bentsen, and L. F. Bussière. 2004. High-quality male field crickets invest heavily in sexual display but die young. *Nature* 432:1024-1027.
- Hutchinson, J. M. C and K. Halupka. 2004. Mate choice when males are in patches: Optimal strategies and good rules of thumb. *J. Theor. Biol.* 231:129-151.
- Ingelby, F. C., J. Hunt and D. J. Hosken. 2010. The role of genotype-by-environment interactions in sexual selection. *J. Evol. Biol.* 23:2031-2045.

Johnston et al. 2001

Johnstone, R. A. 1997. The tactics of mutual mate choice and competitive search. *Behav. Ecol. Sociobiol.* 40:51-59.

Judge, K. A., J. J. Ting, and D. T. Gwynne. 2008. Condition dependence of male lifespan and calling effort in a field cricket. *Evolution* 62:868-878.

Judge, K. A., J. J. Ting, and D. T. Gwynne. 2014. Condition dependence of female choosiness in a field cricket. *J. Evolution. Biol.* 27:2529-2540.

Kaiser, H. F. 1960. The application of electronic computers to factor analysis. *Educ. Psychol. Meas.* 20:141-151

Kempenaers, B. 2007. Mate choice and genetic quality: a review of the heterozygosity theory. In: *Advances in the Study of Behavior*, Vol. 37, pp. 189-278. Elsevier, San Diego.

Kokko, H. and K. Heubel. 2008. Condition-dependence, genotype-by-environment interactions and the lek paradox. *Genetica* 132:209-216.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. USA* 78:3721-3725.

Lawniczak, M. K. N., A. I. Barnes, J. R. Linklater, J. M. Boone, S. Wigby, and T. Chapman. 2007. Mating and immunity in invertebrates. *Trends Ecol. Evol.* 22:48-55.

Loranger, M. and S. M. Bertram. 2016. The effect of male dominance on female choice in a field cricket (*Gryllus assimilis*). *Anim. Behav.* In Press.

Maynard Smith, J. and D. Harper. 2003. *Animal Signals*. Oxford: Oxford University Press.

McGraw, K. J. and G. E. Hill. 2000. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. *Proc. R. Soc. Lond. B.* 267:1525-1531.

- Merilä, J., B. C. Sheldon, and K. Lindström. 1999. Plumage brightness in relation to haematozoan infections in the green- finch: bright males are a good bet. *Écoscience* 6:12-18.
- Møller, A.P. and R. V. Alatalo. 1999. Good genes effects in sexual selection. *Proc. R. Soc. Lond. B* 266:85–91.
- Montroy, K., M. J. Loranger, and S. M. Bertram. 2015. Male crickets adjust their aggressive behaviour when a female is present. *Behavioural Processes* 124:108-114
- Moore, A. J., P. A. Gowaty, and P. J. Moore. 2003. Females avoid manipulative males and live longer. *J. Evol. Biol.* 16:523-530.
- Murray, A-M. and W. H. Cade. 1995. Differences in age structure among field cricket populations (Orthoptera; Gryllidae): possible influence of a sex-biased parasitoid. *Can. J. Zool.* 73:1207-1213.
- Neff, B. D. and T. E. Pitcher. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Mol. Ecol.* 14:19-38.
- Pacheco, K. and S. M. Bertram. 2014. How male sound pressure level influences phonotaxis in virgin female Jamaican field crickets (*Gryllus assimilis*). *Peer J* 2:e437.
- Penn, D. J. and W. K. Potts. 1999. The evolution of mating preferences and major histocompatibility complex genes. *Am. Nat.* 153:145-164.
- Petrie, M., T. R. Halliday, and C. Sanders. 1991 Peahens prefer males with elaborate trains. *Anim. Behav.* 41:323-331.
- Pomiankowski, A. 1988. The evolution of female mate preferences for male genetic quality. *Oxf. Surv. Evol. Biol.* 5:136-184.
- Popov, A. V., and V. F. Shuvalov. 1977. Phonotactic behavior of crickets. *J. Comp. Physiol.*

- 119:111-126.
- Prestwich, K. N. 1994. The energetics of acoustic signaling in anurans and insects. *Am. Zool.* 34:625-643.
- Prestwich, K. N., and T. J. Walker. 1981. Energetics in singing crickets: effect of temperature in three trilling species (Orthoptera: Gryllidae). *J. Comp. Physiol.* 143:199-212.
- Pruett-Jones, S. G., M. A. Pruett-Jones, and H. I. Jones. 1990 Parasites and sexual selection in birds of paradise. *Am. Zool.* 30:287-298.
- Raveh, S., S. Sutalo, K. E. Thonhauser, M. Thoß, A. Hettyey, F. Winkelser, and D. J. Penn. 2014. Female partner preferences enhance offspring ability to survive an infection. *BMC Evol. Biol.* 2014:14.
- Reynolds, J. D. and M. R. Gross. 1990. Costs and benefits of female mate choice: is there a lek paradox? *Am. Nat.* 136:230-243.
- Roberts, M. L., K. L. Buchanan, and M. R. Evans. 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. *Anim. Behav.* 68:227-39.
- Rowe, L. and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B.* 263:1415-1421.
- Saleh, N. W., E. L. Larsen, and R. G. Harrison. 2014. Reproductive success and body size in the cricket *Gryllus firmus*. *J. Insect Behav.* 27:346-356.
- Sandvik, M., G. Rosenqvist, and A. Berglund. 2000. Male and female mate choice affects offspring quality in a sex- role-reversed pipefish. *Proc. R. Soc. Lond. B.* 267:2151-2155.
- Sheldon, B. C. and S. Verhulst. 1996. Ecological immunity: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* 11:317-321.
- Simmons, L. W. 1986. Female choice in the field cricket, *Gryllus bimaculatus* (De Geer). *Anim.*

- Behav., 34:1463-1470.
- Simmons, L. W. 1995. Correlates of male quality in the field cricket, *Gryllus campestris* L.: age, size, and symmetry determine pairing success in field populations. Behav. Ecol. 6:376-381.
- Simmons, L. W., M. Beveridge, N. Wedell, and T. Tregenza. 2006. Postcopulatory inbreeding avoidance by female crickets only revealed by molecular markers. Mol. Ecol. 15:3817-3824.
- Siva-Jothy, M. T. and F. Skarstein. 1998. Towards a functional understanding of “good genes”. Ecol. Letters, 1:178-185.
- Thomson, I. R. C.-A. Darveau, and S. M. Bertram. 2014. Body morphology, energy stores, and muscle enzyme activity explain cricket acoustic mate attraction signaling variation. PLoS ONE, 9(3):e90409.
- Tregenza, T. and N. Wedell. 2000. Genetic compatibility, mate choice and patterns of parentage. Mol. Ecol. 9:1013-1027.
- Tregenza, T. and N. Wedell. 2002. Polyandrous females avoid costs of inbreeding. Nature 415:71-73.
- Tuni, C., M. Beveridge, and L. W. Simmons. 2013. Female crickets assess relatedness during mate guarding and bias storage of sperm towards unrelated males. J. Evol. Biol. 26:1261-1268.
- Wagner, W. E. 1996. Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. Behav. Ecol. 7:279-285.
- Wagner, W. E. 1998. Measuring female mating preferences. Anim. Behav. 55:1029-1042.
- Wagner, W. E. 2011. Direct Benefits and the Evolution of Female Mating Preferences: Conceptual Problems, Potential Solutions, and a Field Cricket. Advances in the Study of

- Behavior. 43:273-319.
- Wagner, W. E. A.-M. Murray, and W. H. Cade. 1995. Phenotypic variation in the mating preferences of female field crickets, *Gryllus integer*. Anim. Behav. 49:1269-1281.
- Wagner, W. E. and M. G. Reiser. 2000. The importance of calling song and courtship song in female mate choice in the variable field cricket. Anim. Behav. 59:1219-1226.
- Walker, T. J. 2011. Texas field cricket, *Gryllus texensis* (Cade and Otte 2000). Singing Insects of North America. <http://entnemdept.ufl.edu/creatures/misc/crickets/gtexensis.html> (15 January 2014).
- Wedekind, C. 1999. Pathogen-driven sexual selection and the evolution of health. In: Evolution in Health and Disease (Ed. by S. C. Stearns), pp. 102-107. Oxford: Oxford University Press.
- Wedekind, C. and S. Furi. 1997. Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? Proc. R. Soc. Lond. B. 264:1471-1479.
- Wedekind, C., T. Seebeck, F. Bettens, and A. J. Paepke. 1995. MHC-dependent mate preferences in humans. Proc. R. Soc. Lond. B. 260:245-249.
- Wedell, N. and T. Tregenza. 1999. Successful fathers sire successful sons. Evolution, 53:620-625.
- Whattam, E. M. and S. M. Bertram. 2011. Effects of juvenile and adult condition on long distance call components in the Jamaican field cricket *Gryllus assimilis*. Anim. Behav. 81:135-144.
- Wiegmann, D. D., L. M. Angeloni, and S. M. Seubert. 2013. Mate choice decisions by searchers. Curr. Zool. 59:184-199.

- Zahavi, A. 1975. Mate selection - a selection for handicap. *J. Theoret. Biol.* 53:205-214.
- Zeh, J. A., and D. W. Zeh. 1997. The evolution of polyandry II: post-copulatory defences against genetic incompatibility. *Proc. R. Soc. Lond. B.* 264:69-75.
- Zuk, M. 1988. Parasite load, body size, and age of wild-caught male field crickets (Orthoptera: Gryllidae: effects on sexual selection. *Evolution* 42:969-976.
- Zuk, M. 1992. The role of parasites in sexual selection: current evidence and future directions. *Adv. Study Behav.* 21:39-68. New York: Academic Press.
- Zuk, M., K. Johnson, R. Thornhill, and J. D. Ligon. 1990. Parasites and male ornamentation in free ranging captive red junglefowl. *Behaviour* 114:232-248.

ACKNOWLEDGEMENTS

We thank five anonymous reviewers, John Hunt, and Molly Morris for their extensive comments on earlier versions of this manuscript. We thank Kathryn Dufour, Dana Kolenich, Zachary Cronk, Sarah Endenburg, Savanna Boutin, Michelle Vala, and Katie Petrie for assisting with laboratory rearing, cricket care, egg counting and morphological measurements. We thank Carleton University for the Development Grant, the Office of the Dean of Science for partially supporting several Deans Summer Research Interns. Natural Sciences and Engineering Research Council (NSERC) of Canada's Discovery Grant #327856, NSERC Undergraduate Student Research Awards, the Canada Foundation for Innovation, and the Ontario Research Fund provided partial support for the research to SMB. National Science Foundation's Division of Integrative Organismal Systems Grant #1121797 also provided partial support for the research to PAG. The authors have no conflict of interest to declare.