

The Effects of the Operational Sex Ratio
on Female and Male Mate Choice in
the Jamaican Field Cricket (*Gryllus assimilis*)

by

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Abstract

The operational sex ratio (OSR; ratio of sexually available females to sexually available males) is a social environment that greatly influences mate choice, but how it concurrently affects mate choice in both sexes in species with mutual mate choice remains poorly understood. In this thesis, I experimentally investigated the potential influence of variation in the OSR (male-biased, even sex ratio, and female-biased) on which sex is the choosiest in the Jamaican field cricket, *Gryllus assimilis*. I also tested the consistency of individual mate choice as quantified using a dichotomous mate choice test and a full-interaction choice test under different OSR levels. OSR did not influence female or male mate choice, but it did influence how potential mates interact. Females and males did not consistently choose the same mate between the two types of mate choices tests, and OSR did not affect the likelihood of consistently choosing the same mate.

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Chapter 2: The Effects of the Operational Sex Ratio on Female and Male Mate Choice in
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Chapter 3: Validating Dichotomous Mate Choice Tests with Full-Interaction Choice
Tests in the Jamaican Field Cricket (*Gryllus assimilis*)

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I conducted the entirety of the experiments, collected all the data, analyzed the results, and wrote the chapters; however, Sue Bertram and Jean-Guy Godin both helped conceive the projects. Both co-authors provided comments and feedback on the chapters.

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Chapter 1

General Introduction

1.1 Mate choice

There are two recognized forms of sexual selection: intrasexual selection, gaining access to a mate through competition with a conspecific, and intersexual selection, attracting a mate with traits, resources, or displays (Darwin, 1871; Andersson 1994). These two forms of sexual selection are not however mutually exclusive, as armaments needed to win fights can also be utilized as ornaments to attract mates (reviews; Berglund et al., 1999; Wong & Candolin, 2005). Intersexual selection is driven by mate choice, a widely studied phenomenon that has been examined in numerous species from diverse taxa (Andersson, 1994). Mate choice is described as non-random mating owing to an individual being selected as a mate based on desirable morphological or behavioural traits, or their ability to access resources (Halliday, 1983; Andersson 1994).

Mate choice is most often studied from the female perspective, with females choosing males as mates based on preferences for particular traits that vary among males (Darwin, 1871; Andersson 1994). Female mate choice is ubiquitous in nature, likely because females often contribute more to their offspring (e.g. parental care, gametes) than males, and as a result they have lower reproductive potential and shorter reproductive windows during a given mating season (Andersson, 1994). Because females are only available as mates for part of the mating season, the sex ratio becomes male biased, setting the stage for males to compete for access and females to be choosy (Emlen & Oring, 1976). Researchers are starting to investigate how often male mate choice occurs and it is becoming widely accepted that male-mate choice often co-occurs in mating systems where females are the primary choosers (Cage & Bernard, 1996; Bonduriansky, 2001; Bateman & Ferguson, 2004; Reading & Blackwell, 2007; Ala-Honkola et al., 2010;

Barry & Kokko, 2010; Jeswiet & Godin, 2011; Edward & Chapman, 2011). In certain systems, sex-role reversal even occurs, where males are the primary choosers and females must compete, display desirable traits and/or provide resources to gain access to mates (Gwynne, 1991). For some species, such as seahorses (*Hippocampus guttulatus*; Naud et al., 2009), pipefish (*Nerophis ophidion*; Rosenqvist, 1990), and Eurasian dotterel (*Charadrius morinellus*; Owens et al., 1994), males are almost always the primary choosers, whereas for other species (e.g. *Metaballus* sp.; Gwynne, 1985) sex-role reversal appears to be due to environmental factors, such as food limitations.

1.2 Importance of the social environment

Variations in social environment can also cause sex-role reversal by limiting one sex over another (Gwynne, 1991). The operational sex ratio (OSR) is the ratio of sexually available females to sexually available males in a given population (Emlen, 1976). In a male-biased environment, females are the limiting sex and thus a limited resource, enabling females to be the choosiest of the sexes, and the larger the shortage of females, the more intense the sexual selection on the males (Emlen & Oring, 1977). While the OSR can cause the more abundant sex to be more competitive and the less abundant sex to be choosier in many species (Lawrence, 1986; Jirotkul, 1999; Souroukis & Murray, 1993; Souroukis & Murray, 1995; Balshine-Earn, 1996; Tregenza & Wedell, 1998; Wagner, 2005; Weissman et al., 2009; Clark & Grant, 2010; Tinghitella et al., 2013), the more abundant sex unexpectedly becomes choosier in other species (Arnqvist, 1992; Klemme et al., 2007; Naud et al., 2009). These studies indicate that conflict amongst conspecifics, such as male-competition and female harassment, can interfere with mate

choice and distort the effects of OSR (Arnqvist, 1992; Klemme et al., 2007; Naud et al., 2009). For example, with a male-biased OSR, females can experience intense sexual harassment from the more abundant males and, as a result, females may mate at random and exhibit very little choice (Arnqvist, 1992). A number of other factors can interact with OSR, such as the cost of breeding and mortality rates, which then influence the impact of OSR on mate choice (Kokko & Monaghan, 2001). Thus, a number of shifts in the social environment, OSR included, greatly influence the identity of the choosing sex and the relative strength of sexual selection on their mates.

1.3 Measuring mate choice

The environment and condition of the choosing individual can impact the results of mate choice tests (Wagner, 1998; Cotton et al., 2006). Various changes in the environment, such as population density (Atwell & Wagner, 2014; Holveck et al., 2015), the choosing individual's age (Atwell & Wagner, 2014), and previous exposure to potential mates (ten Cate, 1985; Shine et al., 2006) can all influence mate choice and the results obtained during mate choice tests.

The particular tests used to quantify mate choice can also determine the outcome obtained (Wagner, 1998). A traditional mate choice test that has been used in numerous studies is the “dichotomous mate choice test”. The choosing individual is referred to as the ‘focal’, who is simultaneously presented with two potential mates to choose from. The focal and the two potential mates are separated from each other with barriers (Wagner, 1998), limiting mating events and potential mates from interacting, which in turn limits the potential confounding effects of intrasexual selection on intersexual

selection (Wagner, 1998; Walling et al., 2010). Dichotomous mate choice tests typically use association time as a measure of mate choice (Wagner, 1998; Walling et al., 2010; Jeswiet and Godin, 2011), although in certain species this may not be an adequate indicator of their preferred mate (Gabor, 1999).

Some studies have sought to validate the use of association time in dichotomous mate choice tests by allowing the focal individual to fully interact with either their preferred or non-preferred mate in a no-choice test, following the dichotomous mate choice test (e.g. Walling et al., 2010; Loranger & Bertram, 2016a). No-choice tests are made up of two individuals, the focal and potential mate, thus eliminating the possibility of intrasexual selection and only test absolute preferences as there is no comparison available (Wagner, 1998). However, a meta-analysis of these tests revealed that the no-choice tests resulted in random mating behaviour, as the focal individuals would usually rather mate with their non-preferred mate than forego mating all together (Dougherty & Shuler, 2015). Other studies have performed dichotomous mate choice test followed by a full-interaction choice test, where both the previously preferred and non-preferred potential mates remain as choices for the focal individual. This full-interaction test allows for the interaction of intrasexual and intersexual selection, as multiple potential mates are present with the focal individual. For certain species, the interaction between male-male competition and female choice seem to work concurrently, with females preferring males in the dichotomous test who then proved themselves to be winners in the full-interaction tests (Kolluru et al., 2015). Alternatively, competition could interfere with mate preferences, as previously preferred mates may end up being losers, which should cause the choosing individual to mate with their previously non-preferred mate. Other forms of

conflict amongst conspecifics that can influence mate choice can also occur the full-interaction tests, such as mate guarding or sexual interference (Howard et al., 1997; Fuller, 2003). For example, in eastern tiger salamanders (*Ambystoma tigrinum tigrinum*), longer males actively interrupt courtship efforts of other males by shoving females away from the courting male in order to court the females themselves (Howard et al., 1997). Although using a no-choice test or a dichotomous choice tests can negate conflict amongst conspecifics, it may over simplify the mating system and lead to exaggerated results for certain traits (Wagner, 1998).

Many studies on fishes have sought to validate whether association time is an accurate indicator of mating behaviour in a full-interaction choice test. While some studies found that focal individuals were consistent with their choice of mates between both tests (Howard et al., 1998; Lehtonen & Lindström, 2008; Cummings & Mollaghan, 2006; Jeswiet & Godin, 2011; Kolluru et al., 2015), others found that association time was not an accurate indicator of the mate chosen during full-interaction tests (Fuller, 2003; Ala-Honkola et al., 2010). Intriguingly, there appears to be no studies that have examined dichotomous mate choice test with a full-interaction choice test in other classes of animals. This is surprising, as previous studies on insects, arachnids, crustaceans, amphibians, reptiles, and birds (see Dougherty & Shuler, 2015) have utilized dichotomous choice tests to quantify mate choice.

1.4 Model study species

To investigate the potential impact of OSR on mating behaviour and to characterize the relationship between the outcome of dichotomous mate choice tests and

full-interaction choice tests, I used the Jamaican field cricket *Gryllus assimilis* as a model study species. This species inhabits grassy habitats in certain Caribbean islands (Jamaica, Hispaniola, Grand Cayman), the United States (southern parts of Florida and Texas), Mexico and Central America (Weissman et al., 2009). Male *G. assimilis* field crickets generally attract females by signaling a long distance acoustic display that has an unusual high sound-pulse rate for a *Gryllus* spp. and that is species specific (Pollack & Kim, 2013); once a female finds a potential mate, the male switches its acoustic mate attraction display to an acoustic short-range display (Alexander, 1961). The courtship signal in *G. assimilis* is unusually variable in terms of pulses per chirp and ticks, however females are highly selective of chirp-pulse rates and are far more likely to mount a male who is able to signal a courtship song (Vedenina & Pollack, 2012). If the female chooses the male, she will climb onto the male's back (mount him), and the male will then wiggle his cerci in place to enable spermatophore transfer (Alexander, 1961). In field crickets, females are typically the primary choosers. Female *G. assimilis* prefer larger males as mates (Loranger & Bertram, 2016a), whereas males, who also exhibit mate choice, do not seem to prefer larger females (Bertram et al., in revision).

Natural variation in the OSR has been assessed for *Gryllus* crickets, with males ratios ranging from 30 – 80% depending on the species and season (Veazey et al., 1976). *Gryllus assimilis* is a flight-capable cricket (Guerra & Pollack, 2007), which has been found in the wild to aggregate *en masse* at artificial light sources (e.g. lamp posts), where the OSR has been shown to vary (Cade, 1979). To my knowledge, there have only been two previous studies on the OSR in field crickets. These studies revealed that male-biased environments increase male-male competition (*Gryllus pennsylvanicus*; Souroukis &

Murray, 1993), whereas female-biased environments reduce female choosiness (*Gryllus pennsylvanicus*; Souroukis & Murray, 1995). However, these studies did not evaluate male mate choice and did not control for total density. Nonetheless, they do suggest that the OSR may play an important role in mate choice in crickets.

Moreover, there appears to only have been one previous study on cricket mate choice using the dichotomous mate choice paradigm. This study on *G. assimilis* evaluated whether mate choice was consistent between a dichotomous choice test and a no-choice test (Loranger & Bertram, 2016a), and found that females are more likely to mount a male if she had previously preferred him during the dichotomous choice test, indicating that association time may be a reliable proxy for actual mate choice in crickets.

1.5 Thesis objectives

My thesis evaluates the effects of three OSR environments (male-biased, even distribution, female-biased) on mate choice and the consistency of mate choice between two different types of mate choice tests. In Chapter 2, I quantify whether OSR affects which sex is the choosiest by testing both females and males for their mate choice. I predict that, in a female-biased environment, males will be the choosiest sex and females will exhibit limited choice. Conversely, I predict that, in a male-biased environment, females will be the choosiest sex and males will exhibit limited choice. In Chapter 3, I test whether mating preferences revealed in a dichotomous choice test are reliable indicators of actual mate choice behaviour in a full-interaction choice test, and whether the OSR affects mate choice. I predict that females will be more likely to mount males that they prefer in dichotomous mate choice tests, and males will be more likely to court

females that they prefer in dichotomous mate choice tests. Additionally, I predict female-biased environments will result in females being less consistent in their choice of mates, whereas male-biased environments will result in males being less consistent in their choice of mates.

Chapter 2

**The Effects of the Operational Sex Ratio on Female and Male
Mate Choice in the Jamaican Field Cricket (*Gryllus assimilis*)**

2.1 Abstract

The operational sex ratio (OSR) is the ratio of sexually available females to sexually available males in a given population and should influence which sex exhibits the strongest mate choice. Theoretically, females in a male-biased OSR environment should be choosiest as they are the most limited sex. However, male mate choice can sometimes co-occur in mating systems wherein females are the primary choosers. I tested these predictions of OSR theory using the Jamaican field cricket (*Gryllus assimilis*) exposed to three different OSR environments (male-biased, even sex ratio, and female-biased). I ran 180 mate choice trials (30 for each sex and OSR environment). Contrary to expectation, the adult OSR environment did not affect female or male choosiness, nor did it influence which sex exhibited the strongest choice. The adult OSR environment did, however, influence the courtship behaviour and aggression of stimulus males, and female sexual interference. These results indicate that the social environment of the choosing cricket influences the behaviour of potential mates, but seemingly not their mating preferences.

2.2 Introduction

Mate choice has important implications for sexual selection and evolution, and has therefore been extensively studied in diverse taxa (Andersson, 1994). Mate choice is defined as non-random mating owing to any behavioural, chemical or morphological trait exhibited by an individual that increases its chances of mating (Halliday, 1983; Andersson, 1994). Traditionally, studies of mate choice have mostly focused on female preferences for males (Darwin 1871; Andersson 1994). Females are deemed the choosers and males the wooers, because females generally invest more energy and time in reproduction (e.g. gametes, parental care) and thus have lower reproductive potential than males (Darwin, 1871; Halliday, 1983). However, even in systems where females exhibit strong mate choice, both male and female mate choice can co-occur (Sargent et al., 1986; Johnstone et al., 1996; Edward & Chapman 2011). Males are more likely to exhibit mate preferences in species where males invest in parental care (Gwynne, 1991), encounter females simultaneously (Barry & Kokko, 2010) and incur mating costs (Bonduriansky, 2001), and where females vary in quality and mate multiply (Bonduriansky, 2001; Edward & Chapman 2011). Examples of males and females co-exhibiting mate preferences have been observed in several vertebrate (e.g. ten Cate 1985; Clayton, 1990; Jeswiet & Godin, 2011; Auld et al., 2016) and invertebrate species, including crabs (e.g. Reading & Backwell, 2007; Reaney, 2009), katydids (e.g. Bateman & Ferguson, 2004; Lehmann, 2012) and crickets (e.g. Souroukis & Murray, 1995; Gage & Bernard, 1996).

Mating preferences can be based on a number of individual traits and external factors, including physical condition, courtship displays and resources (Halliday, 1983). Mate preference results in sexual selection on an individual for their desirable trait or for

their ability to obtain resources required for reproduction by the choosing sex. The intensity of sexual selection should increase when one sex is less abundant than the other, because individuals of the limited sex become a desirable resource (Emlen & Oring, 1977). The abundance of potentially receptive females relative to the abundance of potentially receptive males in the population is the operational sex ratio (OSR) (Emlen, 1976). When the OSR is not even, the less prevalent sex is less available for mating and thus more likely to be the choosier of the sexes. For example, in the fall field cricket (*Gryllus pennsylvanicus*), the OSR is usually male-biased and females exhibit strong mate preferences (Souroukis & Murray, 1995). However, when the OSR is manipulated to become female-biased, females exhibit reduced mate preferences (Souroukis & Murray, 1995). Unfortunately, Souroukis & Murray (1995) did not investigate male mate preferences and so it is unclear whether male crickets exhibit mating preferences in the female-biased environment.

The influence of OSR on multiple aspects of reproduction has been previously investigated (see Appendix, Table 10). Previous studies have addressed the influence of OSR on sexual competition (Lawrence, 1986; Souroukis & Cade, 1993; Clark & Grant, 2010), courtship (Jirotkul, 1999; de Jong et al., 2009; Clark & Grant, 2010), sexual interference (Jirotkul, 1999; Knox & Scott, 2006), mate guarding (Alcock, 1994; Schofl & Taborsky, 2002; Know & Scott, 2006) and territoriality (Kodric-Brown, 1988). Surprisingly, however, studies evaluating the effects of OSR on mate choice are limited and their results inconsistent. In male-biased environments, OSR theory predicts that females should exhibit strong mate choice while males should not be very choosy. While these predictions have been supported by a number of studies (e.g., Souroukis & Murray,

1995; Balshine-Earn, 1996; Wagner, 2005; Weissman et al., 2009; Tinghitella et al., 2013), other studies reveal that females are less choosy (Arnqvist, 1992) and males are very choosy (Naud et al., 2009). Conversely, in female-biased environments, OSR theory predicts that females should exhibit reduced mate choice and males should exhibit strong mate choice. While these predictions have been supported in some studies (e.g., Lawrence, 1986; Souroukis & Murray, 1995; Tregenza & Wedell, 1998; Tinghitella et al., 2013), Klemme et al. (2007) observed that females became even choosier in a female-biased environment. Also contrary to the aforementioned predictions of OSR theory, Wang et al. (2009) found that males were mated more often in female-biased and male-biased environments compared to an even-sex ratio environment. One potential reason for the discrepancy in findings across studies may be the confounding effects of total density, as many of the aforementioned studies did not control for total density in their experimental design. Further, none of the aforementioned studies evaluated OSR in both sexes concurrently, which limits their power to evaluate the overall effects of OSR on mate choice.

Here, I use Jamaican field crickets (*Gryllus assimilis*) to test whether and how the OSR environment concurrently influences female and male mate choice. Male field crickets produce an acoustic long distance signal to attract females, switching to an acoustic shorter-range courtship signal when a female arrives (Alexander, 1961). The crickets then antennate each other to detect chemosensory cues, such as cuticular hydrocarbons, to inform their mate choice (Thomas & Simmons, 2009). If the female decides to mate, she mounts the male and he then transfers his spermatophore to the female (Alexander 1961). Female Jamaican field crickets prefer larger males (Loranger &

Bertram 2016a) and males that produce the loudest acoustic mate attraction signals (Pacheco & Bertram, 2014). Weak male mate choice in field crickets may also be expected as there is variability in female quality (Gray et al., 2001), females gain fitness benefits from mating with multiple males (Kvarnemo & Simmons, 1999), and the production of a spermatophore is costly to males (Berglund, 1994). Male mate choice has been documented in *G. assimilis*, but it remains uncertain what males are selecting for (Bertram et al., in revision). Males should prefer larger females because larger females generally lay more eggs (Loranger & Bertram, 2016b) and, in doing so, choosy males stand to gain a possible fitness advantage.

The sex ratio in *Gryllus* field crickets varies naturally, ranging from 30 to 80% males, depending on the species, time of day, and time of year (Veazey et al. 1976).

Gryllus assimilis is a flight-capable cricket (Guerra & Pollack 2007), and like the Texas field cricket, *Gryllus texensis*, it aggregates near light sources (e.g. lamp posts) at night where sex ratios continuously vary (Cade, 1979). While the effects of OSR have been studied on female mate choice in a *Gryllus* species (*G. pennsylvanicus*, Souroukis & Murray 1995), they have not yet been tested on male mate choice in a *Gryllus* species.

I investigated the effects of three different OSR treatment levels on female and male mate choice in *G. assimilis* (male-biased (6 males to 2 females), even-sex ratio (4 males to 4 females), and female-biased (2 males to 6 females)). I hypothesize that OSR will affect female and male mate choice. I tested three predictions: First, in a male-biased environment, females should exhibit strong mate choice and require more courtship effort from the males prior to mating, whereas males should exhibit very little mate choice and actively court any available female. Second, in a female-biased environment, females

should exhibit reduced mate choice and require less courtship effort from males prior to mating, whereas males should exhibit mating preferences by being selective about which females that they court. Third, in an even-sex ratio environment, females should be more choosy than males as their eggs require more energy to produce and therefore have lower reproductive potential than males.

2.3 Methods

2.3.1 Study species

I tested the effect of varying OSR on female and male mate choice in the Jamaican field cricket, *Gryllus assimilis*. A laboratory population was established at Carleton University in 2008 from wild crickets collected around Stengl Lost Pines Biological station of the University of Texas in Austin, Texas, USA (Bastrop County, Texas, latitude $\sim 30^{\circ} 17' N$, longitude $\sim 97^{\circ} 46' W$, elevation $\sim 145m$) by importing eggs and adults. The crickets are housed in large plastic containers in a greenhouse in the Department of Biology at Carleton University, which is maintained on a 14h L: 10h D illumination cycle and at $25 \pm 2^{\circ}C$. The crickets are provided with *ad libitum* water and food (powdered Harlan Teklad Inc. Rodent diet no. 8640M) and egg cartons for shelter.

2.3.2 Experimental design

I collected adult virgin crickets who were within two days of achieving their imaginal moult, and housed them in one of three OSR treatments: male biased (6 males: 2 females), even-sex ratio (4 males: 4 females), or female biased (2 males: 6 females). The

OSR treatment bins (35.6 x 19.0 x 15.9 cm, L x W x H) held the sexes in separate compartments, but they could contact each other through a perforated divider. Separate compartments ensured that all individuals remained virgins. Each compartment was sized so that it was density controlled (area per individual was kept constant). The treatment bins were housed in acoustic isolation bins, which consisted of large bins (64 x 40 x 42 cm, L x W x H) lined with acoustic foam on all sides. The acoustic foam greatly reduced the chance that individuals would hear male mate attraction signals from nearby treatment bins, potentially confounding the perceived OSR environment. I maintained the crickets in these bins on the same light-dark cycle and temperature as they experienced during development. I kept these treatment crickets in their OSR environment for two weeks and then tested their mating preferences.

Crickets in the OSR treatment bins were used as the ‘focal crickets’ in the mate choice trials (see below). I tested focal crickets for their mating preferences towards ‘stimulus’ crickets. The stimulus crickets were the same age as the focal crickets, and had been housed in medium-sized bins (22 x 15 x 17 cm, L x W x H) with 4 crickets of the same sex. I created one female stimulus cricket bin and one male stimulus cricket bin for every OSR treatment bin.

A total of 69 deaths occurred during the experiment. Death occurred in 48 of the bins and 83% of the individuals that died were male. The highest percentage of deaths occurred in male stimulus cricket bins (36%). When a death occurred, I added a replacement individual to the bin. I collected an adult individual from the general population bin, painted it with red nail polish on its pronotum to allow easy identification later, and placed it into the bin that had lost the individual, thereby ensuring that the OSR

environment was kept constant. These replacement crickets were never used as focal or stimulus individuals in the mate choice trials.

Two days prior to the mate choice trials (day 12), focal and stimulus crickets were assigned ID numbers, weighed, and painted with nail polish on their pronotum to facilitate identification; colours were randomly assigned. Trials were performed two weeks after the crickets entered into their OSR environments (day 14). I chose the focal crickets at random from their respective OSR treatment bins. The stimulus crickets were of the opposite sex and were also chosen at random. I carried out up to 4 trials (2 male choice, 2 female choice) on any given test day, and thus up to 4 focal crickets could originate from a single OSR treatment bin.

I performed mate choice trials between 06:00 and 10:00 hrs because preliminary findings revealed that *G. assimilis* males called most often during this period of day. I ran trials in an acoustically isolating chamber (142 x 79 x 96.5 cm, L x W x H), with a low-intensity light source, a max-min thermometer to ensure than the ambient temperature remained consistently near 25°C (range 23-27°C), and a mount for a video camera. I recorded the trials using an overhead Go-Pro Hero 4 Silver camera, which could be remotely controlled with a Samsung Galaxy 2 tablet via the Go Pro App.

The mate choice trials consisted of four parts: an acclimation period; two consecutive dichotomous-mate choice tests, followed by a full-interaction choice test. The dichotomous-mate choice arena was constructed of corrugated plastic and contained three chambers during the dichotomous-mate choice tests: the focal cricket area (30 x 15 x 30 cm, L x W x H) where the focal cricket could roam, and two adjacent stimulus cricket areas (15 x 15 x 30 cm, L x W x H) for the stimulus crickets to roam (Figure 1).

The focal cricket area was separated from the two stimulus cricket areas by a transparent perforated Plexiglas partition, which allowed the transmission of chemical cues and antennation between focal and stimulus crickets to occur. The focal cricket area also had a zone (7 x 7 cm, L x W) outlined in front of each stimulus cricket area, referred to as the mate choice zones A or B. The stimulus cricket areas were separated by an opaque corrugated plastic divide, which prevented the stimulus crickets from seeing each other. The full-interaction choice test was run in the same arena, but I removed the dividers that separated the focal cricket from the stimulus crickets and the stimulus crickets from one another, creating an open area and allowing for full interactions (30 x 30 x 30 cm, L x W x H).

I performed 180 trials, comprised of 90 male choice trials (30 for each OSR treatment) and 90 female choice (30 for each OSR treatment) trials. Each trial was performed in the following order: a 5-min acclimation period during which all three crickets were in their respective zones in the arena, with the focal cricket temporarily covered by an opaque cup; the cup was lifted, allowing the focal cricket to roam and the first dichotomous mate choice test began; after 15 min, the focal cricket was once again covered by the opaque cup and the locations of the paired stimulus crickets were swapped to avoid any side bias; the cup was then lifted and the second dichotomous mate choice test began; after 15 min, the focal cricket was once again covered by the cup, and the dividers were lifted to create an open-field arena; the cup was lifted and the 15-min full-interaction choice test was carried out.

Following these consecutive choice tests, I collected all three crickets in small plastic bags and euthanized (froze) them for later photography and measurements of

individual body size. I photographed individual frozen crickets dorsal side up with a Panasonic Lumix ZS40 camera. The relative body size of each cricket was measured using *ImageJ* (<https://imagej.nih.gov/ij/>); three traits were measured (in mm), namely, pronotum length, pronotum width, and head width. I tested my photographing and measuring techniques to ensure a high reliability (>99% concordance).

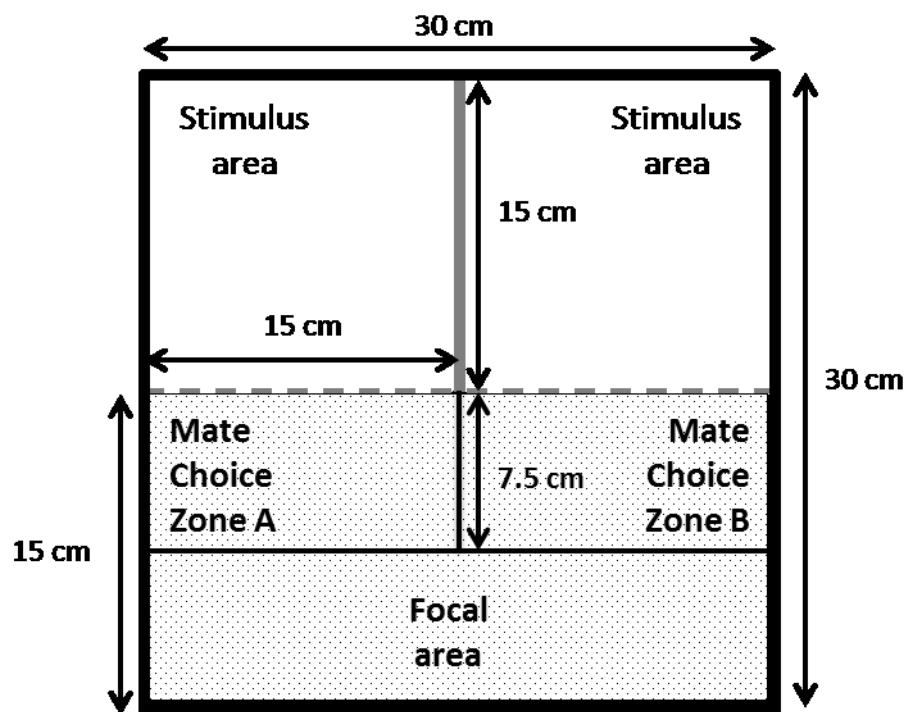


Figure 1: Top view of the dichotomous mate choice arena with dimensions; the dark gray lines indicate the removal divides, the dashed lines represent the perforated divide between the stimulus cricket areas and the focal cricket area, the dotted area shows the entirety of the focal cricket area

2.3.3 Video analysis

To avoid any bias, I scored all the videos blindly of the OSR treatment of the focal crickets. I viewed the dichotomous mate choice videos using a VLC video program and the following behaviours were quantified: first choice zone entered, latency to enter either choice zone, time spent in each choice zone. I converted the full-interaction videos using Go Pro Studio and Final Cut Pro to trim, zoom in, and reduce the speed by 50% to facilitate more accurate scoring of behaviours. I then watched the videos using Mac Media Player and quantified the behaviour described in Table 1. Due to the position of the camera (directly above the arena), it was not possible to confirm if a spermatophore had been transferred to the female and therefore, during photographing, the female crickets were turned dorsal side down to determine whether or not they had a spermatophore attached. If a female had a spermatophore attached and she had mounted a male, the time she spent mounting was also noted as time spent mating. In female mate choice trials, if the female mounted both males and she had a spermatophore attached, it was not possible to determine which male provided the spermatophore, and thus time spent mounting was also noted as time spent mating for both males.

Table 1: Behaviours quantified during the full-interaction choice trials.

Behaviour	Description	Measurement
Latency to court	Time from start of trial for a male to begin courting a female	Time in seconds
Latency to mount	Time from start of trial for a female to mount a male	Time in seconds
Court	Male plants feet after making antennal contact with a female and begins stridulating and/or his courtship signal	Total time spent in seconds
Mount	Female climbs onto a male's back	Number of events Total time spent in seconds Number of events
Same sex sexual behaviour	Male courts an other male; male mounts an other male; female mounts an other female	Yes or No
Maximum aggression score	The maximum aggressive score reached during the trial; see Loranger & Bertram (2016a) for details on scoring	1-5
Overall winner	The winner of the majority of the aggressive encounters	ID of winner
Sexual interference	Stimulus cricket physically rams into, kicks, and/or bites a mated pair	Total time spent in seconds
Titillation	Stimulus male courts a mated pair after having made antennal contact with them	Total time spent in seconds

2.3.4 Statistical analysis

I quantified individual body size using a principal component analysis (PCA) on pronotum length, pronotum width, and head width, because these three traits were inter-correlated. PC1's were strong composite measures of overall body size (female eigenvalue = 2.609 eigenvalue; male eigenvalue = 2.816) and explained 87% and 94% of observed variation for females and males, respectively. All three-body size measures were weighted equally on these PC1's.

I calculated a difference score for the dichotomous mate choice test by subtracting the combined time spent in front of the larger stimulus cricket from the combined time spent in front of the smaller cricket. I also calculated separate difference scores for courting and mounting behaviours in the full-interaction mate choice test by subtracting the value directed towards the larger stimulus cricket from that directed towards the smaller stimulus cricket. A positive difference score indicates a preference for the larger stimulus cricket, whereas a negative difference score indicates a preference for the smaller stimulus cricket (*sensu* Jeswiet & Godin 2011). I used independent one-sample *t*-tests to ascertain whether focal crickets preferred larger stimulus crickets by comparing the mean difference score for each sex and OSR treatment group independently (6 in total) against no preference (i.e. null difference score = 0).

I evaluated female and male choice trials separately. I converted the categorical OSR treatments into continuous variables by diving the total number of males by the total number of females in a treatment bin (male-biased = 0.25, even distribution = 0.5, female-biased = 0.75). I converted OSR into a continuous variable as Kokko and Monaghan (2001) show that OSR is a continuous variable in their model for determining

choosiness. I ran a general linear mixed model for each dependent variable with the following independent continuous variables: OSR treatment, focal cricket PC1, the relative weight of the focal cricket to its OSR bin, the average PC1 of the stimulus crickets, and the difference in PC1 of the stimulus crickets as covariates, and the bin number as a random effect variable (since more than one focal cricket could come from the same bin). The maximum aggression score was initially added to the overall general mixed model as an independent variable; however, because it did not change the significance of any of the results, it was subsequently excluded for the final model. To test for normality I ran Shapiro-Wilk tests and visually inspected the distributions of residuals and no obvious deviations were apparent. I obtained p-values using likelihood ratios tests (*sensu* Winter 2013). Results are presented with the estimates and their standard error (Est. \pm SE), chi squared values with degrees of freedom (χ^2_1), p values, and number of trials (N). All statistical analyses were done using RStudio (R Core Team, 2014) and *lme4* (Bates et al., 2014). I ran comparisons of the different preference scores against the independent variables for each sex and OSR treatment group (6 in total) using the Pearson's correlation test (r). The p values were then corrected using the false discovery rate (BH) procedure (Benjamini & Hochberg, 1995).

In the dichotomous mate choice test, three focal females and three focal males did not move into either of the two choice zones. These trials were excluded from the dichotomous mate choice test analyses. In the full-interaction choice test, two focal females were not courted and five focal males did not court. These trials were similarly excluded from the full-interaction choice test analyses.

2.4 Results

2.4.1 The effects of OSR in the dichotomous mate choice test

The OSR did not influence mating behaviour in the dichotomous mate choice tests. The OSR of the focal female did not affect the speed at which females entered a choice zone ($\text{Est.} \pm \text{SE} = 25.144 \pm 86.549$, $\chi^2_1 = 0.083$, $p = 0.773$, $N=87$), nor did it affect time spent in a choice zone ($\text{Est.} = -116.88 \pm 210.546$, $\chi^2_1 = 0.308$, $p = 0.579$, $N=87$). Similarly, the OSR of the focal male did not affect the speed at which males entered a choice zone ($\text{Est.} = -68.95 \pm 97.393$, $\chi^2_1 = 0.5$, $p = 0.48$, $N=87$), nor did it affect the time spent in a choice zone ($\text{Est.} = 49.279 \pm 200.607$, $\chi^2_1 = 0.06$, $p = 0.806$, $N=87$).

2.4.2 The effects of OSR in the full-interaction choice test

The OSR did not influence male or female choosiness in the open arena mate choice tests. The OSR of the focal female did not significantly influence the female's latency to mount (Figure 2), the number of times she was courted prior to mounting (Figure 3), the number of times she mounted, the time she spent mounting, or whether she mounted both males (Table 2). In the male full-interaction choice trials, the OSR of the focal male did not affect his level of choosiness. The OSR did not significantly influence whether males courted faster (Figure 4), more often, or for longer periods of time (Figure 5) (Table 3).

OSR did, however, affect the behaviour of the stimulus crickets. The OSR of the focal female influenced how male stimulus crickets interacted and the OSR of the focal

male influenced how female stimulus crickets interacted. Stimulus males courted the focal female more frequently and for a longer period of time if her adult OSR environment was female-biased (Table 2), and when corrected using the false-discovery rate (BH; Benjamini & Hochberg, 1995), it was still significant (*total time focal cricket was courted*: p-adjusted = 0.048; *total number of times focal cricket was courted*: p-adjusted = 0.05). Similarly, female stimulus crickets were significantly more likely to interfere with each other when one of them was mounting the focal male if he was from a female-biased environment compared to the other OSR treatments (p-adjusted = 0.048) (Table 3).

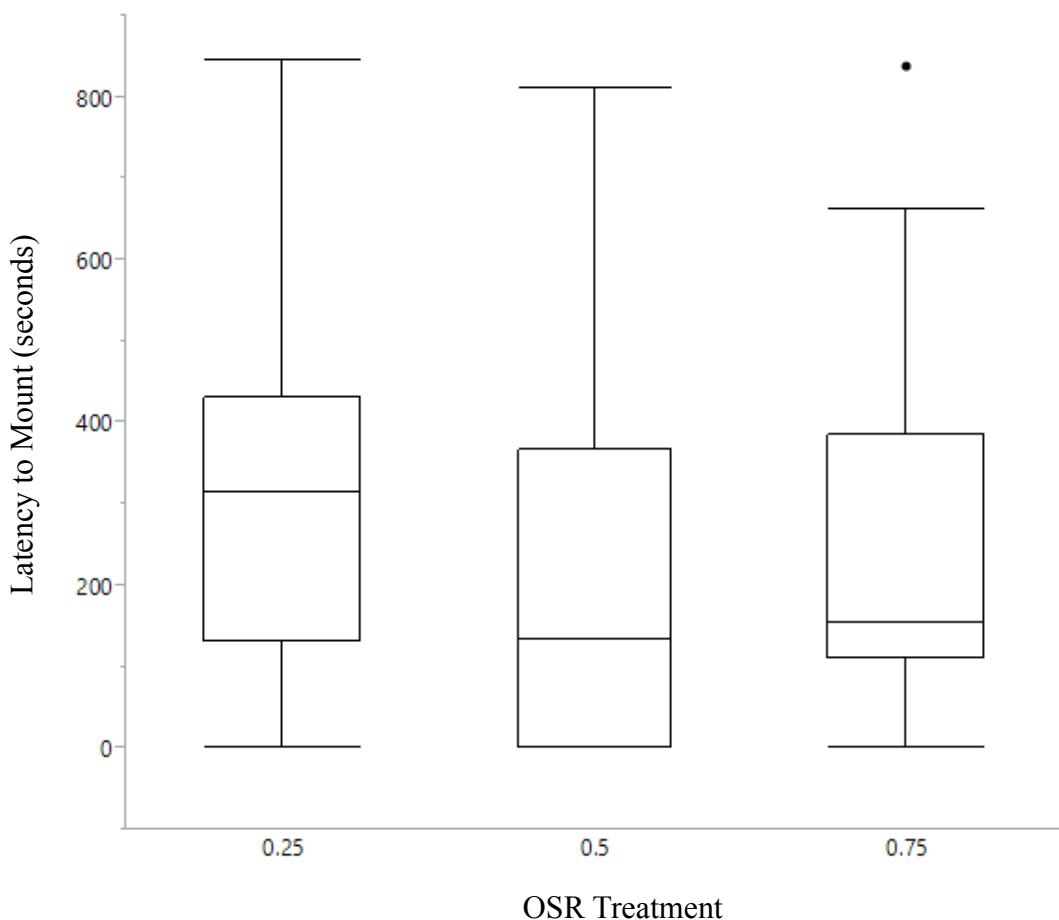


Figure 2: Latency of focal females from different OSR treatments to mount: 0.25 = male-biased, 0.5 = even ratio, 0.75 = female-biased.

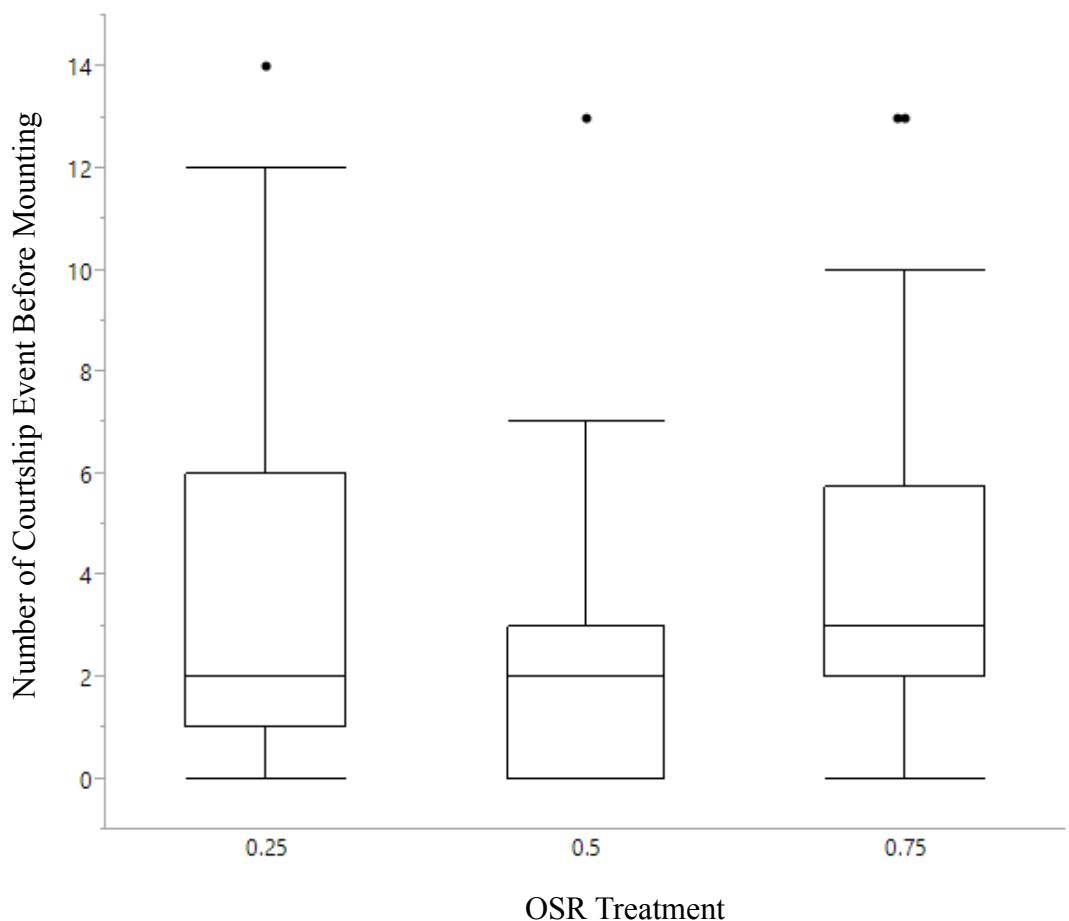


Figure 3: The number of courtship events before a focal female from different OSR treatments would mount; 0.25 = male-biased, 0.5 = even ratio, 0.75 = female-biased.

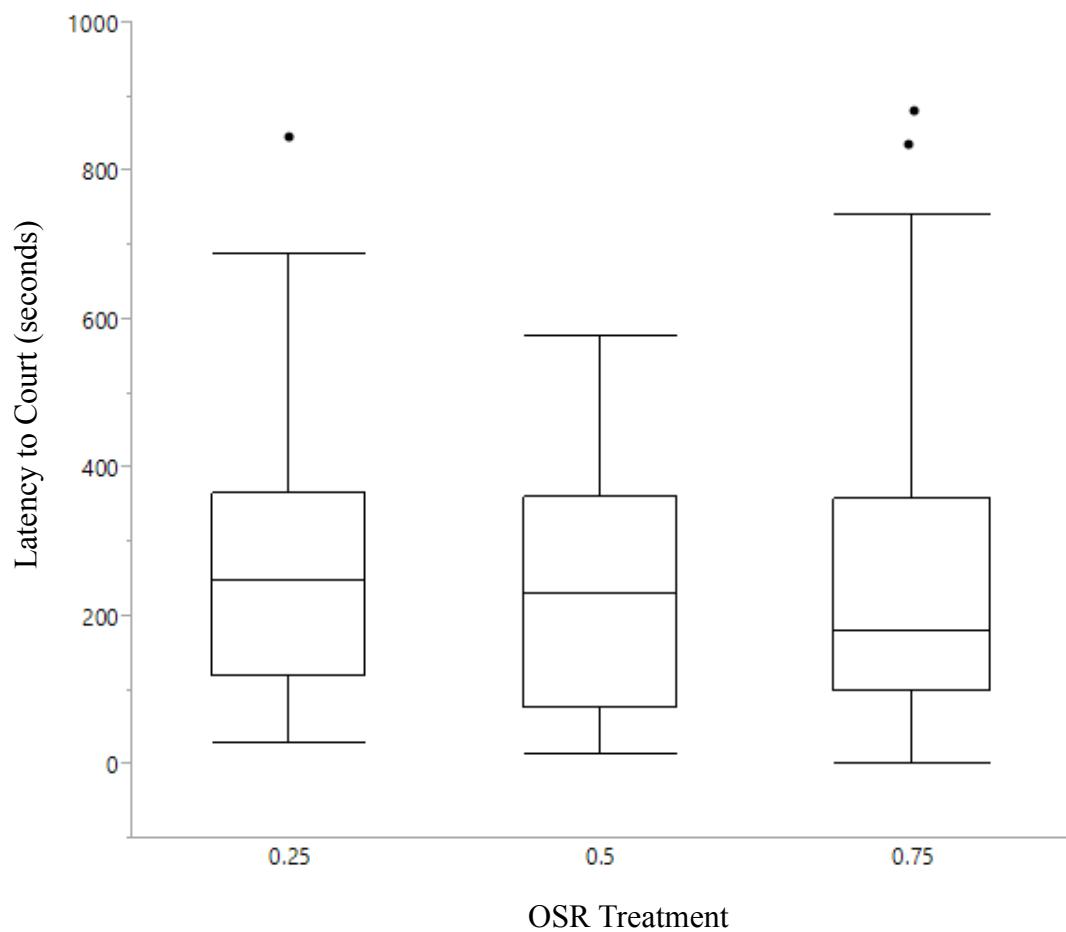


Figure 4: The latency for focal males from different OSR treatments to court; 0.25 = male-biased, 0.5 = even ratio, 0.75 = female-biased.

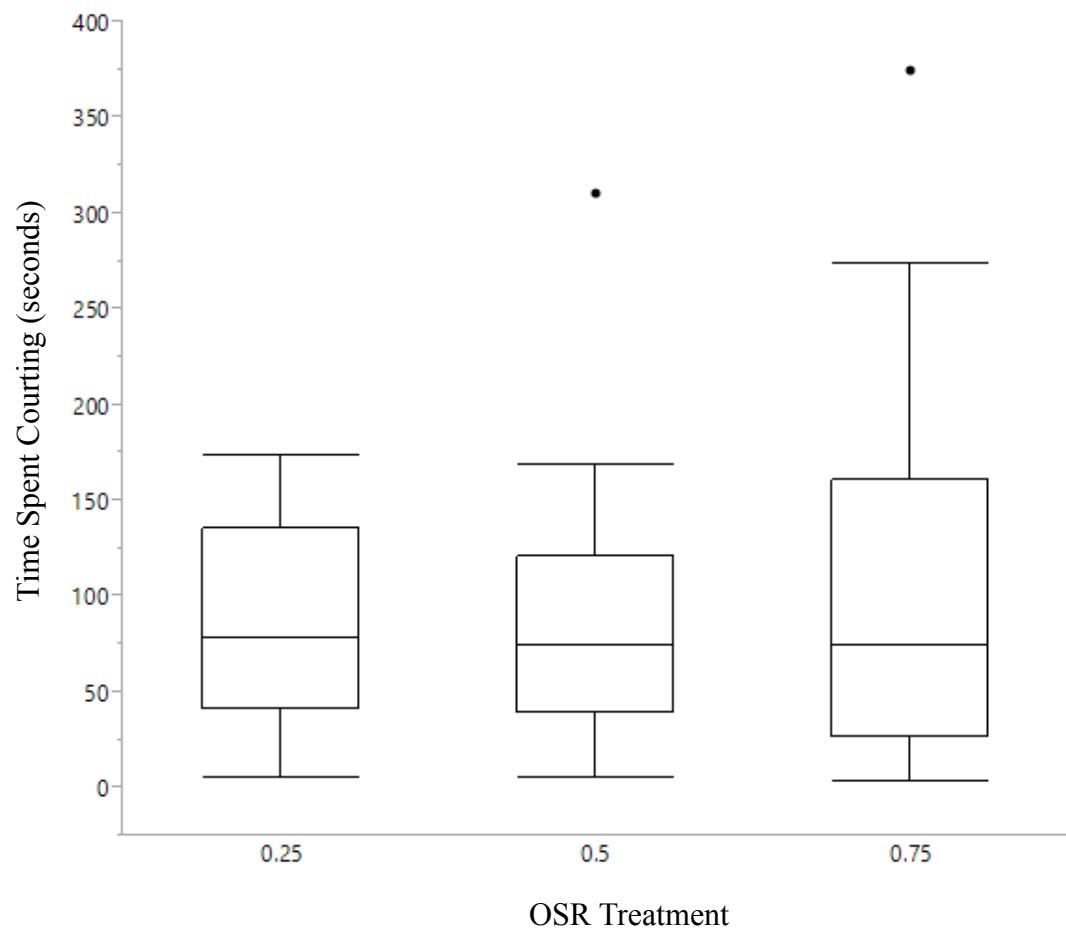


Figure 5: The time focal males from different OSR treatment spent courting: 0.25 = male-biased, 0.5 = even ratio, 0.75 = female-biased.

Table 2: The effects of the OSR on female mate choice in the full-interaction choice trials. Negative model estimate values indicate a trend toward male-biased OSR, and positive values indicate a trend toward female-biased OSR. Boldfaced p-values are statistically significant ($p < 0.05$) and bolded if they are significant once corrected using the false discovery rate (BH).

	Est.	± SE	χ^2_1	p	BH p adjusted
Latency to be courted	-77.548	92.505	0.7	0.403	0.58
Latency to mount	-1.169e+02	1.210e+02	0.928	0.335	0.58
Time from courtship to mounting	-57.87	69.131	0.698	0.404	0.58
Same-sex sexual behaviour	0.3778	0.254	2.174	0.14	0.49
Stimulus crickets maximum aggression score	-1.654	0.694	5.395	0.02*	0.048*
Total time focal cricket was courted	144.79	63.036	5.124	0.024*	0.048*
Total number of times focal cricket was courted	13.1	6.585	3.846	0.05*	0.05*
Number of times focal cricket was courted before mounting	3.651	1.817	0.1	0.753	0.811
Total time focal cricket mounted	6.826	10.113	0.443	0.506	0.644
Number of times focal cricket mounted	0.717	0.704	1.031	0.31	0.58
Both Stimulus crickets mounted	1.085e-01	2.577e-01	0.177	0.674	0.786
Total time focal cricket successfully mated	9.382	10.201	0.842	0.359	0.58
Total time stimulus crickets interfered	0.331	2.637	0.016	0.9	0.9
Total time stimulus crickets titillated	3.785	4.623	0.666	0.414	0.58

Table 3: The effects of the OSR on male mate choice in the full-interaction choice trials. Negative model estimate values indicate a trend toward male-biased OSR, and positive values indicate a trend toward female-biased OSR. Boldfaced p-values are statistically significant ($p < 0.05$) and bolded if they are significant once corrected using the false discovery rate (BH).

	Est.	± SE	χ^2_1	p	BH p adjusted
Latency to court	-49.215	114.556	0.184	0.668	0.729
Latency to be mounted	-58.175	135.064	0.185	0.667	0.729
Time from courting to mounting	98.022	84.99	1.319	0.251	0.588
Same-sex sexual behaviour	-1.559e-01	1.762e-01	2.496	0.114	0.456
Stimulus crickets maximum aggression score	-0.22	0.509	0.187	0.666	0.729
Total time focal cricket courted	63.291	39.46	2.534	0.111	0.456
Total number of times focal cricket courted	1.083	5.595	0.038	0.847	0.847
Number of times courted before being mounted	4.231	2.371	3.006	0.083	0.456
Total time focal cricket was mounted	15.67	14.882	1.101	0.294	0.588
Total number of times focal cricket was mounted	0.623	0.744	0.7	0.403	0.691
Both stimulus crickets mounted	0.19	0.171	1.335	0.248	0.588
Total time focal cricket successful mated	-7.186	11.095	0.419	0.518	0.729
Total time stimulus crickets interfered	2.539	1.195	4.4	0.036*	0.048*

2.4.3 Preference for body size

Overall, mating preferences varied widely among focal females and focal males across all three OSR treatments. Both sexes had certain individuals that showed strong preferences for larger mates and other individuals that strongly preferred smaller mates. Irrespective of OSR treatment, females on average did not exhibit a significant preference for either larger or smaller mates in the dichotomous mate choice trial (Table 4). Similarly, males also did not show a significant preference for larger or smaller mates in the dichotomous mate choice trial (Table 4), although there was a non-significant trend for males to prefer smaller females in the female-biased OSR ($t_{27} = -1.901$, $p = 0.068$).

In the full-interaction choice trial, focal females did not significantly prefer to mount larger or smaller males (Table 4). Similarly, focal males did not significantly prefer to court larger or smaller females (Table 4).

Table 4: Focal female and focal males preference scores for the dichotomous mate choice trials, mounting behaviour, and courtship behaviour in the full-interaction choice trials.

	OSR	df	t	p	BH p adjusted
Focal Female					
Dichotomous Mate Choice Trial	0.25	28	-0.641	0.527	0.791
	0.5	28	0.04	0.968	0.968
	0.75	28	-0.82	0.419	0.791
Mount	0.25	29	0.428	0.672	0.672
	0.5	29	-0.546	0.589	0.672
	0.75	27	1.521	0.140	0.42
Focal Male					
Dichotomous Mate Choice Trial	0.25	29	0.65	0.521	0.521
	0.5	28	1.044	0.305	0.458
	0.75	27	-1.901	0.068	0.204
Court	0.25	28	-0.369	0.715	0.715
	0.5	28	0.371	0.713	0.715
	0.75	26	0.838	0.409	0.715

2.4.4 Comparisons between preference scores and independent variables

There was a significant positive relationship between the mating preference scores of focal females in the dichotomous mate choice test and the difference between stimulus crickets' PC1 values for females from a female-biased OSR (Pearson's correlation, $r = 0.408$, $t_{28} = 2.319$, $p = 0.028$), however when corrected for false discovery rates, the p value was no longer significant (BH p-adjusted = 0.084) (Figure 6). This suggests there is a non-significant trend for focal females from a female-biased environment to prefer a larger male provided there was a relatively large difference in body size between stimulus males. There was no significant relationship for focal females from the other OSR treatments (Table 5). There was no significant relationship in the full-interaction choice test between the focal cricket preference scores and the difference between the stimulus crickets' PC1 values (Table 5). There was no significant relationship between the difference between stimulus crickets' PC1 values value and preference scores during the dichotomous mate choice tests and the full-interaction choice trials for male mate choice trials for focal males from any OSR treatment (Table 6). Overall, these findings suggest that focal crickets are not generally more likely to prefer a mate based on their body size, regardless of the difference in body size between the paired stimulus crickets.

Female preference scores were not significantly correlated with focal female body size (PC1 scores) either in the dichotomous mate choice test (Table 6) or in the full-interaction choice test (Table 5). The dichotomous mate choice test preference scores of the focal males were significantly positively correlated with focal male's PC1 value when males were from a female-biased OSR ($r = 0.482$, $t_{26} = 2.805$, $p = 0.009$, BH adjusted $p = 0.027$) (Figure 7), but not from any other OSR treatments (Table 6). This indicates that

larger focal males from a female-biased environment were more likely to prefer a larger female. There was no significant relationship between focal male PC1 value and preference scores during the full-interaction choice trials (Table 5). Overall, these findings suggest that only large male crickets exposed to a female biased OSR exhibit mating preferences for larger females.

There was a near-significant negative relationship between the dichotomous mate choice test preference scores of focal females from a female-biased environment and the focal females relative weight compared to the average weight of their OSR treatment bin, ($r = -0.368$, $t_{27} = -2.054$, $p = 0.05$, BH p-adjusted = 0.15). However, this relationship disappeared after false discovery rate adjustment, and there was no significant relationship in the other OSR treatments (Table 5). There was a significant positive relationship between the dichotomous mate choice test preference scores of focal males from a female-biased environment and the focal males relative weight compared to the average weight of their OSR treatment bin ($r = 0.488$, $t_{26} = 2.9\sqrt{853}$, $p = 0.008$, BH p-adjusted = 0.024) (Figure 8). This result reveals that relatively heavier males were more likely to prefer larger females provided they are in a female biased environment. The relationship did not occur in the other OSR treatments. There was no significant relationship between focal crickets preference scores in the open arena choice trials and the focal crickets relative weight compared to the average weight of their OSR treatment bins (Table 6). Overall, the relative weight of the focal crickets only affected the likelihood of them preferring a mate based on their body size if they were from a female-biased environment but only during the dichotomous mate choice tests.

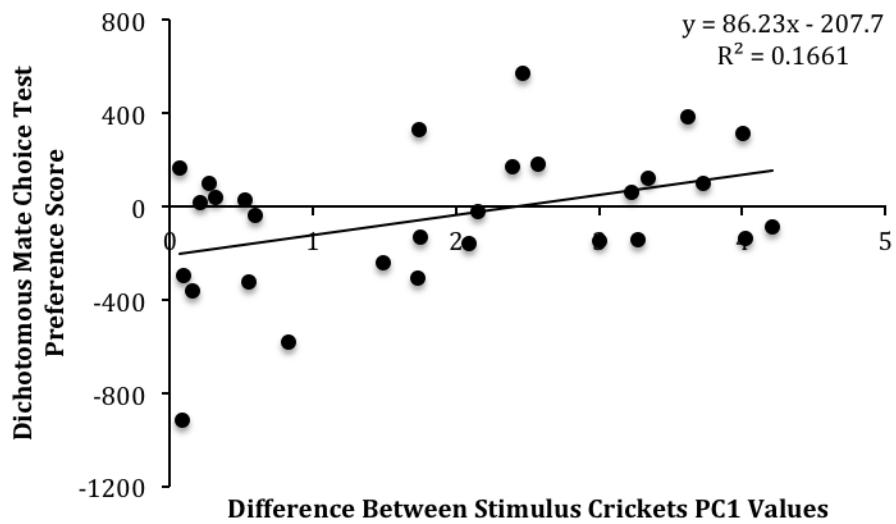


Figure 6: Relationship between the dichotomous mate choice test preference scores and the difference between stimulus crickets PC1 values for females from a female-biased environment: trend line, linear equation and R^2 values displayed.

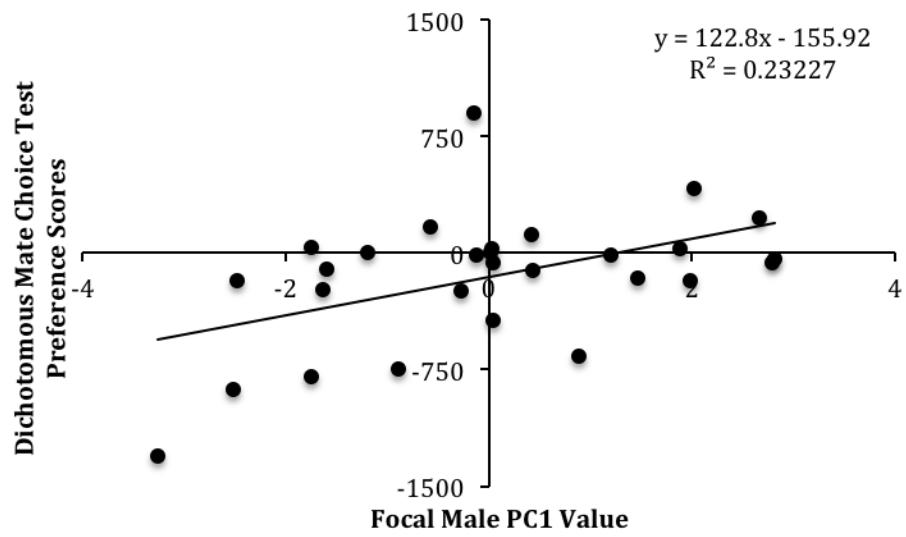


Figure 7: Relationship between the dichotomous mate choice test preference scores and focal male PC1 values for males from a female-biased environment: trend line, linear equation and R^2 values displayed.

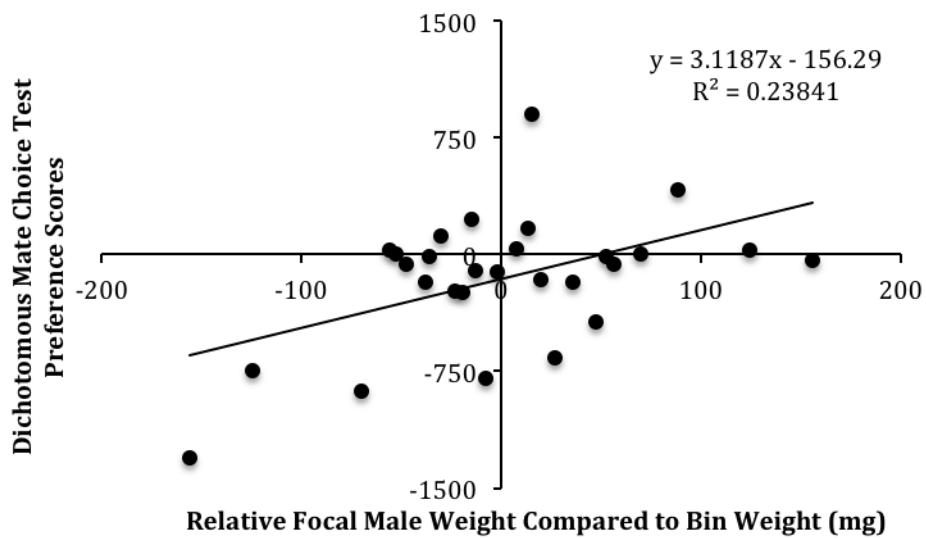


Figure 8: Relationship between the dichotomous mate choice test preference scores and relative focal male cricket weight compared to bin weight for focal males from a female-biased environment. Trend line, linear equation and R^2 values are displayed.

Table 5: Results of focal female Pearson's correlation tests between the different independent variables, the dichotomous mate choice test (DMCT) preference scores, and the courtship preference scores. Significant values ($p < 0.05$) are indicated with an *, and bolded if they are significant once corrected using the Benjamini & Hochberg (BH) false discovery rate.

Independent variables	Preference Score	OSR	df	t	r	p	BH p adjusted
Difference Stimulus PC1	DMCT	0.25	27	-0.008	-0.042	0.967	0.967
		0.5	27	-0.199	-1.057	0.3	0.45
		0.75	27	0.408	2.319	0.028	0.084
	Mount	0.25	28	0.339	1.904	0.067	0.201
		0.5	28	-0.009	-0.049	0.961	0.961
		0.75	26	-0.094	-0.4812	0.634	0.951
Focal PC1	DMCT	0.25	27	-0.226	-1.2038	0.239	0.359
		0.5	27	0.345	1.9069	0.067	0.201
		0.75	27	-0.098	-0.5105	0.614	0.614
	Court	0.25	28	-0.036	-0.188	0.852	0.862
		0.5	28	0.033	0.175	0.862	0.862
		0.75	26	0.15	0.773	0.446	0.862
Relative Focal Weight to Bin Average Weight	DMCT	0.25	27	-0.135	-0.706	0.486	0.729
		0.5	27	-0.057	-0.299	0.767	0.767
		0.75	27	-0.368	-2.054	0.05*	0.15
	Court	0.25	28	0.01	0.056	0.956	0.984
		0.5	28	0.004	0.021	0.984	0.984
		0.75	26	0.054	0.275	0.786	0.984

Table 6: Results of focal male Pearson's correlation tests between the different independent variables, the dichotomous mate choice test (DMCT) preference scores, and the courtship preference scores. Significant values ($p < 0.05$) are indicated with an *, and bolded if they are significant once corrected using the Benjamini & Hochberg (BH) false discovery rate.

Independent variables	Preference Score	OSR	df	t	r	p	BH p adjusted
Difference Stimulus PC1	DMCT	0.25	28	0.092	0.487	0.63	0.957
		0.5	27	-0.053	-0.274	0.786	0.957
		0.75	26	-0.011	-0.055	0.957	0.957
	Court	0.25	27	0.157	0.826	0.416	0.866
		0.5	27	-0.033	-0.171	0.866	0.866
		0.75	25	-0.072	-0.363	0.72	0.866
Focal PC1	DMCT	0.25	28	-0.049	-0.261	0.796	0.836
		0.5	27	-0.04	-0.209	0.836	0.836
		0.75	26	0.482	2.805	0.009	0.027*
	Court	0.25	27	0.343	1.898	0.068	0.204
		0.5	27	0.017	0.087	0.931	0.931
		0.75	25	-0.071	-0.357	0.724	0.931
Relative Focal Weight to Bin Average Weight	DMCT	0.25	28	0.066	0.35	0.729	0.729
		0.5	27	0.12	0.628	0.536	0.729
		0.75	26	0.488	2.853	0.008	0.024*
	Court	0.25	27	0.347	1.921	0.065	0.195
		0.5	27	-0.19	-1.003	0.325	0.488
		0.75	25	0.059	0.294	0.771	0.771

2.4.5 Aggression

Aggressive encounters occurred between the two stimulus males in 94% of female full-interaction mate choice trials. Male fight winners were significantly larger than male fight losers ($t_{82} = -3.377$, $p = 0.001$). Stimulus males were more likely to engage in higher levels of aggressive interactions when the focal female was from the male-biased environment (Est. = -1.654 ± 0.694 , $\chi^2_1 = 5.395$, $p = 0.02$, N=87, BH p-adjusted = 0.048). Aggressive encounters also occurred between females in 15% of male full-interaction mate choice trials. Female fight winners were significantly larger than female fight losers ($t_{12} = -2.214$, $p = 0.047$). The OSR of the focal male did not influence the occurrence of aggressive encounters between female stimulus crickets (Est. = -0.22 ± 0.509 , $\chi^2_1 = 0.187$ p = 0.666, N=87).

2.4.6. Same-sex sexual behaviours

Male same-sex sexual behaviour occurred in 69% of the full-interaction choice trials, while female same-sex sexual behaviour occurred in only 11% of the full-interaction choice trials. The OSR of the focal cricket did not influence the incidence of same-sex sexual behaviour (*Female choice trials*: Est. = 0.3777 ± 0.254 , $\chi^2_1 = 2.174$, $p = 0.14$, N=87; *Male choice trials*: Est. = $-1.559^{e-01} \pm 1.762^{e-01}$, $\chi^2_1 = 2.496$, $p = 0.114$, N=87).

2.5 Discussion

Overall, my results did not support any of my *a priori* OSR-based predictions; the results suggest that OSR does not affect mate choosiness in either females or males and that the OSR does not predict which sex will display the highest level of choice in my study species. Similarly, female house crickets (*Acheta domesticus*) also did not alter their mating decisions based on their previous adult environment (Tinghitella, 2014). My results contradict those of Souroukis and Murray (1995) that revealed female *G. pennsylvanicus* were less choosy in a female-biased environment. However, there were many differences in experimental design between my study and that of Souroukis and Murray (1995). First, because Sourouskis & Murray (1995) did not control total female density, it is difficult to ascertain whether their finding was due to variation in OSR or total density. Second, my experimental crickets were in their OSR environment for the first two weeks of their adulthood, before I tested for their mating behaviour as adults. In comparison, the crickets of Souroukis & Murray (1995) were first introduced into their OSR environment during the mating trials. It is possible that for *Gryllus* crickets, OSR may only impact mate choice when the mating environment is manipulated, rather than their adult environment prior to mating.

Surprisingly, neither females nor males generally exhibited a significant preference for larger mates in the current study. However, individuals in the female-biased OSR environment exhibited some interesting trends. Focal females that were given the option of choosing between stimulus males that differed greatly in body size tended to be choosier (i.e. prefer a larger male) than other focal females. This is surprising, as OSR theory predicts females in female-biased environment should not

exhibit a lot of choice, as males are a limited resource in their environment. Further, larger focal males and males that were relatively heavier were significantly more likely to be choosier (i.e. prefer the larger female) than other focal males. These results are in line with the OSR theory, as males from a female-biased should exhibit stronger preference. Additionally, as higher quality males are more likely to exhibit mate choice (Edward & Chapman, 2011), the choosiest males should be the largest and heaviest.

The adult environment of focal crickets influenced how the stimulus crickets courted, fought, and interfered with each other. Stimulus males courted the focal females more often and for longer periods when the focal females were from a female-biased environment. These findings are consistent with a study on the house cricket (*Acheta domesticus*) that reported that males court females more often when they are from a high-density environment as adults and juveniles (Tinghitella, 2014). It is unclear which cues the stimulus males are using to determine the focal females previous environment. Male crickets (*Teleogryllus oceanicus*) are able to determine a female's previous mating history (Thomas & Simmons, 2007), and female crickets (*Gryllodes sigillatus*) chemically mark their mates with their own scent to avoid re-mating with the same male (Ivy et al., 2005). Cuticular hydrocarbons are the primary chemical cue produced by crickets, and cuticular hydrocarbons are utilized as pheromones to allow crickets to identify sex, kinship, and conspecifics (Otte & Cade, 1976; Warthen & Uebel, 1980; Simmons, 1990; Tregenza & Wedell, 1997). Therefore, it is possible that male stimulus crickets may be more attracted to females from a female-biased because many different females may have chemically marked the focal female, causing her to chemically smell like multiple females.

Stimulus males may also be reacting to the focal female's behaviour, which could be influenced by her previous social environment. Tinghitella (2014) found that females from low-density environments initiated courtship (approached males) more often than females from high-density environments. Although I did not evaluate female courtship behaviour in the current study, females from a female-biased environment may initiate courtship more often than females from a male-biased environment. This idea needs to be tested.

Larger males were more likely to win aggressive encounters. This finding is in line with most other studies on cricket aggression (e.g., Simmons, 1986), and females prefer to mount fight winners (Simmons, 1986; Loranger & Bertram, 2016a). Consistent with OSR theory, stimulus males engaged in higher levels of aggressive competition when focal females came from male-biased environments. Males should be more aggressive in male-biased environments because females are a limited resource. This theory has found support from Japanese medaka (*Oryzias latipes*; Clark & Grant, 2010), male milkweed beetles (*Tetraopes tetraophthalmus*; Lawrence, 1992), and fall field crickets (*G. pennsylvanicus*; Souroukis & Cade, 1993). However, I held stimulus males in all-male environments, not OSR environments, prior to the experiment. Thus, my results suggest that the social environment of the choosing crickets influences the behaviour of its potential mates. Again, males may be reacting to the chemical profiles of females, as male crickets have been observed to escalate aggressive encounters when presented with female odour (Otte & Cade, 1976) and when they are in front of a female audience (Montroy et al., 2016). Females may also be behaving differently, as they were from a male-biased environment and should be choosier, they may have been more reluctant to

mate without first assessing available males through competition. A previous study on house crickets (*Acheta domesticus*) found that males with no prior access to females were more likely to successfully mate if they were able to compete with another male (Brown et al., 2007). To my knowledge, my study is the first to show that a female's OSR environment can influence the aggression levels in male competition.

Focal male OSR environment also influenced how female stimulus crickets interacted with each other. Female stimulus crickets were significantly more likely to interfere with a mated pair by ramming into them if the focal male was from a female-biased environment. This finding is consistent with OSR theory, as females should interfere with other the mating effort of other females when fewer males are available, which is the case in a female-biased environment. Because the stimulus females were held in all-female environment prior to mating, my finding suggests that the OSR environment of a prospective mate can influence the behaviour of non-focal individuals. Stimulus females may be reacting to the focal male's chemical profile, which may have been influenced by his female-biased environment and which may be motivating stimulus females to sexually interfere with one another. It is possible that the stimulus females were reacting to a difference in the focal males' behaviour, although I found no significant difference in male courtship behaviour in the different OSR treatments. Perhaps other male behaviours were different between the OSR treatments that the stimulus females were able to detect but that I did not measure (Otte & Cade, 1976). Female interference has also been documented in the smooth newt (*Triturus vulgaris vulgaris*; Waights, 1996) and the golden snub-nosed monkey (*Rhinopithecus roxellana*;

Qi et al., 2011). To my knowledge, my study is the first to show female sexual interference in crickets and in invertebrates in general.

Female-female competition was observed in 14% of the male full-interaction mate choice trials, and large females were significantly more likely than smaller ones to win these competitive interactions. Female-female competition is rarely documented, although it has been observed in sex-role reversal species, such as the pipefish (*Nerophis ophidion*; Rosenqvist, 1990) and the eurasian dotterel (*Charadrius morinellus*; Owens et al., 1994) where females compete for access to males. Interestingly, however, the OSR of the focal male did not significantly influence the occurrence of female competition, suggesting it is not access to mates that is driving this behavior in crickets. To my knowledge, my study appears to be the first to report female-female competition in crickets.

I also observed female same-sex sexual behaviour in 10% of the male full-interaction mate choice trials; its occurrence was not significantly affected by the OSR of the focal male. Although uncommon, female-female same-sex sexual behaviour has been documented in bonobos (*Pan paniscus*), bottlenose dolphins (*Thursiops* sp.), laysan albatrosses (*Phoebastria immutabilis*), and zebra finches (*Taeniopygia guttata*) (reviewed in Bailey & Zuk, 2009). Future studies should evaluate the incidences of same-sex sexual behaviour in female crickets to investigate whether it occurs for the same reason as male same-sex sexual behaviour. Male same-sex sexual behaviour occurred in 68% of the female full-interaction mate choice trials and was not significantly influenced by the focal female's previous OSR environment. Based on my observations of when same-sex sexual behaviour occurred, same-sex sexual behaviour may be a socially

adaptive behaviour as it appears to be used to diffuse aggressive interactions (Vasey et al., 1998). Alternatively, it may be a simple case of sex misidentification, possibly due to lack of sexual experience (Harari et al., 2000). Male same-sex sexual behaviour in *Gryllus* crickets has been previously reported, and it is hypothesized that its prevalence is due to misidentification and phenotypic correlations between aggressive interactions and mating behaviour (Boutin et al., 2016). As both male and female same-sex sexual behaviour occurred in my study, future work should investigate the factors driving individuals to engage in same-sex sexual behaviour and whether they differ across the sexes.

In the invertebrate literature, OSR seems well supported (Appendix, Table 10), as many studies have found that males are more competitive in male-biased environments (Lawrence, 1986; Souroukis & Murray, 1993; Holveck et al. 2015), male-biased environments lead to choosy females (Souroukis & Murray, 1995; Bateman, 1997; Holveck et al. 2015), and female-biased environments lead to choosy males (Lawrence, 1986; Kvarnemo & Simmons, 1999). However, none of these aforementioned studies properly controlled total density in their experiments, and so it is difficult to dissect whether the affects seen with different the mating behaviours are due to OSR, total density, or an interaction between the two social dynamics. In the few studies that did control total density, OSR has more conflicting results; males do mate guard more in a male-biased environment, as predicted by OSR (Schofl & Taborsky, 2002; Knox & Scott, 2006), however a male-biased environment causes female water striders (*Gerris odontogaster*) to forego mate choice due to an increase in sexual harassment (Arnqvist, 1992), and male mating success in the seed bug (*Nysius huttoni*) is higher in both biased

environments when compared an even sex-ratio environment (Wang et al. 2009).

Additionally, my results found that OSR could not predict the degree of choosiness of a sex when total density was controlled. Overall, this indicates that although OSR seems to affect mating behaviour in a predictable manner in invertebrates, these results are muddled with a possible interaction with total density. As such, OSR alone may not be powerful as a predictor as previously thought. Future studies should attempt a large factorial design to control total density, OSR, and their interaction.

In vertebrate studies, the affects of OSR also seem to interact with total density, but in a less predictable manner (Appendix, Table 10). Studies that did control for total density found females exhibit more choice in male-biased environments (Jirokul, 1999; Tinghitella et al., 2013), but males are not more competitive and exhibit less courtship effort in male-biased environments (Spence & Smith, 2005; de Jong et al. 2009).

Additionally, when total density was not controlled, studies found conflicting results.

Female St Peter's fish (*Sarotherodon galilaeus*) are choosier in male-biased environments (Balshine-Earn, 1996), female annual fish (*Austrolebias reicherti*) exhibit less choice in a female-biased environment (Passos et al., 2014), but female two-spotted gobies (*Gobiusculus flavescens*) were choosier in extreme female-biased environments (Wacker et al., 2013), and male long-snouted seahorses (*Hippocampus guttulatus*) exhibit strong choice in a male-biased environment (Naud et al., 2009). These studies highlight that the predictable affects of OSR may not be as widespread as once thought, but rather may be species dependent. OSR seems to be less effective in predicting male courtship behaviour in fish species, as males seem to be changing their mating tactics in male-biased environments, possibly to avoid sneaker males, or to invest more in sperm

competition (Mills & Reynolds, 2003; Spence & Smith, 2005; de Jong, 2009). This may example why my study did not find any evidence of OSR affecting focal male mating behaviour, as males may be altering the quality of their spermatophores based on their previous OSR environment. As male cricket invest more in their spermatophores with an increase in competition (Gage & Barnard, 1996), I would predict that males from a male-biased environment would be investing more sperm in each spermatophore, as they are less likely to encounter a female, while males from a female-biased environment would invest less sperm in each spermatophore, as they are more likely to mate multiple times.

Modifications to OSR have been suggested to better predict mating behaviour, such as the potential reproductive rate (maximum rate an individual of given sex can produce offspring; Clutton-Brock & Vincent, 1991), collateral investment (relative number of female and male ‘times out’ per reproductive event; Parker & Simmons, 1996) or sex-specific mortality rates (Okuda, 1990; Tershy & Croll, 2000). A more comprehensive model which incorporates all the aforementioned theories as well as OSR is that the cost a single breeding attempt is a more reliable predictor of competition and mate choice as it incorporates life history traits (Kokko & Monaghan, 2001). However, this model requires more indebt knowledge of a system, which is not always available, and to my knowledge, has remained untested. Thus, although less reliable, OSR is useful as a simple model for basic predictions for new system being explored.

In conclusion, my results suggest that the adult OSR environment does not influence female or male mate choosiness, nor does it influence which sex is the choosiest. However, the adult OSR environment does influence how stimulus crickets interact with focal crickets. This finding suggests that the social environment of the

choosing individual influences the behaviour of its potential mates. Future studies should evaluate whether the OSR of the immediate mating environment influences which sex is the choosiest in crickets and whether the ambient OSR influences female courtship effort, male grooming behaviour, and cricket chemical profiles. Additionally, as body size only influenced mating preferences in a small subset of my treatments, other traits, such as weaponry size, aggressive behaviour, and signaling behaviour, should be explored as desirable traits in prospective mates.

Chapter 3

**Validating Dichotomous Mate Choice Tests with Full-
Interaction Choice Tests in the Jamaican Field Cricket
*(Gryllus assimilis)***

3.1 Abstract

Dichotomous mate choice tests, where the choosing individual does not get to interact with its potential mates, are used regularly in sexual selection studies. However, individual mating preferences quantified using dichotomous mate choice tests have rarely been compared with mating preferences quantified using full-interaction choice test. Here, using the Jamaican field cricket (*Gryllus assimilis*), I tested whether the time individuals of both sexes spent associating with ‘preferred’ mates during dichotomous mate choice tests correlated with actual mate choice when females and males were allowed to freely interact. Additionally, because mate choice can be influenced by the condition and social experience of the choosing individual, I tested whether different operational sex ratios (OSR; ratio of sexually available females to sexually available males) affected the consistency of individual mating preferences in both types of tests. Both female and male crickets were not consistent in their mating preferences between the two types of test. The overall lack of consistency between the tests suggests that individual mating preferences obtained using the dichotomous mate choice test in my study species of crickets may not reflect actual mate choice between freely interacting males and females, possibly owing to competition interference in the open-arena test or the lack of chemosensory cues between the sexes in the dichotomous choice test.

3.2 Introduction

Mate choice is an important component of sexual selection (Darwin, 1871). Mate choice occurs when mating is non-random and is often driven by differences in available resources, behavioural traits or morphological traits between prospective mates (Halliday, 1983; Andersson, 1994). Dichotomous mate choice tests are regularly used to quantify mating preferences, where a choosing individual, referred to as a focal individual, is allowed to choose between two potential mates presented simultaneously (Wagner, 1998; Dougherty & Shuker, 2015). Often in this type of choice test, the potential mates are separated from each other and from the focal individual with barriers in order to eliminate sexual competition and to allow the focal individual to exhibit its preferences based on one or more particular trait in the prospective mate (Wagner, 1998). Dichotomous mate choice designs use association time spent with either of the two prospective mates presented as a proxy measure of mate choice (Walling et al., 2010). However, because the focal individual never physically interacts with the available potential mates, it is difficult to determine if dichotomous mate choice tests accurately reflect mating preference and thus actual mate choice (Wagner, 1998; Dougherty & Shuker, 2015).

Some researchers have sought to test the validity of using association time as a proxy for mating preference by immediately following each dichotomous mate choice test with a no-choice test, using either the preferred or non-preferred potential mate drawn at random (Walling et al., 2010; Loranger & Bertram, 2016a). These studies reveal that females are more likely to mate with their preferred male, as indicated in a preceding dichotomous choice test (Walling et al., 2010; Loranger & Bertram, 2016a). However, a meta-analysis of these types of comparisons revealed that preference scores for a mate

were stronger during the dichotomous choice tests than during no-choice tests because focal individuals were more likely to mate at random during the no-choice tests (Dougherty & Shuker, 2015). Random mating may occur because focal individuals may be selected to mate with a non-preferred individual versus foregoing mating all together (Dougherty & Shuker, 2015). This study highlights the importance of available options during mate choice tests, as the drive to mate may overshadow preferences for certain traits.

A less common way of testing the validity of using association time as a proxy for mating preference is to perform a full-interaction choice test where the focal individual is given complete access to both potential mates after a dichotomous choice test and their mating behaviour is observed. In the handful of studies that have compared association times to full-interaction mating preferences, 71% show consistency between the two tests, while 29% show conflicting results (Table 7). When the results are in conflict, competitive behaviour and mate guarding seem to explain the lack of relationship (Fuller, 2003; Ala-Honkola et al., 2010). Oddly, these studies seem to have been run almost exclusively on fish species to date. The only other study I could find that was not on a fish species was on two species of zebra finches (*Taeniopygia guttata guttata* and *T. g. castanotis*). This latter study revealed that for both species, female's association time with a particular preferred male in a dichotomous choice test was correlated with the male she later mated with in the aviary, but this study gave focal females a choice of 10 potential mates (Clayton, 1990). The lack of consistency between studies and the limited taxonomic diversity in the tested species suggest the need for further tests.

In the current study, I tested for individual consistency in mating preferences in both males and females, where consistency refers to the same mate being preferred across tests. This was assessed separately by the dichotomous choice test and the full interaction test, using the Jamaican field cricket (*Gryllus assimilis*) as a model species. Field crickets use acoustic, tactile, and chemical cues to choose their mates (Thomas & Simmons, 2009). Typically, males signal acoustically to attract females, once a female is attracted and comes into physical contact with the male, the male switches to short range courtship calling (Alexander, 1961). Females inspect the males, potentially using auditory, chemosensory, and visual cues, and if the female accepts the male's courtship attempts, she mounts the male and he transfers a spermatophore to her (Alexander, 1961). Because females mount males, females control mating and forced copulation is impossible. Male *G. assimilis* may also exhibit some form of mate choice, as they exhibit repeatable preferences for one female over another (Bertram et al., in revision).

The condition of the choosing individuals, such as age, body size, or parasite load, can influence their mating decisions (Cotton et al., 2006). The social context experienced by individual animals can also influence mate choice results (Cotton et al., 2006). For example, male zebra finches raised with conspecific females prefer conspecifics, but males raised with heterospecific females prefer heterospecifics (ten Cate, 1985). The operational sex ratio (OSR: the ratio of sexually available females to sexually available males, Emlen, 1976) can also influence the behaviour of focal individuals. OSR can influence which sex is the choisiest because a mating bias for one sex over another will limit the availability of the other sex in sexual encounters, resulting in the limited sex becoming a valuable resource (Emlen & Oring, 1977). For example, in the fall field

cricket, *Gryllus pennsylvanicus*, the OSR is typically male-biased and females typically exhibit strong mate choice (Souroukis & Murray, 1995). However, when the OSR is experimentally shifted to being female-biased, females exhibit little choice in their mates (Souroukis & Murray, 1995). Further, the butterfly, *Bicyclus anynana*, is often reared in dense, male-biased cages for experimental studies. Holveck et al. (2015) revealed that these conditions result in more aggressive males, which hinder female mate choice, potentially biasing estimates of the strength and direction of selection acting on mating in laboratory-reared butterflies (Holveck et al., 2015). These results highlight the importance of elucidating how the social environment influences mate choice in different contexts.

Here, I quantified the mating preferences of individual crickets of both sexes separately using dichotomous mate choice and full interaction choice tests to ascertain whether focal individuals chose the same individual across both tests. Prior to testing, I exposed crickets to one of three different OSR treatments: male-biased, even sex ratio, and female-biased for a two-week period and then tested both females and males for their individual mating preferences. I evaluated if the OSR treatments affected individual consistency in mating preferences between the two types of test. Given that Loranger & Bertram (2016a) found consistent results between association time in dichotomous mate choice tests and mounting behaviour in no-choice tests using the same species, I hypothesized that association time during the dichotomous mate choice would predict (i.e. correlate with) focal cricket mating behaviour during the full-interaction choice test. Furthermore, I hypothesized that OSR would affect the consistency of individual mating preferences between the two tests, because if the focal individual experienced a social

environment where it was the more abundant sex, it should exhibit more random mating behaviour. I predicted that (i) females in the full-interaction choice tests should mount their previously preferred male first and spend more time mounting this male, (ii) males in the full-interaction choice tests should court their previously preferred female first and spend more time courting this female, (iii) females from the female-biased OSR treatment should be less consistent in their mate choice between tests, and (iv) males from the male-biased OSR treatment should similarly be less consistent in their mate choice between tests.

Table 7: Consistency in the results of studies that evaluated concordance between association time recorded during a dichotomous mate choice test and mating behaviour during a full-interaction choice test.

Species	Choosing Sex	Results	References
Japanese medaka <i>Oryzias latipes</i>	Female	Consistent	Howard et al., 1998
Sand gobies <i>Pomatoschistus minutus</i>	Female	Consistent	Lehtonen & Lindström, 2008
Northern swordtail <i>Xiphophorus nigrensis</i>	Female	Consistent	Cummings & Mollaghan, 2006
Black morph <i>Girardinus metallicus</i>	Female	Consistent	Kolluru et al., 2015
Rainbow darter <i>Etheostoma caeruleum</i>	Female	Inconsistent	Fuller, 2003
Guppy <i>Poecilia reticulata</i>	Male	Consistent	Jeswiet & Godin, 2011
Least killifish <i>Heterandria formosa</i>	Male	Inconsistent	Ala-Honkola et al., 2010

3.3 Methods

3.3.1 Study Species

The laboratory population of Jamaican field crickets that I used in my study was established in 2008, from wild-caught crickets in Bastrop County, Texas, USA (latitude ~ $30^{\circ}17'N$, longitude ~ $97^{\circ}46'W$, elevation ~145m). I housed the laboratory population in a temperature-controlled ($25 \pm 2^{\circ}C$) greenhouse on a 14h L:10h D illumination cycle. The crickets were housed in large bins (64 x 40 x 42 cm, L x W x H) with a 10 x 15 cm mesh opening on the lid for light exposure. I provided them with *ad libitum* food (powdered Harlan Teklad Inc. Rodent diet no. 8640M), water (containers with gravel filled with water), and shelter (egg cartons).

3.3.2 Experimental design

I randomly placed individual crickets into either OSR treatment bins or stimulus cricket bins 1-2 days after they had reached adulthood. I used three OSR treatment bins: male biased (6 males to 2 females), even sex ratio (4 males to 4 females) and female biased (2 males to 6 females). Males and females were separated within each OSR treatment bins using a perforated divider to ensure the crickets remained virgins. Each OSR treatment bin was place inside an acoustic isolating bin (large bins lined with acoustic foam, with a light source embedded). For each OSR treatment, I created a male stimulus bin and a female stimulus bin. Each stimulus bin contained four crickets of the same sex. I provided all crickets with *ad libitum* food and water, and egg cartons for shelter. If a death occurred in any of the bins, I collected a replacement cricket of the

same sex from the colony population to keep the OSR constant. I placed a dot of red paint on the replacement cricket's pronotum to ensure it could be easily identified, and then introduced it to the bin where the death occurred. I did not use these red painted crickets in the experiment.

I kept the crickets in their respective bins for two weeks prior to testing their individual mating preferences. On day 12, I assigned the crickets individual identification numbers, weighed them, and painted them on their pronotum with a drop of randomly chosen coloured nail polish (but not red). The paint was necessary to facilitate individual identification. I ran a total of 180 trials (=90 male choice and 90 female choice trials) between May and October 2015. I carried out all trials between 06:00 - 10:00 hrs daily because preliminary data showed that *G. assimilis* males signal most often during this period of day. I ran all trials in an acoustic isolating chamber, consisting of a large wooden box (142 x 79 x 96.5 cm, L x W x H) lined with two layers of acoustic foam (1.27 cm and 2.53 cm thick), equipped with a light source (LED lamp with a gray filter to reduce light intensity), camera holster, and thermometer and maintained at $25 \pm 2^{\circ}\text{C}$. I recorded the trials with a Go Pro Hero 4 Silver camera mounted overhead and monitored the behaviour of the experimental crickets via the Go Pro App on a Samsung Galaxy 2 tablet.

I constructed the mate choice arena (30 x 30 x 30 cm, L x W x H) out of corrugated plastic. It had two removable dividers, which subdivided the arena into three chambers: two stimulus cricket chambers (15 x 15 x 30 cm, L x W x H) and one focal cricket chamber (30 x 15 x 30 cm, L x W x H; see Figure 1 in Chapter 2). These dividers were kept in place during the dichotomous mate choice trials. The divider that separated

the two adjacent stimulus cricket chambers was opaque to prevent the stimulus crickets from seeing each other, whereas the divider separating the stimulus crickets from the focal cricket was perforated and transparent to allow the focal cricket the opportunity to inspect the stimulus crickets (and vice versa) using chemical (antennation), acoustic, and visual cues while simultaneously preventing mating. I demarcated two 15 x 7.5 cm (L x W) mate choice zones in the focal cricket area, directly in front of the stimulus cricket areas. These choice zones were used to determine the preference of the focal cricket for either of the stimulus crickets.

Each trial involved three crickets: one focal (either male or female) and two stimulus crickets of the opposite sex. I chose the focal cricket at random from one of the OSR treatment bins, and then haphazardly selected two stimulus crickets of the opposite sex from one of the stimulus cricket bins. These stimulus crickets had to have contrasting colours on their pronotum to allow their individual identification during the behavioral trials, and the stimulus cricket was not a replacement cricket with red paint. I carried out up to four trials per day (two male mate choice trials and two female mate choice trials), with the focal crickets originating from the same OSR treatment bin. Each focal cricket's trial lasted 50 min, with the following consecutive components: one 5-min acclimation period, two 15-min dichotomous mate choice trials, and one 15-min full-interaction choice trial in series. In the second dichotomous mate choice trial, I switched the location of the stimulus crickets to control for any potential side bias by the focal cricket. To transition from the dichotomous choice trials to the full-interaction choice trials, I removed the dividers, enabling all three crickets to freely interact with one another. At the end of the 50-min trial, I euthanized (froze) the crickets for later photography and

morphological measurements. I photographed individual frozen crickets dorsal side up using a Panasonic Lumix ZS40 camera, and quantified their pronotum length, pronotum width, and head width using *ImageJ*. I made repeated measurements confirm that they were highly repeatable (>99% concordance).

3.3.3 Video analysis

I quantified cricket behaviour in 540 video films (=180 dichotomous + 90 full interactions x 2 focal sexes). So as to avoid any bias, I scored all the videos blindly of the OSR treatment of the focal animals. I viewed the dichotomous mate choice videos using *VLC*, and recorded the latency of the focal cricket to enter a choice zone, the particular choice zone entered first, and the time spent in each choice zone (= association time). I converted full-interaction choice trials with *Go Pro Studio* and *Final Cut Pro* to trim, zoom in, and reduce film speed by 50% to facilitate the quantification of behaviour, and viewed them using Mac Media Player. I quantified male latency to court, female latency to mount, male courting behaviour (frequency, frequency prior to mounting, and duration), female mounting (frequency and duration), maximum aggression score (*sensu* Loranger & Bertram, 2016a), loser of the majority of the aggressive encounters, sexual interference, titillation (acoustic sexual interference), and same-sex sexual behaviour (see Table 1 in Chapter 2 for descriptions).

3.3.4 Statistical analysis

I quantified cricket body size using a principal components analysis (PCA) with all three morphological measurements. The first principal component (PC1 size) had all three morphological measures weighted equally and strongly. The eigenvalues for both sexes were above 1 (Female eigenvalue = 2.609; Male eigenvalue = 2.816), and explained 87 and 94% of the observed variation for females and males, respectively.

I tested whether focal individuals were consistent in their mating preferences between the two sequential dichotomous mate choice trials by examining whether the focal individual spent more time spent in front of the same stimulus cricket in each trial. To test for individual consistency, I coded the focal cricket with a (1) if its mating preference for a particular stimulus cricket (i.e. the stimulus cricket it spent $\geq 50\%$ of its total association time with) was consistent across both dichotomous mate choice trials, or with a (0) if it switched its preference or showed no preference. I referred to focal crickets as exhibiting ‘dichotomous time consistency’ if they were consistent with the time they spent associating with their preferred mate. I carried out this analysis separately for each sex and OSR treatments. However, if there was no difference in the results between the three OSR treatments, I combined them for the final test of consistency.

I tested for trial consistency between the dichotomous mate choice and the full-interaction mate choice tests in three different ways. First, using only focal crickets that exhibited dichotomous time consistency, I tested to see if focal males first courted or spent more time courting their preferred cricket, and if focal females first mounted or spent more time mounting preferred cricket in the full-interaction mate choice trial. Second, using all focal individuals, regardless of whether or not they made consistent

choices in the dichotomous mate choice tests, I tested whether focal males courted (first and spent more time) and whether focal females mounted (first and spent more time) the stimulus cricket they had spent more time associating with overall (summed across both dichotomous mate choice trials). I tested for consistency using a Chi-squared test, testing for deviation from random. Third, I created preference scores for the dichotomous choice test (summed across the two dichotomous choice tests) by subtracting time spent in front of their non-preferred stimulus mate from the time spent in front of preferred stimulus mate. I then created preference scores for the full-interaction test by subtracting the time spent courting (focal males) or the time spent mounting (focal females) the previously non-preferred mate from the time spent courting or mounting the previously preferred mate, respectively. A negative score would indicate a switch in preference. I then ran Pearson's correlation test (r) on these two preference scores, testing each sex and OSR treatment separately (6 in total). I tested for false discovery rate using the Benjamini-Hochberg (BH) procedure (Benjamini & Hochberg, 1995).

In the dichotomous mate choice trials, 6 of the 180 focal crickets tested did not enter a choice zone in either test. In the full-interaction choice trial 7 of the 180 focal crickets tested did not interact with the stimulus crickets. I excluded these 13 individuals from the analysis.

3.4 Results

Focal females and focal males did not exhibit consistent mating preferences between the two sequential dichotomous mate choice trials, nor were they consistent in their mating preference across the two choice test types (Table 8). If I had observed

consistency between the dichotomous mate choice trials, I would have expected to see far more than 50% of focal individuals exhibiting a consistent choice. Rather, only 36% (31 of 85) of the focal females consistently spent more time with the same stimulus cricket between dichotomous mate choice trial, and only 38% (31 of 82) of focal males consistently spent more time with the same stimulus cricket between both dichotomous mate choice trials (Table 8). These odds are not different from those expected by chance alone.

Of the focal females and focal males that exhibited association time consistency in the dichotomous mate choice trials, males did not consistently court the same stimulus cricket first or spent more time courting them, and females did not consistently mount their preferred stimulus cricket first or spent more time mounting them in the full-interaction choice trials (Table 8). Further, focal females did not consistently spend more time mounting or first mount the stimulus males they had spent more overall time associating with during the dichotomous mate choice trials (regardless of whether they were dichotomous time consistent), nor did the focal males consistently spend more time courting or first court the stimulus females they had previously spent more overall time associating with during the dichotomous mate choice trials (regardless of whether they were dichotomous time consistent) (Table 8). Additionally, there was no difference between the three different OSR treatments for either males or females, indicating that OSR did not affect the likelihood that a cricket consistently choosing the same mate across tests; thus, Table 2 presents the combined results.

Finally, there were no significant correlations between the mating preference scores of focal individuals obtained in the two sequential dichotomous mate choice trials

and the mating preference scores based on focal male courtship or focal female mounting behaviour in the full interaction test, regardless of the OSR treatment (Table 9). In the female choice trials, there is an indication of a trend towards females being more consistent in their choice of males if the females are from an environment with more males: females from a male-biased environment were closer to significance ($p = 0.152$) than females from an even-biased environment ($p = 0.182$), or females from a male-biased environment ($p = 0.705$) (Table 9). A similar trend is found in male choice trials, where males tend to be more consistent if the males are from an environment with more females, although the results do not approach significance: males from a female-biased environment ($p = 0.692$), versus males from even sex ratio ($p = 0.790$), and male-biased environment ($p = 0.906$). These non-significant trends might indicate that with a larger sample size, a significant relationship might be found between OSR and mating consistency. Overall, my findings indicate that neither female nor male crickets consistently choose the same mate between dichotomous mate choice trials and full-interaction choice trials, irrespective of their social (OSR) environment.

Table 8: Results for Chi-square tests of consistency in mating preference for focal females and focal males between the two sequential dichotomous mate choice trials and between the dichotomous mate choice trials and the full-interaction choice trials.

		Obs/Exp*	χ^2_1	p	BH p adjusted
Between Both Dichotomous Mate Choice Trials					
	Consistent Time Spent	31/43	1.946	0.163	0.51
Between Dichotomous Mate Choice Trials and the Full-interaction Choice Trials					
Female	Consistent Time Spent & Time Mount	13/16	0.31	0.578	0.723
	Consistent Time Spent & First Mount	12/16	0.571	0.45	0.723
	Overall Time Spent & Time Mount	41/43	0.048	0.827	0.827
	Overall Time Spent & First Mount	32/43	1.613	0.204	0.51
Between Both Dichotomous Mate Choice Trials					
	Consistent Time Spent	31/41	1.39	0.239	0.928
Between Dichotomous Mate Choice Trials and the Full-interaction Choice Trials					
Male	Consistent Time Spent & Time Court	15/16	0.008	0.928	0.928
	Consistent Time Spent & First Court	15/16	0.008	0.928	0.928
	Overall Time Spent & Time Court	40/41	0.012	0.912	0.928
	Overall Time Spent & First Court	42/41	0.012	0.91	0.928

* Observed/Expected number of trials

Table 9: Results of Pearson's correlation (*r*) tests of consistency in mating preference for focal females and focal males between the dichotomous mate choice trials and the mating preference scores based on either male courtship or female mounting behaviour for each OSR treatment separately.

Sex	OSR	df	t	r	p	BH p adjusted
Female	Male-biased	27	1.474	0.273	0.152	0.273
	Even-sex ratio	27	1.369	0.255	0.182	0.273
	Female-biased	25	0.384	0.076	0.705	0.705
Male	Male-biased	27	0.119	0.023	0.906	0.906
	Even-sex ratio	26	0.269	0.053	0.790	0.906
	Female-biased	23	-0.401	-0.083	0.692	0.906

3.5 Discussion

My results suggest that neither female nor male crickets are consistent in their mating preferences (as measured by association time) between both dichotomous mate choice trials. Further, association times in dichotomous mate choice tests do not predict mating behaviour in full-interaction choice tests. These findings suggest dichotomous mate choice tests may not be appropriate for field crickets. There are a few possible explanations why dichotomous mate choice tests may not predict mating behaviour in full-interaction choice tests. These include: (i) lack of tactile/chemical cues, (ii) differences in acoustic behaviour, (ii) conflict amongst conspecifics, and (iv) male quality. I address each of these ideas in turn.

First, tactile and chemical cues in cricket courtship behaviour may play a larger role in mating decisions than previously thought, and the dichotomous mate choice arena may not allow focal individuals to have enough access to tactile and chemical cues to make accurate mating decisions. Chemical cues are known to influence cricket mating behaviour (Thomas & Simmons, 2009; Thomas & Simmons, 2010). For example, females are significantly less likely to mount a male if they are unable to tactiley investigate the male due to the removal of their antennae (*Teleogryllus commodus*; Loher & Rence, 1978; *Gryllus bimaculatus*; Adamo & Hoy, 1994; *Teleogryllus oceanicus*; Balakrishnan & Pollack, 1997). Further, while females deprived of chemical cues are able to distinguish males from females, they are unable to distinguish conspecific males from heterospecific males (*Gryllus interger* and *Gryllus lineaticeps*; Leonard & Hedrick, 2009). Males are not only able to determine the previous mating history of potential

mates, but they can also adjust the quality of their spermatophore based on female mating history (*Teleogryllus oceanicus*; Thomas & Simmons, 2007).

Given that tactile investigation seems to influence cricket mating behaviour, my dichotomous mate choice trials may not have provided crickets with enough opportunity to explore potential mates through touching. The perforated divide was intended to allow crickets to antennate with prospective mates, however this could only be achieved when the stimulus cricket and the focal cricket were directly beside the divide. I did not quantify how often the focal cricket antennated with both stimulus crickets during dichotomous mate choice tests because antennation was rarely observed. The general lack of antennation suggests that the dichotomous mate choice arenas may not provide enough tactile cues.

Second, differences in acoustic behaviour may also be a possible explanation for the lack of consistent mate choice between dichotomous mate choice tests and full-interaction choice tests. Although I did not evaluate the parameters of male songs, I did note when males signaled. Only 3 out of 540 (<1%) males (stimulus and focal) signaled acoustically during the dichotomous mate choice tests, while 523 out of 540 (97%) of males signaled acoustically during the full interaction choice tests. Other studies using this species and dichotomous mate choice tests have also noted that males are extremely unlikely to call during dichotomous mate choice tests (Loranger & Bertram, 2016a; Bertram et al., 2016), which further suggests that dichotomous mate choice tests may hinder the focal cricket's ability to perceive the necessary cues to choose a mate.

Third, the lack of consistency in mating preferences between the dichotomous and full-interaction choice test may be due to conflict amongst conspecifics, such as

competition, harassment, or sexual interference. In the full-interaction female choice trials, male-male competition occurred in 94% of the tests, male sexual interference (physical and/or acoustic) occurred in 34% of tests, and same-sex sexual behaviour occurred in 69% of tests. In the full-interaction male choice trials, female-female competition occurred in 15% of trials, female sexual interference occurred in 4% of trials, and same-sex sexual behaviour occurred in 11% of trials. These behaviours between potential mates may have adversely influenced any tendency for consistent mating preferences in focal crickets. In water striders (*Gerris odontogaster*), females in a male-biased environment are unable to exhibit mate choice due to the increase in sexual harassment from the more abundant males in the environment (Arnqvist, 1992).

Additionally, in the rainbow darter (*Theostoma caeruleum*), mate guarding seemed to have a greater affect on male mating success than female mate choice (Fuller, 2003). Conflict amongst conspecifics is more likely to explain the lack of consistency for focal females rather than the lack of consistency seen in focal males, as stimulus males were far more likely to engage in aggressive encounters and sexually interfere with one another than stimulus females.

Lastly, the lack of consistency seen with focal males could also be due to variation in focal male quality. The focal individuals were chosen at random, and so focal males greatly varied in body size (4.529 to 6.044 mm, head width). However, high quality males are more likely to exhibit mate choice (Edward & Chapman, 2011). For examples, in the beaugregory damselfish males (*Stegastes leucotictus*), larger males were more likely to exhibit mate choice (Itzkowitz & Haley, 1999), and in the two-spotted goby males (*Gobiusculus flavescens*), males with access to better resources were more

likely to exhibit mate choice (Amundsen & Forsgen, 2003). Additionally, in Chapter 2, I found that only large and heavy males from a female-biased environment exhibited a preference for larger females. Bertram et al. (in revision) also revealed that larger male *G. assimilis* were more likely to make consistent mating preferences than smaller males. Thus, if only high quality males (i.e. larger and heavier) can exhibit choice, it is unsurprising that I did not find consistent results of mate choice in my male choice trials.

Overall, this lack of consistent mate choice across dichotomous mate choice tests and from a dichotomous mate choice test to a full-interactions test in my study is a cautionary note suggesting that testing for mating preference in other species utilizing dichotomous mate choice tests may yield unreliable results. A study on jumping spiders (*Cosmophasis umbratica*) also cautioned against overreliance on association time as an indicator for mate choice, specifically with species where close visual proximity is insufficient in ascertaining necessary information from a potential mate (Lim et al., 2007). In a study using zebra finch (*Taeniopygia guttata*), Rutstein et al. (2007) used three different methods to test for mate choice: dichotomous mate choice, no choice test, and aviary mate choice (full interaction with multiple pairs). They found conflicting results on female assortative mating behaviour depending on the mate choice test used. Their no-choice results were most similar to the aviary mate choice test, which was meant to simulate natural conditions, and so they recommended the no-choice test for the zebra finch system as physical contact appeared to be an important component in choosing a mate. This method of testing various mate choice test in order to determine the most adequate method for obtaining reliable results for a specific species should therefore be more widely validated across taxa.

In conclusion, the dichotomous mate choice test may not be an appropriate test of individual mating preferences for crickets because focal individuals do not have access to the full natural range of cues from their prospective mates that are necessary for accurate and repeatable assessment of mate quality. Further investigation into the importance of chemosensory and tactile cues during cricket courtship behaviour in mate choice should be conducted to provide more insight into the utility of non-interactive mating preference tests in crickets.

Chapter 4

General Conclusions

4.1 Discussion of salient findings

Operational sex ratio (OSR) theory predicts that the less abundant sex in a population should exhibit the strongest mate choice (Emlen & Oring, 1977). Here, I tested this general prediction by experimentally varying the OSR and concurrently quantifying the mating preferences of males and females in the Jamaican field cricket, *Gryllus assimilis*, as a model study species. I exposed newly-moulted virgin adults to either a male-biased, even-sex ratio, or female-biased OSR environments for two weeks prior to testing their individual mating preferences when presented with two stimulus crickets of the opposite sex that differed in body size. I predicted that the most limited sex (i.e. males in a female-biased OSR and females in a male-biased OSR) would be the choosiest sex. My results indicate that the adult OSR environment does not affect female or male choosiness, nor does it influence which sex is the choosiest. Females from a male-biased environment did not require more courtship prior to mounting a male, nor did they take longer to mount males. Likewise, females from a female-biased environment did not mount potential male mates faster, and they were not more likely to mount both males. Similarly, males from a female-biased environment did not selectively court females, nor did they take longer to court to potential mates. Further, males from a male-biased environment did not court females more quickly and they did not spend more time courting females.

The results of my study indicate that the adult OSR prior to mating does not affect cricket mate choice. My findings lie in stark contrast to the only previous study on OSR in crickets. Research on *Gryllus pennsylvanicus* revealed that the OSR environment during mate choice trials influenced female choosiness (Souroukis & Murray, 1995). The

fundamental difference between my study and the Souroukis and Murray (1995) study was that the OSR was manipulated during the mating trial rather than during their adult environment prior to mating. It is therefore possible that current OSR drives differences in mating behaviour, but past OSR has no effect.

The naturally occurring OSR of *Gryllus* species varies greatly, ranging from 30 to 80% (Veazey et al., 1976). Given this fluctuation in OSR, it is possible that only the immediate OSR influences mate choice. Immediate OSR may have a strong influence on *G. assimilis*, as they are flight capable crickets (Zera et al., 1999), that aggregate near light sources, and the sex ratio of field crickets at light sources varies thorough out the night (Cade, 1979). With a variable OSR, the sex that exhibits the most choice could be in constant flux. Additionally, as the crickets aggregate in the hundreds to these light sources, the perceived OSR of an individual cricket rather than the overall OSR could have a greater influence on their mating behaviour. For example, the overall OSR at the light source could be an even sex ratio. However, if a newly arrived male only encounters other males initially, he may perceive his immediate mating environment as being male-biased and, as a result, increase his courtship effort when he finally encounters a female more so than otherwise. If the immediate OSR is important in field crickets, it is then not surprising that my experiment did not find any effects of OSR on focal cricket behaviour, as the males were all kept in the same type of OSR during their trials (1 focal male to 2 stimulus females), and likewise the females were also in the same type of OSR during their trials (1 focal female to 2 stimulus males).

Additionally, it is possible that the juvenile environment may affect later adult mating behaviour. The social environment of juveniles, specifically the acoustic

environment, influences maturation rates in both females and males, and how females later allocate resources towards egg production (Kasumovic et al., 2011). These findings suggest that other components of the juvenile social environment, such as OSR, could influence the courtship and mating behaviour of adult crickets, as the variation in the number of males in the environment would influence the acoustic environment. If juveniles are reared in an environment where the adult OSR is biased, the juveniles of the less abundant sex may develop more rapidly as to gain mating opportunities with the adults of their environment, or they may exhibit more choice once they reach adulthood. Additionally, as juveniles are unable to signal because their wings are under-developed, it is highly possible that they are more sensitive to chemosensory cues, allowing them to assess the adult sex ratios of the population as well. This potential chemosensory sensitivity may also allow them to determine the sex ratio of the juvenile population, permitting them to anticipate the future OSR and adjust their development accordingly.

In fruitflies, *Drosophila melanogaster*, female and male pupae can communicate via pheromones, which acts to regulate gamete compatibility (Pontier & Schweisguth, 2015). Juveniles reared in an environment where they are the less abundant sex may be very choosy of their mates when they reach adulthood and may take longer to reach adulthood in order to optimize their body size to increase fitness, as they anticipate having many potential mates. Thus, the OSR of the adult population in which the juveniles are reared may influence the juvenile's future mating behaviour and development, but the perceived future OSR of the juveniles may also be important.

Interestingly, the behaviour of potential mates was affected by the OSR previously experienced by the choosing crickets. When stimulus males were presented

with a focal female from male-biased environment, the stimulus males engaged in higher levels of aggression. When stimulus males were presented with a focal female from a female-biased environment, the stimulus males courted the focal female more often and for longer periods of time. And when stimulus females were presented with a focal male from a female-biased environment, they were more likely to sexually interfere with the mating efforts of their rival female. These behaviours would be predicted by OSR theory had the stimulus crickets been exposed to variation in OSR, but they were not; the stimulus crickets were housed in sex-specific bins during the two weeks prior to mating. Thus, the latter results suggest that the stimulus crickets responded to the focal crickets, which had been previously exposed to a particular OSR environment. Stimulus crickets may have been cued to the competitive environment of the focal crickets as the focal cricket may have been behaving differently owing to their OSR environment. For example, focal females may have been approaching males more frequently to initiate courtship events if she was from a female-biased environment, which may explain the increase in stimulus male courtship efforts. I did not measure female courtship effort, however in a house cricket (*Acheta domesticus*) study, females from low-density populations did approach males more frequently (Tinghitella, 2014). OSR would predict that females should approach males more often in an attempt to instigate courtship efforts from the males when the females are from a female-biased environment because males are a limited resource.

Another possibility is that the adult OSR of the focal cricket may have influenced the pheromone (cuticular hydrocarbon) profile. Cuticular hydrocarbons composition is influenced by diet in *Drosophila melanogaster* (Fedina et al., 2012), and by the breeding

status of parents in the burying beetle, *Nicrophorus vespilloides* (Steiger et al., 2007). If the social environment influences the composition of cricket pheromones, this might explain why stimulus crickets altered their behaviour based on the previous OSR of the focal cricket they were presented with. An investigation into the factors influencing the composition of cuticular hydrocarbons in crickets warrants further examination.

Using the same data set, I also sought to validate the use of the dichotomous mate choice test by testing whether association time in a such a test accurately predicts actual mate choice during a full-interaction choice test, and whether the strength of this relationship between these two types of test varied with OSR treatments. I predicted that the stimulus male the focal female associated with during the dichotomous mate choice tests would also be the male she would then mount first and(or) the male she would spend more time mounting during the full-interaction test. Likewise, I predicted that the stimulus female a focal male associated with most during the dichotomous mate choice tests would also be the female he would then court first and(or) the female he would spend the most time courting during the full-interaction test. Lastly, I predicted that females from a female-biased OSR would be more likely to mount males they had not previously preferred, and that males from a male-biased OSR would be more likely to court a female they not previously preferred.

My results indicate that, regardless of their OSR environment, both females and males did not consistently chose the same mate across the two dichotomous choice tests, and that association time was not an accurate indicator of mate preferences during a full-interaction choice test. Focal individuals did not associate more with the same potential mate across both dichotomous mate choice tests more than chance would predict. The

few focal individuals that consistently associated more with their preferred mate across both dichotomous mate choice trials were not more likely to court or mount that individual first or for a longer period of time during the full-interaction tests.

Additionally, the overall time spent associating with a potential mate during both dichotomous mate choice trials did not predict which mate would be courted or mounted first, or which mate would be courted or mounted for a longer period of time. Finally, there was no relationship between the preference scores during the dichotomous mate choice test, and preference scores for courtship or mounting behaviour during the full-interaction tests. Together, these results suggest that dichotomous mate choice tests do not accurately predict mating behaviour during full-interaction choice tests.

My findings suggest that dichotomous mate choice tests may not be an appropriate test to quantify mating preferences in field crickets. Crickets may require more tactile and chemical (cuticular hydrocarbons) than they can glean from dichotomous mate choice tests in order to exhibit complete mate choice. A study using *Gryllus bimaculatus* found that males would not court females when the female's cuticular hydrocarbons were removed, but the males would resume their courtship effort if the females' pheromones were replaced (Tregenza & Wedell, 1997). Additionally, if females were unable to tactility investigate their mate, they were far less likely to mount a male (*Teleogryllus commodus*; Loher & Rence, 1978; *Gryllus bimaculatus*; Adamo & Hoy, 1994; *Teleogryllus oceanicus*; Balakrishnan & Pollack, 1997). Furthermore, in the Australian field cricket, *Teleogryllus oceanicus*, both females and males exhibit sexual selection of their mates' cuticular hydrocarbons composition (Thomas & Simmons, 2009; Thomas & Simmons, 2010). If both females and males are exhibiting choice based on

their mates' pheromones, eliminating most physical contact with potential mates during mate choice trials could remove their ability to effectively investigate available mates and exhibit a choice all together. This might explain why crickets in my experimental were not exhibiting consistent choice of mates between the dichotomous mate choice test and the full-interaction choice test, as they were not able to fully access their mates in the dichotomous mate choice trials.

In a recent study on *G. assimilis* Loranger & Bertram (2016a) revealed consistent mating behaviour between the dichotomous mate choice test and the no-choice test (Loranger & Bertram, 2016a). However, in the latter study, only 49% of the no-choice tests resulted in mounting, while mounting occurred in 92% of my female choice trials and 89% of my male choice trials in my study. This difference suggests that having the options of two potential mates in the area influences female's likelihood of mating. Additionally, although females were more likely to mount a preferred male, it is unclear whether or not the preferred male was also the larger male. As the females were more likely to mount a larger male in the no choice trials (Loranger & Bertram, 2016a), any consistency between tests may have been masked by a preference for size, as females may forego mating with a male if he is small, and presumably of lower quality. Females did not show a preference for winners over losers in the dichotomous mate choice trials, but they did prefer to mount winners to losers in the no-choice trials (Loranger & Bertram, 2016a), indicating once again the importance of chemosensory cues in cricket mating behaviour.

Another possible explanation for why Loranger & Bertram (2016a) found consistency between dichotomous mate choice and no-choice studies where I found no

consistency between dichotomous mate choice and full-interaction studies was that we used slightly different arenas. The predominant difference was in the space allocated to the stimulus crickets. In my study, stimulus crickets had 15 x 15 cm (L x W) space in which to roam, while the Loranger and Bertram (2016a) study gave the stimulus crickets a substantially smaller 6 x 5 cm (L x W) space to roam. Additionally, another study using *G. assimilis* and dichotomous choice tests also gave their stimulus crickets a 6 x 5 cm (L x W) space to roam and found that their focal females were consistently choosing the same male stimulus crickets between both dichotomous mate choice trials significantly more than expected by random choice (Bertram et al., 2016). This is a reduction in size by nearly 1/3 of the stimulus crickets' area used in my study, which may have prompted the stimulus crickets to be closer to the partition separating them from the focal cricket, allowing antennal contact between the stimulus and focal crickets to occur more frequently. Focal crickets would have been able to better access the pheromones of their potential mates, and therefore make informed decisions during the dichotomous mate choice tests. This indicates that dichotomous mate choice tests may be able to properly access mate preferences in crickets, but only if the space allocated to stimulus crickets is small, forcing them to remain close to the barrier between them and the focal cricket, allowing the focal cricket to access chemosensory cues needed for mate choice.

My study did not reveal a preference for body size. The previous study that evaluated male mate choice in *Gryllus assimilis* also found that males do not seem to prefer larger females (Bertram et al., in revision), although female *G. assimilis* prefer larger males (Loranger & Bertram, 2016a; Bertram et al., in revision). The negative findings of my study may be the result of the randomization method I used to select the

stimulus crickets. While this situation is akin to the trait distribution individuals would naturally encounter, it dramatically decreases the likelihood of observing mating preference relative to the more standard methodology of using stimulus crickets selected from the two ends of the size distribution. In support of the argument that randomly selecting crickets decreased the likelihood of finding mating preferences, I found that females from a female-biased environment tended to prefer larger males when there was a greater difference between the stimulus males in the dichotomous mate choice test. Size may therefore be important, but only if there is a large size discrepancy between potential mates. Additionally, only larger and heavier males from a female-biased environment showed a preference for larger females, indicating that only high-quality males are able to exhibit choice (Edward & Chapman, 2011). Bertram et al (in revision) had a similar finding, where large males were much more likely to exhibit consistent mating preferences across both dichotomous mate preference trials than smaller males. Overall, these findings suggest that using random stimulus crickets may make finding an effect of body size difficult.

4.2 Future directions

Investigations into whether the OSR during mating events affects mate choice should be examined in *Gryllus assimilis*. The only study to date to vary OSR in crickets during mating events found an effect, but their study also simultaneously varied total density, so it is difficult to tease apart these two effects (Souroukis & Murray, 1995).

OSR during juvenile development may also influence adult mating behaviour. Additionally, the perceived future OSR of juveniles may also influence development and

mating behaviours. Future studies should evaluate the effects of both the adult OSR and juvenile sex ratios on juvenile development and future mating behaviours.

Future studies should also investigate female courtship effort in terms of females approaching males, as females from a female-biased OSR may approach males more often in order to promote courtship effort from the males. Additionally, these potential studies should also further investigate whether OSR affects competition and sexual interference, which should increase in sex that is the most abundant sex.

Investigating whether *Gryllus assimilis* can exhibit mate choice based on the pheromones of potential mates should also be investigated. Additionally, if they are choosing mates based on their pheromone profiles, an experiment should be conducted to test whether a preferred cricket will still be chosen as a mate if its pheromones are experimentally removed.

A study using different dichotomous mate choice arenas should also be conducted, where the stimulus areas vary in sizes. This could determine how much space should be allocated to allow the stimulus individuals some freedom to roam, but without hindering the focal individuals ability to access chemosensory cues. This could better determine whether dichotomous mate choice tests are adequate in determining mate choice in crickets.

As female crickets did not prefer larger males in the current study, future studies should use males from both extremes of the body size spectrum in order to determine whether male body size is an important trait for females when choosing mates. Also, only large and heavy males showed a preference for larger females, indicating that experiments on male mate choice may only yield results if the males chosen as the focal

individuals are of high quality. An experiment should be conducted using a set of large males and a set of small males, with females from the extremes of the size spectrum, in order to determine whether female body size is important to males when male size is controlled. Other morphological traits could also be evaluated during mate choice tests, such as mandible sizes. Mandibles are part of the crickets' armaments, and males that win more aggressive encounters tend to have relatively larger mandibles (Judge & Bonanno, 2008). As winners are more likely to successfully mate (Simmons, 1986), cricket mandibles may be used as ornaments to honestly signal their potential competitive success.

Overall, my study found that the adult OSR prior to mating does not influence male or female mate choices. However, OSR could influence the cuticular hydrocarbon composition of focal crickets, which may have influenced the behaviour of the stimulus crickets. My methods may not have been able to detect an affect of OSR on mate choice because the immediate OSR for crickets, or at least their perceived OSR, may be what is most influential on their mating behaviour. My experiments also indicate that the use of dichotomous mate choice tests may be inappropriate with crickets because crickets may utilize contact-based cuticular hydrocarbons as traits to choose mates.

Appendix

Table 10: Comparison of studies evaluating the effects of OSR on different mating behaviours and whether or not total density was controlled (DC): Competition (Comp), sexual interference (Interf), mate guarding (Guard), courtship (Court), mating success (Success), and mate choice (Choice).

Species	Sex	DC	Comp	Interf	Guard	Court	Success	Choice	Reference
Mammals									
Bank vole <i>Clethrionomys glareolus</i>	F	Almost					Contest		Klemme et al., 2007
Fishes									
Threespine stickleback	F	Yes					Support		Tinghitella et al., 2013
<i>Gasterosteus aculeatus</i>									
St Peter's fish <i>Sarotherodon galilaeus</i>	F/M	No					Support		Balshine-Earn, 1996
Japanese medaka <i>Oryzias latipes</i>	F/M	No	Support			Support			Clark & Grant, 2010
	F	No	Support						Grant & Foam, 2002
Guppy <i>Poecilia reticulata</i>	F/M	Yes		Support		Contest	Support	Support	Jirokul, 1999
Zebrafish <i>Danio rerio</i>	M	Yes	Contest			Contest			Spence & Smith, 2005
Two spotted goby <i>Gobiusculus flavescens</i>	F/M	Yes	Contest			Contest			de Jong et al., 2009
	F/M	No	Support				Contest		Wacker et al., 2013
Annual fish	F	No					Support		Passos et al., 2014
<i>Austrolebias reicherti</i>									
European bitterling <i>Rhodeus sericeus</i>	M	No	Contest			Contest	Contest		Mills & Reynolds, 2003
Rosy bitterling <i>Rhodeus ocellatus</i>	F	No	Contest		Support	Support			Liao et al., 2014
Pipefish <i>Syngnathus typhle</i>	M	Yes					Support		Berglund, 1994
Long-snouted seahorse	M	No	Contest				Contest		Naud et al., 2009

Hippocampus guttalatus

Invertebrates

Carrion beetle <i>Necrophila americana</i>	M	Yes	Support	Knox & Scott, 2006
Firebugs <i>Pyrrhocoris apterus</i>	M	Yes	Support	Schofl & Taborsky, 2002
Milkweed beetles <i>Tetraopes traophthalmus</i>	M	No	Support	Support Lawrence, 1986
Fall field cricket <i>Gryllus pennsylvanicus</i>	M	No	Support	Souroukis & Murray, 1993
	F	No		Support Souroukis & Murray, 1995
Water Stider <i>Gerris odontogaster</i>	F	Yes		Contest Arnqvist 1992
Burying beetle <i>Nicrophorus vespilloides</i>	M	No		Contest Hopwood et al., 2016
Bushcricket <i>Kawanaphila nartee</i>	M	No		Support Kvarnemo & Simmons, 1999
Bushcricket <i>Steropleurus stali</i>	F	No		Support Bateman, 1997
Fiddler crab <i>Uca mjoebergi</i>	M	No		Support Reading & Backwell, 2007
Butterfly <i>Bicyclus anynana</i>	F/M	No	Support	Support Holveck et al., 2015
Seed bug <i>Nysius huttoni</i>	M	Yes		Contest Wang et al., 2009

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