Winter 12-2011

EFFECT OF DIET QUANITITY AND QUALITY ON FEMALE SAMPLING BEHAVIOUR AND MATING PREFERENCES IN A FIELD CRICKET

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by

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A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Biological Sciences

Under the Supervision of Professor William E. Wagner, Jr.

Lincoln, Nebraska

December, 2011

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University of Nebraska, 2011

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Understanding the adaptive significance of variation in female mating behaviour is important because variation may often be favored by selection instead of a change in mean mating behaviour, particularly in variable environments. Females are known to adjust their mating behaviour to a variety of intrinsic and extrinsic factors including nutrition. There are multiple reasons why female behaviours might vary with nutrition; we tested two of these hypotheses: the search cost hypothesis and the direct benefits hypothesis. These hypotheses are not mutually exclusive, but they make contrasting predictions under some conditions. Low nutrition females may have less available energy to support the costs of searching for and sampling males. If sampling males is energetically costly, the search cost hypothesis predicts that low nutrition females will sample fewer males, and because they invest less in sampling, will show less biased mate choices. Alternatively, in species with male provided direct benefits, low nutrition females often benefit more from mating with preferred males. When this is the case, the direct benefits hypothesis predicts that low nutrition females will show stronger preferences for traits correlated with benefit quality and thus show more biased mate choices. The direct benefits hypothesis does not make an obvious prediction about female sampling behaviour, although greater sampling might be required to identify high benefit

males. We tested these hypotheses using the variable field cricket, *Gryllus lineaticeps*. In this species, females receive fecundity benefits from mating with high chirp rate males but only in low nutrition females. We manipulated either diet quantity or quality (protein to carbohydrate ratio) and measured female mate preference. In the diet quality experiment, we also measured sampling behaviour. Diet quality influenced female sampling behaviour: females provided a high quality diet sampled more extensively. However, neither diet quantity nor diet quality influenced female preference. These results partially support the search cost hypothesis. Possible explanations for why diet treatments did not influence female chirp rate preference are discussed.

AUTHOR'S ACKNOWLEDGEMENTS

Festivus, bad movie nights, sitting by the pool, adventures on O Street, "Crappy Beer Nights", and most recently tribal belly dancing. I would like to specially thank Danielle Tufts and Kathy Roccaforte for always being there for both the good and the bad.

Lastly, I would like to thank my family for being supportive in my journey from Minnesota to Nebraska to attend graduate school. I give a heartfelt thank you to my parents, Deb and Jerry, for encouraging my love of biology. One of my favorite childhood toys will always be the homemade bug barn. I thank my sisters, Emily and Stacie, and my brother-in-law (Stacie's husband) Michael for helping me to keep everything in perspective. I also thank my guinea pig, Roscoe, for being ridiculously cute and for making me smile every day.

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INTRODUCTION

  Understanding the adaptive significance of variation in female mating behaviour is important because variation may often be favored by selection instead of a change in mean mating behaviour, particularly in variable environments (Henson & Warner 1997; Jennions & Petrie 1997; Widemo & Sæther 1999). Female mate choices are affected by the interaction between sampling behaviour (the number of males whose traits they assess), their preferences (the traits they prefer), and environmental factors (Wagner 1998). Females are known to adjust their mating behaviour to a variety of intrinsic and extrinsic factors that can vary during an individual's lifetime (Jennions & Petrie 1997). Females may use one of many possible sampling strategies during mate choice that can influence female sampling behaviour (Janetos 1980). Sampling behaviour has been shown vary due to increased costs related to increased sampling effort such as travel costs between males (Milinski & Bakker 1992) and parasite load (Lopez 1999, Buchholz 2004). Females may benefit from changing their sampling behavior under certain conditions depending on the costs and benefits. Female mate preferences have been shown to vary due to predation risk during mate choice (Forsgren 1992; Hedrick & Dill 1993; Johnson & Basolo 2003; Vélez & Brockmann 2006), parasite load (Poulin 1994; Lopez 1999), operational sex ratio (Souroukis & Murray 1995; Jirotkul 1999), previous experience with males (Collins 1995; Marler et al. 1997; Hebets 2003), and nutrition (Bakker et al. 1999; Hingle et al. 2001; Cratsley & Lewis 2003; Hunt et al. 2005; Fisher & Rosenthal 2006; Hebets et al. 2008; Immonen et al. 2009). In some cases, females are known to benefit from expressing different preferences based on intrinsic or extrinsic factors (Qvarnström et al. 2000; Pfennig 2007; Reaney & Backwell 2007). For example,

female spadefoot toads prefer heterospecific calls over conspecific calls in environments where hybrid offspring have a better chance of surviving than non-hybrid offspring (Pfennig 2007).

There are multiple reasons why female mating behaviour might vary with nutrition; we tested two of these hypotheses: the search cost hypothesis and the direct benefits hypothesis. These hypotheses are not mutually exclusive, but they make contrasting predictions under some conditions. Low nutrition females may have less available energy to support the costs of searching for and sampling males (Gray 1999; Cotton et al. 2006). If sampling males is energetically costly, the search cost hypothesis predicts that low nutrition females will sample fewer males, and because they invest less in sampling, will show less biased mate choices. The mate choice prediction of the search cost hypothesis is supported by studies on species where females may not receive male provided direct benefits (Bakker et al. 1999; Hingle et al. 2001; Hunt et al. 2005). Alternatively, in species with male provided direct benefits, low nutrition females often benefit more from mating with preferred males (Brown 1997; Wagner & Harper 2003; Engqvist 2009). When this is the case, the direct benefits hypothesis predicts that low nutrition females will show stronger preferences for traits correlated with benefit quality and thus show more biased mate choices. The direct benefits hypothesis does not make an obvious prediction about female sampling behaviour, although greater sampling might be required to identify high benefit males. The mate choice prediction of the direct benefits hypothesis is supported by some studies on species in which females gain direct benefits from males (Cratsley & Lewis 2003; Immonen et al. 2009). We are not aware of any studies that have tested the sampling predictions of the two hypotheses.

Study System and Background

We tested the predictions of the search cost and direct benefits hypotheses using the variable field cricket, *Gryllus lineaticeps*. This species is found in southern Oregon, California, and Baja California (Weissman et al. 1980). In general, male field crickets attract females to their burrows by producing a calling song (Alexander 1961). Previous studies on *G. lineaticeps* have shown that females prefer males that produce higher chirp rate songs (Wagner 1996; Wagner & Basolo 2007). If a female is attracted to a calling song, she will approach the male and both crickets enter the burrow. During mating, males will transfer a spermatophore containing sperm and seminal proteins. Approximately 30 to 90 minutes after mating, females may remove and consume the spermatophore, although the benefits of spermatophore consumption appear to be minimal in field crickets (Simmons 1988). In *G. lineaticeps*, females receive fecundity benefits from mating with high chirp rate males, even when prevented from consuming spermatophores, but only in low nutrition females (Wagner & Harper 2003; Tolle & Wagner 2011). All females thus appear to receive beneficial seminal products from preferred males, but the value of these seminal products depends on nutrition.

Field crickets are omnivores; they feed on grasses, seeds and dead insects (Gangwere 1961; Monteith 1971). These food sources vary in both quantity and quality. The quantity of food resources may vary spatially and temporally due to multiple environmental factors such as amount of rainfall, temperature and habitat disturbance. The quality of food items may also vary spatially and temporally due to variation within and among food types in their nutritional composition; plants have low protein to

carbohydrate ratios while insects have high protein to carbohydrate ratios (Schoonhoven et al. 2005). For example, native California grasses have a crude protein content of 2.5-12.7% (Dee & Box 1967; Adams et al. 1999) and grasshoppers and crickets have a crude protein content of 55% to ≥70% (Ueckert et al. 1972; Wang et al. 2004). Protein and carbohydrate are two very important macronutrients for all organisms and are often limiting in environments (Behmer & Joern 2008). Studies on grasshoppers, which are generalist feeders and closely related to crickets, have shown that when females are given a choice, they select a preferred protein to carbohydrate ratio and perform best (e.g., develop faster and have higher growth rates) on diets at or near the preferred ratio (Behmer & Joern 2008). This preferred diet usually consists of some intermediate amount of protein and carbohydrate.

We tested the search cost and direct benefits hypotheses using manipulations of diet quantity and diet quality in a field cricket. In the first experiment, we manipulated juvenile and adult diet quantity and measured adult female chirp rate preferences. In the second experiment, we first measured the diet preferences of a set of females by allowing them to select a preferred protein to carbohydrate ratio. We then manipulated diet quality (protein to carbohydrate ratio) for a different set of females and measured female sampling behaviour based on female movement and chirp rate preference.

GENERAL METHODS

Cricket Populations

For the diet quantity experiment, we used second- and third-generation offspring from field-inseminated female crickets collected from Tucker's Grove Park, Santa

Barbara, CA, USA. The crickets used came from the families reared for a previously

described study (Tolle & Wagner 2011). For the diet quality experiment, we used third-

generation offspring from field-inseminated female crickets collected from Santa Barbara

Shores County Park, Goleta, CA, USA. The two locations are 10 km apart and have

similar environmental characteristics.

Laboratory Rearing Conditions

Families were produced from cricket matings that were arranged to reduce

inbreeding (for details see Wagner & Basolo 2007). Each family was reared in a large

clear plastic container (25 x 15 x 17 cm), each of which was outfitted with egg carton

shelter, a paper towel substrate, a water vial plugged with cotton, and Purina Cat Chow.

For the diet quantity experiment, all experimental juvenile females were acoustically

isolated in an environmental chamber maintained at 32° C on a reversed 14:10 h

light:dark cycle. For both experiments, all experimental mature females were acoustically

isolated in a room at 23° C on a reversed 14:10 h light:dark cycle. As females were

isolated before sexual maturity, all females used were virgins.

Preference Chamber

We tested female sampling behaviour (diet quality experiment) and preference

(both experiments) in a 2.2 X 2.2 X 2.7 m acoustically isolated chamber lined with foam

to minimize echoes. Red lighting (four bars of fluorescent lights covered with red film)

was used inside the chamber to facilitate observations. Synthetic male songs were played

from a Macintosh Quadra 840 AV using SoundEdit16 (diet quantity experiment) or Sony

Walkman D-NR430 CD players (diet quality experiment) and were broadcast from KLH

970 speakers connected to Optimus SA-155 amplifiers. In each corner of the floor of the

chamber, there was a 0.26 m diameter circle denoting the zone where the female was said

to be associating with the song. The speaker(s) broadcasting the male song(s) were set in

the center of these circles and placed so that they were 0.31 m away from the wall of the

chamber.

Preference Trial

To begin a trial, the female cricket was acclimated under a cup in the center of the

chamber for 10 minutes during which the appropriate song(s) were broadcast. At the end

of the 10 minutes, we lifted the cup to release the female. The female cricket was given

10 minutes from the time she first moved to travel about the preference chamber. During

the trial, data was collected by viewing crickets on a Panasonic CT-1384Y television

outside of the chamber connected to a Panasonic WV-BP100 video camera located on the

ceiling of the chamber. We recorded preference as the total amount of time the female

cricket spent near the male song(s) (in the circle around the speaker(s)) for both

experiments. For the diet quality experiment we also recorded the first circle entered and

the number of switches between speakers plus one for the first speaker visited (sampling

behaviour). Chamber temperature was maintained at $23.5 \pm 1.5^{\circ}$C.

Male Songs

Synthetic male songs were created by digitizing a natural cricket pulse and

copying it eight times to create a 120 ms long chirp (pulse duration = 11 ms, dominant

frequency = 5.17 kHz); songs of different chirp rate were then created (for details see Wagner & Basolo 2007). We used male songs with three different chirp rates: a low chirp rate song (1.8 chirps/s), an intermediate chirp rate song (3.0 chirps/s), and a high chirp rate song (4.2 chirps/s) (Wagner & Reiser 2000).

Data Analysis

For the diet quantity experiment, all females that did not spend time in the circle around the speaker during either or both trails were excluded in the data analysis because they were considered non-responsive. For the diet quality experiment, data from female crickets that spent less than 10 seconds total (an arbitrary *a priori* number) in the circles around the speakers during one of the trials was excluded because females were considered to be non-responsive. All analyses were performed in Stata IC 10 for Macintosh.

EXPERIMENT 1: EFFECT OF DIET QUANTITY ON CHIRP RATE PREFERRENCE

Acknowledgements

This experiment was conducted by Cassandra M. Martin.

Methods

We fed female crickets different quantity diets as both juveniles and adults and tested their chirp rate preferences At the third stadium, female nymphs were transferred from family containers to individual containers (15 x 8 x 11 cm) with shelter, substrate, water, and a small Petri dish with either a low quantity or high quantity nutrition diet.

These diets were small chunks of moist food that were replaced every other day throughout the experiment. Both diets were composed of 40% wheat bran, 37% wheat germ, 14% casein, 9% yeast, and 2.5% aqueous agar base (Zera & Larsen 2001). The low quantity diet was cut with 50% non-nutritive cellulose. After they matured, some of the crickets were placed on the low quantity diet and the others were placed on the high quantity diet. Thus, we had four diet combinations of juvenile/adult diet: high/high, low/low, high/low, and low/ high.

We tested female chirp rate preferences in the chamber described above using a single-speaker response design. The songs were presented at 76 dB SPL (re: 20 μPa) at 30 cm from the speaker measured using a Radio Shack 33-4050 analog sound level meter. For each test of female chirp rate preference, a female was tested sequentially with two songs: the intermediate chirp rate song in an initial trial and then with one of the test chirp rate songs (low, intermediate, high) in the preference trial. The initial trial was conducted as described above with the intermediate chirp rate song. Immediately following this initial trial, we conducted a preference trial by repeating the above procedure in the exact same manner except for presenting the female cricket with a haphazardly assigned chirp rate (low, intermediate, high). The presentation of an initial intermediate song allowed us to control for differences in responsiveness (time spent in the circle) in the preference trial (see Wagner & Basolo 2007 for full explanation).

A total of 168 females were tested after the diet treatment: 28 females on the high/high diet, 46 on the high/low diet, 45 on the low/low diet, and 49 on the low/high diet. We discarded data for 2 females on the high/high diet, 14 on the high/low diet, 9 females on the low/low diet, and 12 females on the low/high diet for being non-

responsive. We used a linear regression to test the effect of the following variables on the time female crickets spent near the male song in the preference trial: test chirp rate, juvenile diet, adult diet, and all interactions. All categorical variables were coded as dummy variables. We included the time spent near the intermediate chirp rate song in the initial trial as a covariate in the model to control for differences in female responsiveness. Time spent near the song in the preference trial was not normally distributed (Shapiro-Wilk: $P < 0.001$), so we applied a box-cox transformation (time$^{0.1193828}$). Female responses were not normally distributed after transformation (Shapiro-Wilk: $P = 0.001$), so we used a bootstrap analysis with 1000 replications on the transformed data. In our regression, we used a backward stepwise removal procedure to remove non-significant interaction terms from the final model.

Results

There was no effect of diet quantity on chirp rate preference; all interactions between chirp rate and diet treatment were non-significant (all $P > 0.338$). We also found no effect of diet alone; neither juvenile ($F_{1,125} = 0.04$, $P = 0.849$) nor adult ($F_{1,125} = 2.28$, $P = 0.131$) diet quantity affected the time spent near the male song. However, there was an effect of chirp rate on the time spent near male song ($F_{2,125} = 3.09$, $P = 0.045$, Fig. 1). Regardless of diet, females spent more time near the high chirp rate song than the low chirp rate song ($F_{1,125} = 6.06$, $P = 0.014$, p critical = 0.016). There was no difference in the time spent near low and intermediate chirp rate songs ($F_{1,125} = 0.50$, $P = 0.478$, p critical = 0.016) and no different in the time spent near intermediate and high chirp rate songs ($F_{1,125} = 2.44$, $P = 0.118$, p critical = 0.016). There was a strong effect of female

responsiveness in the initial trial on time spent near the male song in the preference trial

($F_{1,125} = 23.18$, $P < 0.001$); females who were more responsive in the initial test with an

intermediate chirp rate spent more time near the speaker in the subsequent trial.
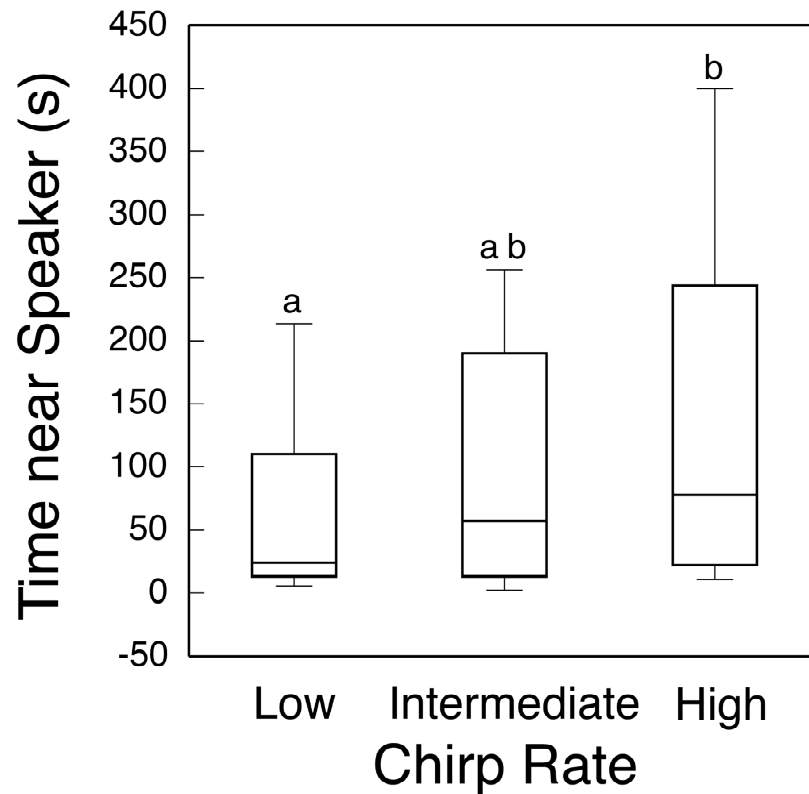
Figure 1.1. Box plot for time near each speaker versus chirp rate. Diet did not influence female chip rate preferences therefore data was combined across diet treatments. Whiskers are the 10th and 90th percentile. Different letters represent significant differences ($P < 0.05$).

EXPERIMENT 2: EFFECT OF DIET QUALITY ON SAMPLING BEHAVIOUR AND CHIRP RATE PREFERENCE

Acknowledgement

This experiment was conducted by Heidi L. Bulfer.

Methods

Experiment 1 suggested that diet quantity does not affect female chirp rate preferences. We therefore examined the effect of diet quality on female chirp rate preference, as well as the effect of diet quality on female sampling behaviour. Before their penultimate molt, female nymphs were transferred from family containers to individual containers (15 x 8 x 11 cm) with shelter, substrate, water, and *ad libitum* Purina cat chow. Each day, we checked containers to determine the day females reached sexual maturity. We first determined the protein to carbohydrate ratio preferred by females. We then placed females on preferred and non-preferred diets and tested the effect on female behaviour. Diets consisted of varying percentages of protein (3:1:1 mixture of casein, peptone and egg albumen) and digestible carbohydrate (1:1 mixture of sucrose and white dextrin) to produce the appropriate protein:carbohydrate (P:C) ratio. Diet ratios are the percent of dry mass that is composed of protein and digestible carbohydrate. Diets also contained 2.4% Wesson's salt, 0.5% linoleic acid, 0.5% cholesterol, 0.3% ascorbic acid, 0.2% vitamin mix (see Dadd 1961) and an appropriate amount of cellulose as filler (Simpson & Abisgold 1985). A diet constituent worksheet and protocol was obtained from the Behmer Lab located at Texas A&M University,

College Station, TX. Diets were homogenized and ground to a fine powder so individuals could not pick out particular protein, carbohydrate, or other food particles.

In order to determine if females have a preferred protein to carbohydrate ratio, we provided individual females with a dish containing a high protein diet and a dish containing a high carbohydrate diet, measured the amount consumed from each dish, and calculated the ratio of protein to carbohydrate consumed. To do this, we weighed mature, virgin female crickets and placed each cricket in a large container (25 x 15 x 17 cm) with one high protein (P:C = 28:14) food dish and one high carbohydrate (P:C = 7:35) food dish on opposite ends of the container. Approximately 2 grams of food was placed in each food dish, which was a sufficient amount for the entire feeding period. Females were also provided a cardboard egg crate shelter, a paper towel substrate and a water vial plugged with cotton. The water vial was haphazardly placed on one end of the container next to a food dish. The diets are hygroscopic, so control dishes for both diets were set out each day to control for changes in moisture content from the environment (Simpson & Abisgold 1985). Control dishes were placed in a large container with no cricket. We measured the amount of food consumed from each food dish on days 1, 4, 8, and 12. This created three time periods to determine if the preferred P:C ratio changed with female age. For each diet, the percent weight gain for the daily control dish was calculated for each time period. We then added the percent increase of the control dish to the calculation of the amount consumed from the experimental dish to control for increase in moisture content. Using the know protein to carbohydrate ratios of the diets and the amount consumed from each dish, we calculated the amount of protein and carbohydrate consumed by each female. The diet preference of 23 females was measured. We used

repeated measures ANOVA to determine if the preferred P:C ratio varied with age and linear regression to examine the relationship between protein and carbohydrate consumption; the slope of the relationship is the preferred ratio of protein to carbohydrate. In addition, we examined the correlation between female initial mass and the mass of food consumed.

To determine if female sampling behaviour and preferences vary with diet quality, we tested females before and after a diet treatment in the chamber described above using a three-speaker choice design. The songs were presented at 70 ± 0.5 dB SPL (re: 20 µPa) at 30.5 cm from each speaker measured using a Casella CEL-254 Digital Impulse Sound Level Meter (impulse RMS). We selected females that were 8 days of adult age, weighed females, and tested their sampling behaviour and chirp rate preference using three chirp rate songs: low, intermediate, and high. Females were individually placed in the testing arena with one speaker in each of three corners (for details see Beckers & Wagner 2011). The three songs were randomly assigned to one of the three speakers and changed after each trial. Trials were conducted as described above with all three songs playing simultaneously.

After the initial test, we provided each female with one of three diets, either the preferred diet with a P:C percent dry mass ratio of 21:28 (see results) or a high carbohydrate (P:C=7:35) or high protein (P:C=35:7) diet. Since crickets are omnivores, a high carbohydrate diet represents a diet high in plant material and a high protein diet represents a diet high in insects (see introduction). Each female was given approximately 1.5 grams of food, which was sufficient for the entire feeding period. After 10 days on the diet, we retested female chirp rate responses and sampling behaviour using the same

procedure described above. After the final test, we recorded female weight. A total of 73 females were tested before the diet treatment; 8 of these females were non-responsive and discarded. This left 65 females that were tested after the diet treatment: 20 females on the high carbohydrate diet, 22 females on the preferred diet, and 23 females on the high protein diet. We discarded data for 2 females on the high carbohydrate diet, 2 females on the preferred diet, and 5 females on the high protein diet for being non-responsive in the after diet treatment test.

The effect of diet on mass gain was analyzed using an ANOVA. We used Tukey-Kramer post hoc comparisons. Sampling behaviour was analyzed with a linear mixed model with a Poisson distribution and maximum likelihood estimation. Diet, time period (before and after the diet manipulation), and the interaction between diet and time period were fixed factors, and female identity was a random factor. The effect of diet on female chirp rate preferences was analyzed with a linear mixed model with a Gaussian distribution and maximum likelihood estimation. Diet, time period, and the interaction between diet and time period were fixed factors, and female identity was a random factor. Female choice was measured as the first speaker circle entered. Because female responses were not normally distributed, we used a bootstrap analysis with 1000 replications for significance testing. In our analysis, we used a backward stepwise removal procedure to remove all non-significant interaction terms.

We also cold anesthetized, decapitated and dissected a subset of females to measure ovary size as the wet mass in grams of the ovaries. Ovary size data was collected for 21 females from the diet quality experiment: 8 females on the high carbohydrate diet, 7 females on the preferred diet and 6 females on the high protein diet. Additional data

were collected for each treatment group to increase sample size. These additional data were collected using females that were 8 days after adult moult and placed on one of the three diets for 10 days. Ovary size was measured as described above. Ovary size was collect for a total of 32 females: 12 females on the high carbohydrate diet, 11 females on the preferred diet and 9 females on the high protein diet. Ovary size data was analyzed using an ANCOVA with total individual mass as a covariate. We used Tukey-Kramer post hoc comparisons.

Results

First, we examined the protein:carbohydrate (P:C) ratio preferred by females. The preferred P:C ratio for mature female crickets did not vary with age (repeated measures ANOVA: $F_{2,44} = 3.08$, $P = 0.056$). We pooled the data for the whole time period and found that females preferred to eat a diet with an approximate 3:4 ratio of protein to carbohydrate (Linear regression: y = 1.27x + 0; $F_{1,22} = 983.00$, $P < 0.000$). This is equivalent to a P:C diet percent of dry mass ratio of 21:28. This preferred ratio was provided to females assigned a preferred diet in the diet manipulation experiment. Total amount of food consumed was positively correlated with female weight (Pearson's correlation: $r = 0.53$, $P = 0.010$). While larger females consumed more food, the ratio of protein to carbohydrate consumed was consistent among females (Fig. 2).
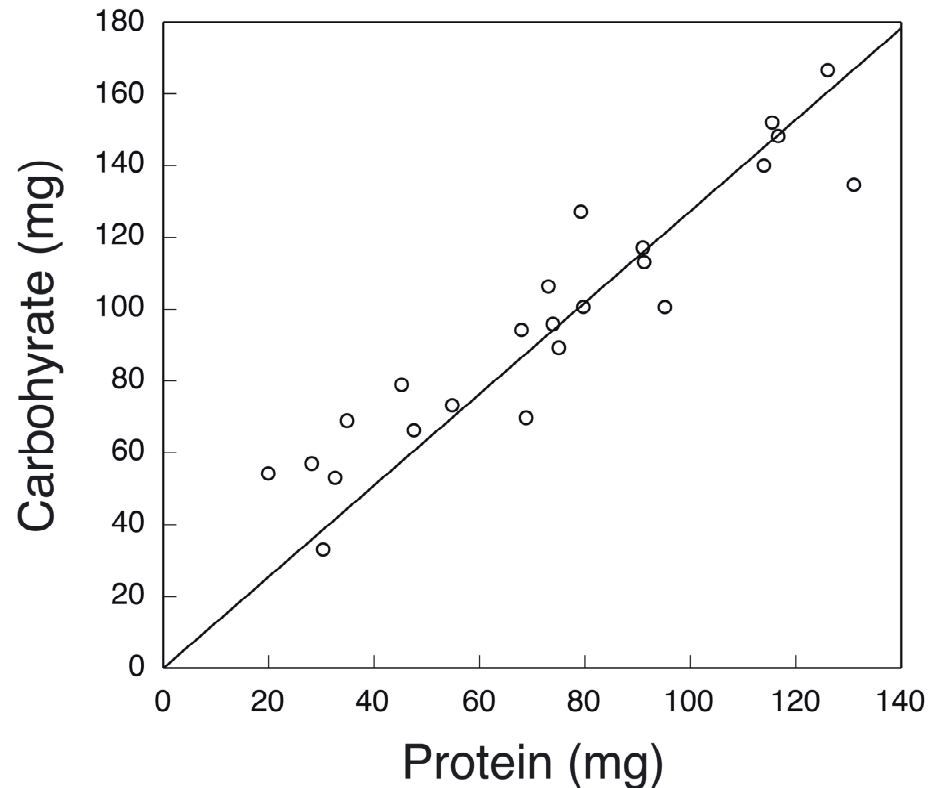
Figure 1.2. The total amount of protein versus carbohydrate consumed when females are given a choice between two diets. Open circles represent individual female cricket diet preference and the solid line shows the line of best fit (y=1.27x + 0).

Second, we manipulated the diets of females and examined the effect of diet quality on female mass, ovary size, and behaviour. There was a significant effect of diet on female change in mass (ANOVA: $F_{2,53} = 6.87$, $P = 0.002$); females on the high carbohydrate diet gained significantly less mass compared to females on either the preferred diet or the high protein diet (Fig. 3a). There was also a significant effect of diet on ovary mass after controlling for total mass (ANCOVA: diet $F_{2,28} = 4.05$, $P = 0.029$; total mass $F_{1,31} = 44.16$, $P < 0.001$); females on the high protein diet had significantly smaller ovaries (Fig. 3b)
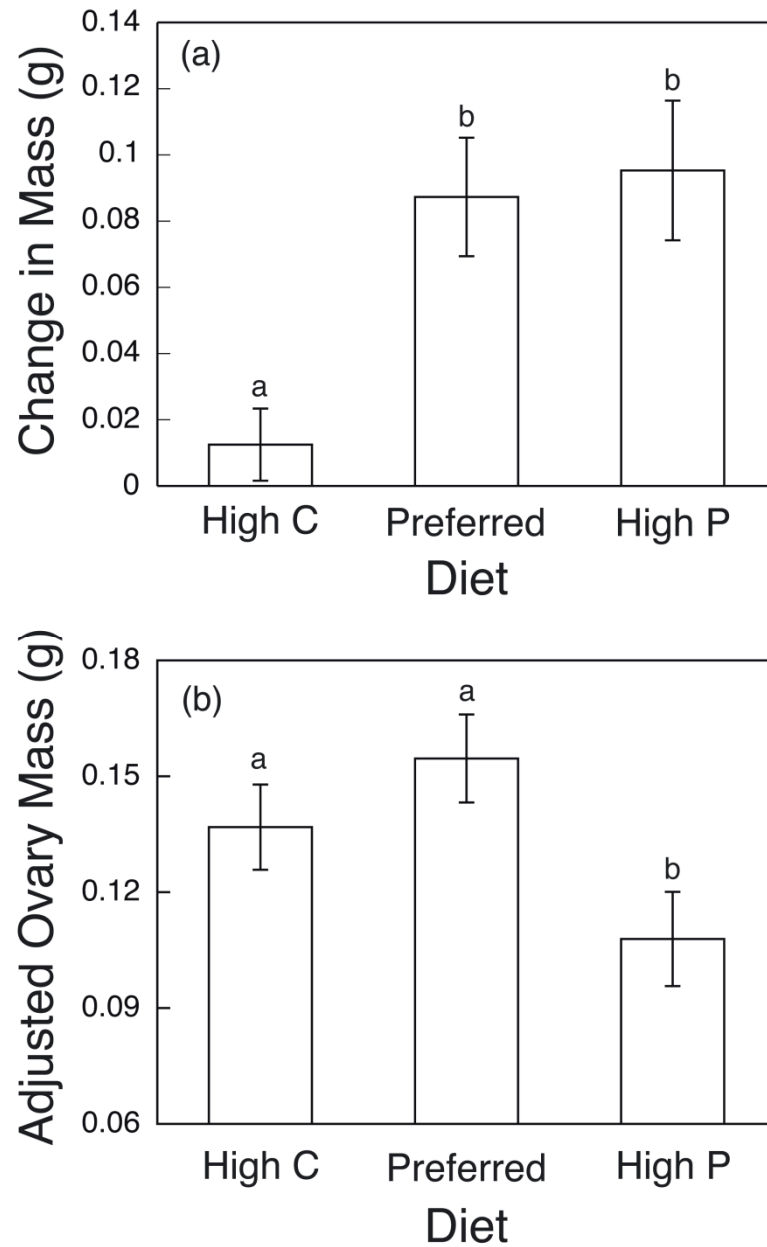
Figure 1.3. Effect of the diet treatment on female (a) mean change in weight and (b) mean adjusted ovary size. High C is the high carbohydrate diet and High P is the high protein diet. Error bars are standard error. Different letters represent significant differences ($P < 0.05$).

In three speaker choice tests, the number of speakers that a female approached during a trial was significantly affected by the interaction between time period (before and after the diet manipulation) and diet treatment (Table 1). Females on the preferred and high carbohydrate diets sampled approximately the same number of speakers before and after the diet manipulations, whereas females on the high protein diet sampled substantially fewer speakers after the diet treatment (Fig. 4).

Table 1.1 Results of a linear mixed model examining effects on female sampling behaviour. Regression coefficients are presented for the fixed effects. The random factor of female identity was not significant and therefore not included. Significant results are in bold.

Fixed effects	Coefficient	SE	X^2_1	P
Diet	0.792	0.296	7.16	0.007
Period	0.807	0.421	3.70	0.054
Diet x period	**-0.442**	**0.189**	**5.47**	**0.019**
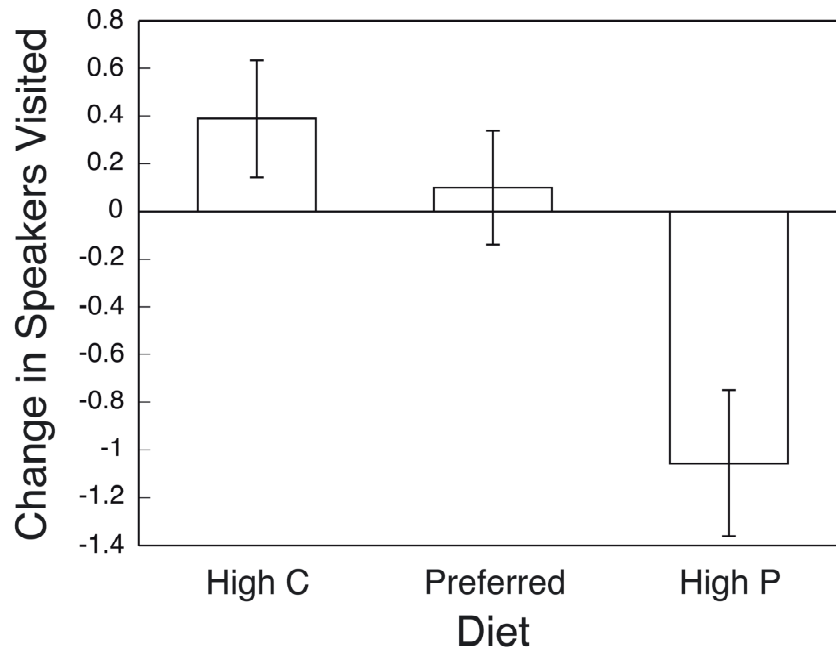
Figure 1.4. Box plot for the changed in the number of speakers visited after the diet treatment. High C is the high carbohydrate diet and High P is the high protein diet. The dark lines represent the median. Whiskers are the 10th and 90th percentile. Different letters represent significant differences ($P < 0.05$).

The first speakers visited by females on the different diets was not significantly different either before the diet treatment (Fisher's exact test: $P = 0.425$; Fig. 1.5a) or after the diet treatment (Fisher's exact test: $P = 0.253$; Fig. 1.5b). Female responses were not significantly affected by the interaction between chirp rate, time period, and diet (mixed linear model: $X_1^2 = 0.16$, $P = 0.687$), indicating that female preferences after the diet manipulations were not affected by diet treatment (i.e., females placed on the two non-preferred diets did not show a significant increase or decrease in the strength of their chirp rate preference compared to females placed on the preferred diet). The three-way interaction was thus dropped from the statistical model. Female responses were, however, affected by the interaction between time period and diet (Table 2); females in all of the

treatment groups spent less time around speakers after the diet manipulations, but females on the high protein diet reduced their responses the least and females on the preferred diet reduced their responses the most (Fig. 4). Female responses were also significantly affected by chirp rate: independently of time period and diet treatment, females spent more time around speakers broadcasting higher chirp rates than speakers broadcasting lower chirp rates (Fig. 5).
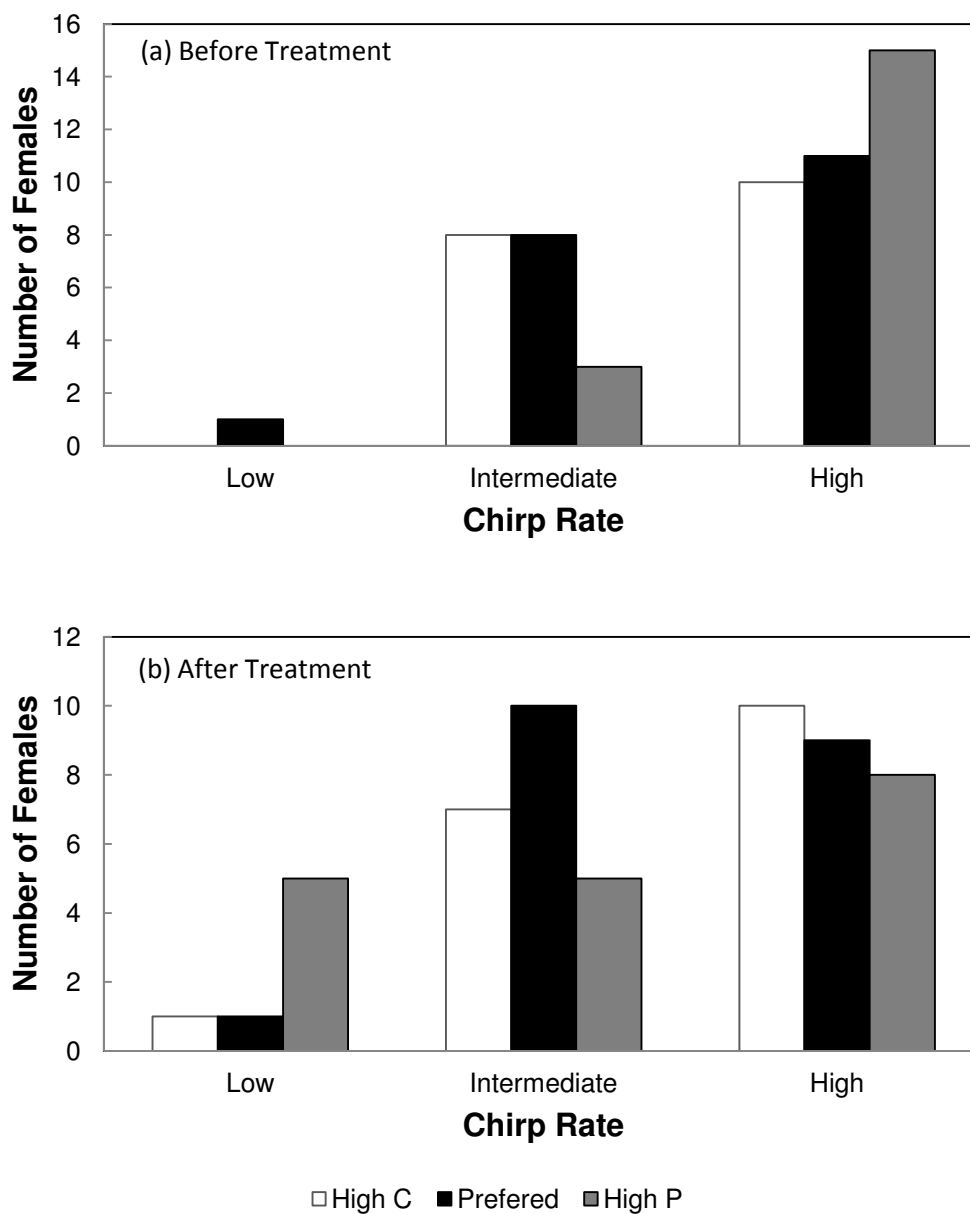
Figure 1.5. Histograms for the first speaker visited (a) before and (b) after the diet treatment. High C is the high carbohydrate diet and High P is the high protein diet.

Table 1.2. Results of a linear mixed model examining effects on female preference. Regression coefficients are presented for the fixed effects. The effect of the chirp rate x period x diet interaction was not significant (see results) and was dropped from the final model. The random factors of female identity and error were not significant and therefore not included in the table. Significant results are in bold.

Fixed effects	Coefficient	SE	X^2_1	P
Chirp rate	**-0.196**	**0.054**	**13.15**	**< 0.001**
Period	-0.190	0.071	7.04	0.008
Diet	-0.119	0.049	5.80	0.016
Chirp rate x period	0.043	0.027	2.56	0.110
Chirp rate x diet	0.011	0.160	0.44	0.508
Period x die	**0.057**	**0.027**	**4.42**	**0.035**

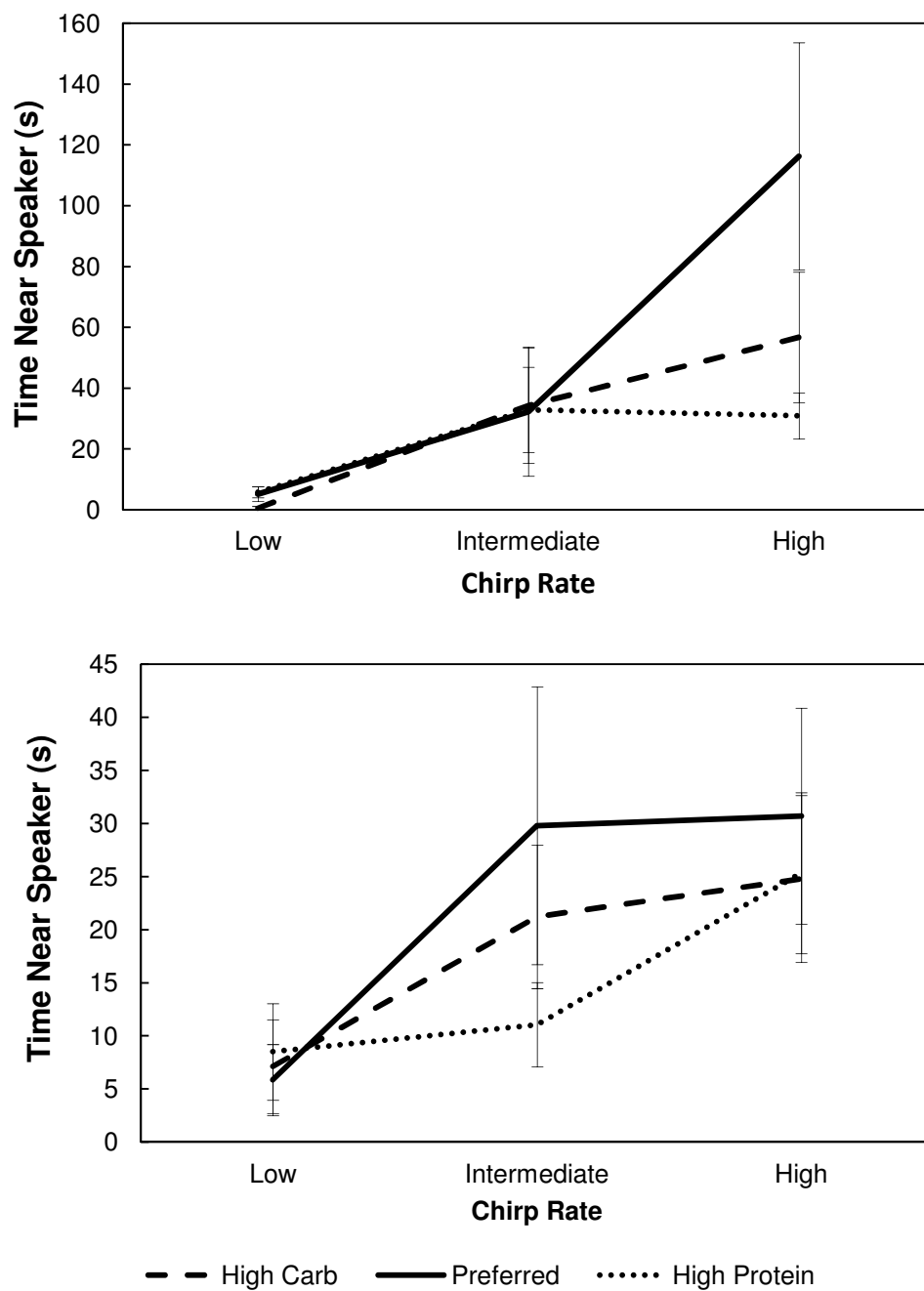**Figure 1.6.** Mean time near each speaker versus chirp rate for females on the three diets (a) before and (b) after diet treatment. High C is the high carbohydrate diet and High P is the high protein diet. Error bars are the standard error.

DISCUSSION

Females often show variation in sampling behaviour and mate preference (Henson & Warner 1997; Jennions & Petrie 1997; Widemo & Sæther 1999). One source of this variation may be available resources or nutrition (Jennions and Petrie 1997). There are multiple reasons why female behaviours might vary with nutrition; we tested two of these hypotheses. The search cost hypothesis predicts that low nutrition females will sample fewer males, and because they invest less in sampling, will show less biased mate choices. The direct benefits hypothesis predicts that low nutrition females will show stronger preferences for traits correlated with benefit quality and thus show more biased mate choices. The direct benefits hypothesis does not make an obvious prediction about female sampling behaviour, although female may need to sample more males to identify high benefit males. Our results suggest that diet quality affected female sampling behaviour; high protein females visited fewer speakers after the diet treatment. However, neither diet quantity nor diet quality significantly influenced female chirp rate preference. These results are surprising, and we discuss possible explanations below. Our results also suggest that females are able to select a preferred protein to carbohydrate ratio and diets that differ from the preferred diet influence female weight gain and ovary size.

Female Diet Choice

We found that when given a choice between two diets, female crickets will select a common protein to carbohydrate ratio by adjusting their consumption of different food items. The ability to select protein to carbohydrate ratios has been shown in a wide variety of taxa including mammals (Musten et al. 1974; Kyriazakis & Emmans 1991),

birds (Kaufman et al. 1978), spiders (Greenstone 1979), and insects (Cohen et al. 1987; Waldbauer & Friedman 1991; Telang et al. 2001; Behmer & Joern 2008). Diet selection is important because individuals may be able to choose food items at different life stages to maximize growth and/or reproductive output (Cohen et al. 1987; Hamilton et al. 1990); insects fed at or near their preferred diet develop faster and have higher growth rates compared to individuals fed a non-preferred diet (Cohen 2001; Behmer and Joern 2008). Although our results show that females did not differ in the preferred protein to carbohydrate ratios with age after maturity, female nymphs may have a different preferred protein to carbohydrate ratio that could maximize growth during this stage.

Effect of Diet Quality on Female Mass and Ovary Size

Females on the high carbohydrate diet gained significantly less mass compared to females on the preferred or high protein diet. These results are similar to studies done on other species of crickets. Female black field crickets, *Teleogryllus commodus*, reared on a low protein diet gained significantly less weight compared to females on a high protein diet (Hunt et al. 2005), and, female house crickets, *Acheta domesticus*, fed a low diet, lacking essential vitamins and minerals, had smaller body sizes compared to females on a high nutrition diet (Patton 1967). Our results suggest that females have smaller body size on the non-preferred high carbohydrate diet than on the preferred diet.

Protein has been shown to be a limiting factor for egg production in birds (Ramsay & Houston 1998; Roberts et al. 2007), reptiles (Henen 2002), and arthropods (Checkley 1980; Hingle et al. 2001; Carey et al. 1998; Aluja et al. 2001). However, our results show that high protein diet females had significantly smaller ovaries relative to

their body weight compared to females on the preferred or the high carbohydrate diet. In a closely related species of field cricket, *Gryllus firmus*, egg number is proportional to ovary mass (Roff 1994). This suggests the high carbohydrate diet contained a sufficient amount of protein to produce eggs, and that excess protein negatively affects ovary development and thus egg production. These results are similar to a study on female German cockroaches where females given a high protein diet produced smaller oothecae with significantly fewer eggs compared to females on a low protein diet (Hamilton & Schal 1988). One possible explanation for these results is that females on the high protein diet did not have sufficient carbohydrate to allocate towards reproduction. They may, for example, allocate available carbohydrate to survival until they locate a high carbohydrate food source and can allocate resources to egg production. This hypothesis could be tested by switching high protein females to a high carbohydrate diet. Alternatively, it has been suggested that high protein diets are toxic to many insects; a high protein diet may cause individuals to produce excess uric acid, ammonia, and tryptophan, all of which are toxic at high concentrations (Hamilton & Schal 1988).

Together, these results suggest that females perform best on the preferred diet: they gained more mass on the preferred diet than on the high carbohydrate diet, and they had larger ovaries on the preferred diet than on the high protein diet, which should result in higher fecundity.

Effect of Diet Quantity and Quality on Female Mate Preference

We tested two hypotheses that could explain the effect of diet quantity and quality on female mate preference: the search cost hypothesis and the direct benefits hypothesis.

The search cost hypothesis predicts that low nutrition females will show less biased mate choices, while the direct benefits hypothesis predicts that low nutrition females will more biased mate choices. There was no detectable effect of juvenile or adult diet quantity on female chirp rate preference; regardless of their diet, females in all four treatment groups showed a similar preference for higher chirp rate song. There was also no detectable effect of adult diet quality on female chirp rate preference; regardless of their diet, females in all three treatment groups showed a similar preference for higher chirp rate song. Females thus appear to show consistent chirp rate preferences across a variety of diets (see also Wagner 1996; Wagner & Basolo 2007). Our results thus provide little support for either hypothesis, which is unexpected.

Numerous studies have examined the effect of diet on female mate preference. Some have found that females on low nutrition diets show weaker preferences (Bakker et al. 1999; Hingle et al. 2001; Hunt et al. 2005), while others have found that females on low nutrition diets show stronger preferences (Cratsley & Lewis 2003; Fisher & Rosenthal 2006; Immonen et. al. 2009). Some have also found no effect of diet on female preference (Gray 1999; Syriatowicz & Brooks 2004; Archard et al. 2006). There are many possible explanations for why nutrition did not affect female chirp rate preferences in our study. Nutrition may have little effect on the costs and benefits of being choosy in *G. lineaticeps* (i.e., both the search cost and the direct benefits hypotheses may be false). For example, the energetic cost of sampling males might be small, particularly when females use terrestrial locomotion to search for males over relatively small geographic scales. There is evidence that diet quantity affects the benefits of being choosy in *G. lineaticeps* (Wagner & Harper 2003), which might argue against this interpretation. It is

possible, however, that these benefits are insufficient to affect the evolution of preference plasticity. Alternatively, nutrition may affect both the costs and benefits of being choosy in *G. lineaticeps*, but the costs may be balanced by the benefits (i.e., both the search costs and the direct benefits hypotheses may be true). For example, low nutrition females might be less able to afford the energetic costs of sampling males, but if male-provided direct benefits have a greater effect on female fitness in a low nutrition environment, then the costs may be balanced by the benefits. It seems unlikely that costs and benefits of being choosy would exactly balance across the four diet quantity treatments and the three diet quality treatments. It is possible, however, that small imbalances might not have been detectable with the experimental designs and sample sizes we used.

Another possibility is that nutrition may affect the costs and/or benefits of being choosy, but females may compensate or incur costs that mitigate these effects (i.e., one or both of the search cost and direct benefits hypotheses may be true, but other factors may counter their effects). Diet is known to affect a variety of female mating behaviours, such as willingness to mate repeatedly with the same male (Brown 1997; Fox and Moya-Laran□o 2009), and willingness to mate with multiple males (Aluja et al. 2009). Low nutrition females might compensate for their increased search costs by mating more frequently with the males they select, and by searching for and mating with fewer males. Similarly, low nutrition females might benefit more from being choosy, but environmental factors correlated with nutrition, such as the risk of predation, might have counteracting effects. Diet may also influence female preference for characteristics of male songs that we did not test. In *G. lineaticeps,* females prefer male songs that contain longer chirps, and long chirp duration is positively correlated with female life span

benefits (Wagner & Harper 2003). We might expect low nutrition females to show stronger preferences for longer chirp durations to increase their life span, which would give these females more time to find additional resources to increase ovary size and egg number before mating again.

Effect of Diet Quality on Sampling Behaviour

In contrast to the lack of effect of diet quantity and quality on female preference, we did find that diet quality affected female sampling behaviour; females on the high protein diet visited fewer speakers after the diet treatment compared to females on the high carbohydrate and preferred diets. These results may partially support the search cost hypotheses, which predicts that females will show weaker preferences in a low nutrition environment because they can less afford the cost of sampling males. However, because there was no effect of a high protein diet on female preference, there is no evidence that the change in female sampling affected female choosiness. One possibility that we cannot exclude is that the reduced sampling of high protein females would result in less choosiness under natural conditions. We used a three-speaker choice design to test the effect of diet quality on female preference. If females primarily encounter males sequentially, high protein females might be less likely to continue searching when they encounter a low chirp rate male because they have less energy to put into sampling males. Another explanation is that high protein females may have less motivation to find a mate because they have smaller ovaries with fewer eggs to fertilize. These females may first need to find a food item with high carbohydrates to allocate towards ovary and egg development before being motivated to mate.

General Conclusions

Diet quantity and quality did not influence female chirp rate preference in *G. lineaticeps*. These results provide little support for the search cost and no support for the direct benefits hypotheses, but it is possible that nutritional effects on the costs and benefits of being choosy have counteracting effects on the evolution of preference plasticity. It is also possible that other factors related to nutrition have counteracting effects on the evolution of preference plasticity. While the search cost and direct benefits hypotheses make clear and simple predictions about environmental effects on preference expression, a complex interaction of factors likely affects nutrition-related preference plasticity in many animals.

REFERENCES

Adams, T. E., Vaughn, C. E. & Sands P.B. 1999. Geographic races may exist among perennial grasses. *California Agriculture*, 53, 33-38.

Alexander, R. D. 1961. Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera: Gryllidae). *Behaviour,* 17, 130-223.

Aluja, M., Diaz-Fleischer, F., Papaj, D. R., Lagunes, G. & Sivinski, J. 2001. Effects of age, diet, female density, and host resources on egg load in *Anastrepha ludens* and *Anastrepha oblique* (Diptera: Tephritidae). *Journal of Insect Physiology*, 47, 975-988.

Aluja, M., Rull, J., Sivinski, J., Trujillo, G. & Perez-Staples, D. 2009. Male and female condition influences mating performance and sexual receptivity in two tropical fruit flies (Dipterra: Tephritidae) with contrasting life histories. *Journal of Insect Physiology*, 55, 1091-1098.

Archard, G. A., Cuthill, I. C. & Partridge, J. C. 2006. Condition dependent mate choice in the guppy: a role for short-term food restriction? *Behaviour*, 143, 1317-1340.

Bakker, T. C. M., Ku□nzler, R. & Mazzi, D. 1999. Condition-related mate choice in sticklebacks. *Nature*, 401, 234.

Beckers, O. M. & Wagner, W. E., Jr. 2011. Mate sampling strategy in a field cricket: evidence for a fixed threshold strategy with last chance option. *Animal Behaviour,* 81, 519-537.

Behmer, S. T. & Joern, A. 2008. Coexisting generalist herbivores occupy unique nutritional feeding niches. *Proceedings of the National Academy of Sciences*, 105, 1977-1982.

Brown, W. D. 1997. Female remating and the intensity of female choice in black-horned tree crickets, *Oecanthus nigricornis*. *Behavioural Ecology*, 8, 66-74.

Buchholz, R. 2004. Effects of parasitic infection on mate sampling by female wild turkeys (Meleangris gallopavo): should infected females be more or less choosy? Behavioral Ecoloby, 15, 687-694.

Carey, J. R., Liedo, P., Muller, H., Wang, J. & Vaupel, J. W. 1998. Dual modes of aging in Mediterranean fruit fly females. *Science*, 281, 996-998.

Checkley, D. M., Jr. 1980. The egg production of a marine planktonic copepod in relation to its food supply: laboratory studies. *Limnology and Oceanography*, 25, 430-446.

Cohen, R. W. 2001. Diet balancing in the cockroach *Rhyparobia madera*: does serotonin regulate this behavior? *Journal of Insect Behavior*, 14, 99-111.

Cohen, R. W., Heydon, S. L., Waldbauer, G. P. & Friedman, S. 1987. Nutrient self-selection by the omnivorous cockroach *Supella longipalpa. Journal of Insect Physiology*, 33, 935-940.

Collins, S. A. 1995. The effect of recent experience on female choice in zebra finches. *Animal Behaviour*, 49, 479-486.

Cotton, S., Small, J. & Pomiankowski, A. 2006. Sexual selection and condition-dependent mate preferences. *Current Biology*, 16, R755-R765.

Cratsley, C. K. & Lewis, S. M. 2003. Female preference for male courtship flashes in *Photinus ignites* fireflies. *Behavioural Ecology*, 14, 135-140.

Dadd, R. H. 1961. The nutritional requirements of locusts. IV. Requirements for vitamins of the B complex. *Journal of Insect Physiology*, 6, 1-12.

Dee, R. G. & Box, T. W. 1967. Commercial fertilizers influence crude protein content of four mixed prairie grasses. *Journal of Range Management*, 20, 96-99.

Engqvist, L. 2009. Should I stay of should I go? Condition- and status-dependent courtship decisions in the scorpionfly *Panorpa cognate*. *Animal Behaviour*, 78, 491-497.

Fisher, H. S. & Rosenthal, G. G. 2006. Hungry females show stronger mating preferences. *Behavioral Ecology*, 979-981.

Forsgren, E. 1992. Predation risk affects mate choice in a gobiid fish. *The American Naturalist*, 140, 1041-1049.

Fox, C. W. & Moya-Laranõo, J. 2009. Diet affects female mating behaviour in a seed-feeding beetle. *Physiological Entomology*, 34, 370-378.

Gangwere, S. K. 1961. A monograph of food selection in Orthoptera. *Transactions of the American Entomological Society*, 87, 67-230.

Gray, D. A. 1999. Intrinsic factors affecting female choice in house crickets: time cost, female age, nutritional condition, body size, and size-relative reproductive investment. *Journal of Insect Behaviour*, 12, 691-700.

Greenstone, M. H. 1979. Spider feeding behaviour optimises dietary essential amino acid composition. *Nature,* 282, 501–503.

Hamilton, R. L., Cooper, R. A. & Schal, C. 1990. The influence of nymphal and adult dietary protein on food intake and reproduction in female brown-banded cockroaches. *Entomologia Experimentalis et Applicata*, 55, 23-31.

Hamilton, R. L. & Schal, C. 1988. Effects of dietary protein levels on reproduction and food consumption in the German cockroach (Dictyoptera: Blattellidae). *Annals of the Entomological Society of America,* 81, 969-976.

Hebets, E. A. 2003. Subadult experience influences adult mate choice in an arthropod: exposed female wolf spiders prefer males of a familiar phenotype. *Proceedings of the National Academy of Sciences,* 100, 13390-13395.

Hebets, E. A., Wesson, J. & Shamble, P. S. 2008. Diet influences mate choice selectivity in adult female wolf spiders. *Animal Behaviour*, 76, 355-363.

Hedrick, A. V. & Dill, L. M. 1993. Mate choice by female crickets is influenced by predation risk. *Animal Behavior*, 46, 193-196.

Henen, B. T. 2002. Reproductive effort and reproductive nutrition of female desert tortoises: essential field methods. *Integrative & Comparative Biology*, 42, 43-50.

Henson, S. A. & Warner, R. R. 1997. Male and female alternative reproductive behaviors in fishes: a new approach using intersexual dynamics. *Annual Review of Ecology and Systematics*, 28, 571-592.

Hingle, A., Fowler, K. & Pomiankowski, A. 2001. The effect of transient food stress on female mate preference in the stalk-eyed fly *Cyrtodiopsis dalmanni. Proceedings of the Royal Society B*, 268, 1239-1244.

Hunt, J., Brooks, R. & Jennions, M. D. 2005. Female mate choice as a condition-dependent life-history trait. *American Naturalist*, 166, 79-92.

Immonen, E., Hoikkala, A., Kazem, A. J. N. & Ritchie, M. S. 2009. When are vomiting males attractive? Sexual selection on condition-dependent nuptial feeding in *Drosophila subobscura. Behavioral Ecology*, 20, 289-295.

Janetos, A. C. 1980. Strategies of female mate choice: a theoretical analysis. *Behavioral Ecology and Sociobiology*, 7, 107-112.

Jennions, M. D. & Petrie, M. 1997. Variation in mate choice and mating preferences: a review of the causes and consequences. *Biological Review*, 72, 283-327.

Jirotkul, M. 1999. Operational sex ratio influences female preference and male-male competition in guppies. *Animal Behaviour,* 58, 287-294.

Johnson, J. C. & Basolo, A. L. 2003. Predator exposure alters female mate choice in the green swordtail. *Behavioral Ecology*, 14, 619-625.

Kaufman, L. W., Collier, G. and Squibb, R. L. 1978. Selection of an adequate protein-carbohydrate ratio by the domestic chick. *Physiology & Behavior*, 20, 399-344.

Kyriazakis, I. & Emmans, G. C. 1991. Diet selection in pigs: dietary choices made by growing pigs following a period of underfeeding with protein. *Animal Production*, 52, 337-346.

Lopez, S. 1999. Parasitized female guppies do not prefer showy males. *Animal Behaviour,* 57, 1129–1134.

Marler, C. A., Foran, C. & Ryan, M. J. 1997. The influence of experience on mating preferences of the gynogenetic Amaozon molly. *Animal Behaviour*, 53, 1035-1041.

Milinski, M. & Bakker, T. C. M. 1992. Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. *Proceedings of the Royal Society of London, Series B*, 250, 229-233.

Monteith, L. G. 1971.Crickets as predators of the apple maggot, *Rhagoletis pomonella* (Diptera: Tephritidae). *The Canadian Entomologist*, 103, 52-58.

Musten, B., Peace, D. & Anderson, G. J. 1974. Food intake regulation in the weanling rat: self-selection of protein and energy. *The Journal of Nutrition*, 104, 563-572.

Patton, R. L. 1967. Oligidic diets for Acheta domesticus (Orthoptera: Gryllidae). *Annals of the Entomological Society of America*, 60, 1238-1242.

Pfenning, K. S. 2007. Facultative mate choice drives adaptive hybridization. *Science*, 318, 965-967.

Poulin, R. 1994. Mate choice decisions by parasitized female upland bullies, *Gobiomorphus breviceps. Proceedings of the Royal Society London B*, 263, 183-187.

Qvarnström A., Prät, T. & Sheldon, B. C. 2000. Adaptive plasticity in mate preference linked to differences in reproductive effort. *Nature*, 405, 344-346.

Ramsay, S. L. & Houston, D. C. 1998. The effect of dietary amino acid composition on egg production in blue tits. *Proceedings of the Royal Society of London B*, 265, 1401-1405.

Reaney, L. T. & Backwell, P. R. Y. 2007. Temporal constraints and female preference for burrow width in the fiddler crab, *Uca mjoebergi. Behavioral Ecology and Sociobiology*, 61, 1515-1521.

Roberts, S. A., Xin, H., Kerr, B. J., Russell, R. & Bregendahl, K. 2007. Effects of dietary fiber and reduced crude protein on nitrogen balance and egg production in laying hens. *Poultry Science,* 86, 1716-1725.

Roff, D. A. 1994. Evidence that the magnitude of the trade-off in a dichotomous trait is frequency dependent. *Evolution*, 48, 1650-1656.

Schoonhoven, L. M., Jermy, T. & van Loon, J. J. A. 2005. *Insect-Plant Biology*. 2nd edn. Oxford: Oxford University Press.

Simmons, L. W. 1988. The contribution of multiple mating and spermatophore consumption to the lifetime reproductive success of female field crickets (*Gryllus bimaculatus*). *Ecological Entomology*, *13,* 57-69.

Simpson, S. J. & Abisgold, J. D. 1985. Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. *Physiological Entomology*, 10, 443-452.

Souroukis, K. & Murray, A. 1995. Female mating behavior in the field cricket, *Gryllus pennsylvanicus* (Orthoptera: Gryllidae) at different operational sex ratios. *Journal of Insect Behavior*, 8, 269-279.

Syriatowicz, A. & Brooks, R. 2004. Sexual responsiveness is condition-dependent in female guppies, but preference functions are not. *BMC Ecology*, 4, 5-12.

Telang,. A., Booton, V., Chapman, D. E. & Wheeler, D. E. 2001. How female caterpillars accumulate their nutrient reserves. *Journal of Insect Physiology*, 47, 1055-1064.

Tolle, A. E. & Wagner, W. E., Jr. 2011. Costly signals in a field cricket can indicate high or low-quality direct benefits depending upon the environment. *Evolution*, 65, 283-294.

Ueckert, D. N., Yang, S. P. & Albin, R. C. 1972. Biological value of rangeland grasshoppers as a protein concentrate. *Journal of Economic Entomology*, 65, 1286-1288.

Vélez, M. J. & Brockmann, H. J. 2006. Seasonal variation in female response to male calling song in the field cricket, *Gryllus rubens. Ethology*, 112, 1041-1049.

Waldbauer, G. P. & Friedman, S. 1991. Self-selection of optimal diets by insects. *Annual Review of Entomology*, 36, 43-63.

Wang, D., Bai, Y., Li, J. & Zhang, C. 2004. Nutritional value of the field cricket (*Gryllus testaceus Walker*). *Insect Science*, 11, 275-283.

Wagner, W. E., Jr. 1996. Convergent song preferences between females field crickets and acoustically orienting parasitoid flies. *Behavioural Ecology*, 7, 279-285.

Wagner, W. E., Jr. 1998. Measuring female mating preferences. *Animal Behaviour*, 55, 1029-1042.

Wagner, W. E., Jr. & Basolo, A. L. 2007. The relative importance of different direct benefits in the mate choices of a field cricket. *Evolution*, 61, 617-622.

Wagner, W. E., Jr. & Harper, C. J. 2003. Female life span and fertility are increased by the ejaculates of preferred males. *Evolution*, 57, 2054-2066.

Wagner, W. E., Jr. & Reiser, M. G. 2000. The importance of calling song and courtship song in female mate choie in the variable field cricket. *Animal Behaviour*, 59, 1219-1226.

Weissman, D.B., Rentz, D. C. F., Alexander, R. D. & Loher, W. 1980. Field crickets (*Gryllus* and *Acheta*) of California and Baja California Mexico (Orthoptera: Gryllidae: Gryllinae). Trans. *American Entomological Society*, 106, 327-356.

Widemo, F. & Sæther, S. A. 1999. Beauty is in the eye of the beholder: causes and consequences of variation in mating preferences. *Trends in Ecology & Evolution*, 14, 26-31.

Zera A. J. & Larsen, A. 2001. The metabolic basis of life history variation: genetic and phenotypic differences in lipid reserves among life history morphs of the wing-polymorphic cricket, *Gryllus firmus*. *Journal of Insect Physiology*, 47, 1147-1160.