THE ROLE OF FOOD PROTEIN-CARBOHYDRATE CONTENT ON NUTRIENT REGULATION STRATEGIES AND WING MORPH DETERMINATION IN THE WING POLYMORPHIC CRICKET *GRYLLUS FIRMUS*

A Thesis

by

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ABSTRACT

Field crickets in the genus *Gryllus* are wing polymorphic and have been used for many years as a model for trade-offs between dispersal and reproduction. There are two main morphs of adult crickets. The first has long hindwings but small ovaries and is capable of flight while the other has short hindwings and cannot fly but has much larger ovaries and therefore higher reproduction. This trade-off is well studied in adult crickets but very little work of any sort has been done with the nymphs. Previous studies have shown that the morph the nymphs will become is influenced by their genetics as well as environmental cues such as population density. The experiments in this thesis examine how the nymphs regulate their protein and carbohydrate intake and the extent to which food protein and carbohydrate content influences wing morph determination. Two experiments, using three cricket lines, were used. Two of these lines were selected to produce either long winged or short winged individuals; the third line was unselected and representative of field populations. First a choice experiment was conducted to determine the protein:carbohydrate (P:C) ratio nymphs from the different lines self-selected. The second experiment was a no-choice experiment that tested how the nymphs from the different lines regulated their protein-carbohydrate intake when they were restricted to a single diet as well as how those diets affected their performance and final wing morph. The results from these experiments are compared to nutrient regulation strategies in the adults of each morph, and discussed within the context of how food protein-carbohydrate content influences wing morph determination.

DEDICATION

This thesis is dedicated to my family; without their support I doubt I would have been able to finish.

ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

Many crickets in the genus *Gryllus* are wing polymorphic and exist as two main types either mixed in one population or separate populations of the two morphs. These morphs are characterized by their wing length. The first main morph is long winged (LW), these crickets have long hindwings and large wing muscles and so are capable of flight. However, these wing muscles take a large amount of resources to maintain in the forms of both energy as well as protein. These crickets, like all organisms, have finite resources available to them, so this leads to trade-offs between flight capability and other resource intensive processes such as reproduction. LW crickets therefore have smaller ovaries and consequently much lower reproduction during their early adulthood than the other morphs of crickets (Zhao and Zera 2002). In response to this trade-off, LW crickets histolize their wing muscles about a week after molting to adulthood which allows them to shift their efforts to reproduction instead of flight. The other main morph is short winged (SW). It is characterized by hindwings that are too small to allow for flight, small wing muscles, and large ovaries. Since these crickets don't have large wing muscles to maintain they have higher rates of reproduction during early adulthood compared to LW crickets (Zera and Denno 1997, Zera and Larsen 2001). Another possible benefit of not maintaining wing muscles is that SW crickets appear to be more stress tolerant than LW crickets (Zera 1994, Zera et al. 2009). The trade-off between flight and reproduction has been well studied in these crickets for many years, however, the exact influences that cause a particular cricket to become one morph or the other aren't fully known. Genetics are known to play a large role and lines of crickets can be selected to produce one morph or the other. However, even after years of selection under controlled population densities these lines only produce 85-90% of the selected morph (Zera and Tiebel 1988, Zera and Harshman 2001, Zera and Larsen 2001, Zera 2005). The remaining variation in wing morph is likely due to environmental influences.

Another potential source of environmental variation that could play a role in determining morph is the nutritional environment they experience as nymphs. Interestingly, adult female crickets of the two morphs have been shown to select significantly different ratios of protein to carbohydrates when offered diets that varied in their protein and carbohydrate content (Clark et al. 2013). These two nutrients, and the ratio they occur in the diet, may have potential to influence the wing morph they become. As summarized in Behmer (2009) all organisms need a suite of nutrients to grow and reproduce. Protein (for amino acids), lipids, and carbohydrates are required in large amounts. Different organisms require different amounts/ratios of these nutrients, however, nutrients are never found in a pure form in nature and all foods consist of a mixture of different nutrients (Joern et al. 2012). In order to achieve the highest rates of growth and reproduction organisms must be able to obtain these key nutrients in specific ratios, and they can do this by selectively eating from multiple different food sources. Many organisms are quite adept at assessing the nutrient content of their food and regulating the intake of these different nutrients accordingly (Behmer and Joern 2008, Roeder and Behmer 214). However, if they try to control the ratio of every nutrient in their diet it would be impossible because foods with either the ideal ratio of nutrients for that organism or ones that are complementary to each other in their ratios of every nutrient don't exist. Because of this, organisms typically only regulate for a few major nutrients, such as the macronutrients. They do this by selectively eating from among different foods (that contain varying ratios of key nutrients). Many organisms are quite good at selecting the correct mixtures of foods that yield their preferred amounts of each nutrient and they have multiple methods for selecting their diets. They can use both preingesitve and postingestive methods for their selections (Simpson and Raubenheimer 2000). Preingestive methods are ones that allow them to determine the nutrient content of their food before they eat it. These methods include taste receptors that are able to detect particular nutrients such as amino acids or sugars and are able to be tuned to the current nutritional needs of the organism by becoming more sensitive when that nutrient is needed and less sensitive when it isn't (Abisgold and Simpson 1988). Postingestive methods are how organisms deal with excess nutrients that have already been eaten. These methods typically involve excreting the excess nutrients or storing them in the body for later use (Zanotto et al. 1993). Postingesive methods are especially important because in nature certain nutrients are likely to be scarcer than others and so in order to obtain enough of a rarer nutrient it is likely that the organism will have to eat an excess of a more common nutrient.

In this paper the nutrient regulation of the cricket nymphs will be studied using the Geometric Framework. As seen in Behmer (2009) this framework is a method for examining how an organism regulates one nutrient in relation to others. Since organisms need a certain mixture of nutrients they have to regulate their intake of foods that contain differing amounts of various nutrients in order to obtain the nutrients in correct proportions to each other. The ratio of nutrients the organisms eat (when allowed to choose their own diets) can be measured in the lab. This is done by observing how the organism feeds on a pair of foods that differ in their content of the nutrients of interest and are individually suboptimal for the organism's needs, but together are nutritionally complementary. By measuring how much of each of the foods are eaten the total amount of each of the nutrients, as well as the ratio between them, can be calculated. The self-selected ratio is known as the intake target, and represents the amount of each nutrient the organism has to eat in order to attain its ideal fitness. This intake target is related to the nutrient target which is the amount of each nutrient that the organism needs for optimal performance. However, because not all the nutrients that are eaten are absorbed, and some of the nutrients that are absorbed are used for metabolism and maintenance of tissues, the intake target will always be higher than the nutrient target. The geometric framework will be used in this experiment to determine the protein to carbohydrate intake target selected by the nymphs. This target will then be the basis for an experiment testing their performance on different diets as well as how their wing morph responds when they aren't able to select their ideal ratio of nutrients.

CHAPTER II

THE ROLE OF FOOD PROTEIN-CARBOHYDRATE CONTENT ON NUTRIENT REGULATION STRATEGIES AND WING MORPH DETERMINATION IN THE WING POLYMORPHIC CRICKET GRYLLUS FIRMUS.

Overview

To understand how the nutrient content of their food influences the performance and development of the cricket nymphs during their last two nymphal instars I used two different experiments. The first was a choice experiment that was used to determine the intake target of three lines of crickets. In this experiment all three lines of crickets self-selected similar protein to carbohydrate ratios and there were no significant differences between the ratios chosen by lines that were selected to produce either LW or SW individuals. The second experiment was a no-choice experiment that examined the responses to the three cricket lines when they were restricted to one of a series of diets that contained varying amounts of protein and carbohydrates. In this experiment the crickets practiced similar regulation strategies to their adult counterparts from earlier studies. LW crickets were more selective about maintaining a specific protein to carbohydrate ratio in their diets and were less willing to eat excess of one nutrient in order to obtain the scarcer nutrient while SW crickets ate similar total amounts of nutrients across the different diets. The crickets from a line that wasn't selected to produce a specific morph, and so is better representative of a field population, practiced nutrient regulation strategies that are more similar to those from the LW line. They ate less as the foods became more imbalanced from their ideal protein and carbohydrate ratio. All three of the lines showed similar patterns across the different diets with regards to their growth and performance. Mass gain, lipid content, development time, and survival were all improved on diets that were closer to the intake target that was selected by the crickets in the choice experiment. In both the choice and no-choice experiment significantly more crickets became SW as adults than would have been expected. The exact cause of this shift is unknown though it could be due to stress during the experiment.

Introduction

Wing polymorphic crickets in the genus *Gryllus* have been studied for many years as a model of the tradeoff between dispersal and reproduction. Many species in this genus are known to be wing polymorphic as adults with the morph of an individual cricket being determined by genetics as well as environmental factors such as population density (Zera and Denno 1997). There are two main adult morphs found in these crickets, though a third morph is formed later in the lifespan of some of the crickets. The first main morph that is present during the early adulthood of these crickets is long winged (LW). These crickets have large hindwings and wing muscles that are capable of flight and dispersal. However, these large muscles require both protein (to build) and energy (for maintenance). This comes at the cost of having less protein and energy to devote to ovary development and reproduction. As LW crickets age they tend to histolize their wing muscles and become a second long winged morph termed LW(h) which renders them flightless but allows them to devote more energy to reproduction (Zhao and Zera 2002). The second main morph is short winged (SW). These crickets have short hindwings that are too small to allow for flight, small non-functional flight muscles, and larger ovaries during early adulthood than crickets of the LW morph. Since they do not have to pay the cost of developing and maintaining large wing muscles SW crickets have been shown to have much higher reproduction during their early adulthood than LW crickets (Zera and Denno 1997, Zera and Larsen 2001).

There are several factors that play a role in determining the adult morph of these crickets. One of the most important factors is genetics. Lines of crickets that have been selected for a certain morph for many generations will show greater than 85% purity (Zera and Larsen 2001, Zera 2005). But environmental factors also play a large role in determining adult morph. For example, population density is known to affect the ratio of the morphs that are produced in a population with high densities favoring SW crickets and low densities producing more LW crickets (Zera and Tiebel 1988, Zera and Harshman 2001, Zera and Larsen 2001). An additional potential source of environmental influence on this polymorphism is the nutritional environment that the crickets experience as nymphs. Protein and carbohydrates have the potential to influence the adult morph because they impact both nymph and adult growth processes. Nymphs require carbohydrates to fuel their growth as well as protein to develop new tissues, especially those that will be LW adults since they are forming their large wing muscles. Adult crickets also require carbohydrates (for fueling flight and maintaining wing muscles in LW crickets) and protein (for egg development and reproduction).

Another reason that protein and carbohydrates are likely to influence adult morph is that adult females of the different morphs have been shown to choose different ratios of protein to carbohydrates when offered diets that varied in the proportions of the two nutrients. LW females chose a diet that has a higher carbohydrate ratio compared to SW females (Clark et al. 2013). Therefore, it seems likely that the amounts of these nutrients that are eaten by the crickets as nymphs would lead to one adult morph being more successful in that particular environment than the other.

To understand the nutritional influence on the cricket morphs there must be a way to show how the crickets select the nutrients and the effect of specific nutrients on their physiology. The Geometric Framework is useful in analyzing how each nutrient is balanced, relative to each other. As reviewed in Behmer (2009), the geometric framework is a method used to understand the particular balance of nutrients needed by an individual. It works by allowing an organism to mix its intake of two different foods that are imbalanced in the nutrient or nutrients of interest. By measuring how the organism chooses to feed on the different foods it is possible to find the intake target of the organism. This intake target is relative to the total amount of a nutrient the organism is trying to obtain in order to achieve its optimum growth rate, but it will always be greater because not all of the nutrients that are eaten can be absorbed during digestion and those that are absorbed must also be used for maintenance of organs as well as fueling the organism's activities.

Because the tradeoff between dispersal and reproduction is well defined in these crickets, and they are easy to rear, they are frequently used as a model organism to study wing morph polymorphisms and their resulting trade-offs with reproduction (Zera and Harshman 2001). To date, almost all experiments have been performed with adults. As a result, this has led to a lack of knowledge on factors that may play a role in determining the final morph of an individual cricket. In this study I assessed the effect of food protein carbohydrate content on wing morph determination. This was done in two experiments. The first was a choice experiment to determine whether the protein to carbohydrate (P:C) intake target of the nymphs is the same as that of the adults, as has been recently determined (Clark et al. 2013). However, nymphs cannot be assumed to have the same intake target as adults because they will be putting their resources toward growth and development instead of reproduction. This choice experiment was followed by a no choice experiment to determine if the protein carbohydrate content in their diets would affect their final wing morph. In this experiment nymphs were confined to one of a series of diets that varied in their protein and carbohydrate content for their final two instars. Because the crickets were only offered a single diet, they couldn't self-regulate their intake of protein and carbohydrates. This allowed for the observation of the effects of the diets on their final wing morph as well as their responses to imbalanced diets with respect to their feeding patterns, development time, and performance.

Materials and Methods

Experimental Insects

Three cricket lines were used in this study. The first two lines were produced from eggs from two large outbred populations maintained at the University of Nebraska-Lincoln that were selected to produce either LW or SW individuals. Each line was part of an artificial selection experiment where a pair of populations was selected to produce one of the morphs. This selection experiment had three separate populations selected for each morph, with the lines used in this study coming from block 2 (Zera and Larsen 2001, Zera 2005). The differences between the pair of populations in one block has been shown to be similar to that of the differences between the populations in the other two blocks (Zera 2005) so the differences observed in one block are likely to be representative of general differences between LW and SW populations. The third line of crickets was also from the Zera lab and wasn’t selected for a specific adult morph. Eggs were collected from the three populations and shipped to Texas A&M University where they were reared in groups of approximately 100 in 17L plastic containers for the first 14 days after hatching and then in groups of about 40 in the same containers: rearing crickets at these densities doesn’t affect the final wing morph of the adult crickets (Zera and Tiebel 1988). Crickets from these eggs were used to establish a stock population at Texas A&M which was the source population for the crickets used in these experiments. They were fed stock diet containing: wheat bran, wheat germ, powdered whole milk, and nutritional yeast (Zera and Larsen 2001) and were provided with distilled drinking water in a plastic tube plugged with cotton. They were maintained in an incubator under a

16:8 hour day/night cycle at 28°C during both their rearing and the experiments. For all the experiments, newly molted individuals that were two instars from adulthood were collected, weighed, and placed in 6-3/4 inches long by 4-13/16 inches wide and 2-3/8 inch tall experimental arenas that were covered with paper on the outside to prevent the crickets from seeing the behaviors of those in other arenas and contained either two pre-weighed dishes containing a pair of experimental diets (choice experiments) or a single dish of diet (no-choice experiments). The arenas also contained half of a 1oz opaque condiment cup to provide shelter, and a plastic tube plugged with cotton containing distilled water.

Experimental Diets

Choice Experiments

The diets used in this experiment varied in their digestible protein and carbohydrate content. They are based on synthetic diets originally designed to be used for grasshoppers (Dadd 1961, Simpson and Abisgold 1985, Behmer et al. 2001) and were prepared following the protocols outlined in (Behmer et al. 2002). There were three diets used in the choice experiment 7% protein and 35% carbohydrates (p7: c35), p28: c14 and p35: c7. All of the diets contained the same amount of total macronutrients (42%) and the same amounts of all other ingredients (e.g. cellulose, cholesterol, vitamins).

No-choice experiments

The six diets used in this experiment followed the same protocols and recipes as those from the choice experiment but they differed in their p:c ratios. Five of these diets had a total macronutrient content of 42% of the total diet, p8:c34, p12:c30, p16:c26, p20:c22, and p24:c18 while the sixth had 21% total macronutrient content p8:c13. These diets were chosen based on the intake target found in the choice experiment. Two of the diets had p:c ratios that matched the intake target from the choice experiment p16:c26 and p8:c13. The four other diets had p:c ratios that were nutritionally suboptimal compared with the nymph's intake target. Two were more protein biased then the intake target, p20:c22 and p24:c18 and the other two were more carbohydrate biased, p12:c30 and p16:c26.

Experimental Protocol

Choice Experiment

This experiment revealed the self-selected p:c intake target of the nymphs. Prior to the start of this experiment the rearing containers were checked three times per day for signs of molting. When a freshly molted penultimate instar cricket was found it was collected and placed in an experimental arena (described above) that contained two food dishes containing one of two pairs of artificial diets. The diet pairings used in this experiment were: 1) p7:c35 with p28:c14 and 2) p7:c35 with p35:c7. These diet pairings represent foods that alone are nutritionally suboptimal but are complimentary, thus the crickets can eat from both of them to cover a wide range of the p: c ratios that the crickets would be likely to encounter in nature. Two pairings were used to ensure that the crickets were actively selecting their diets as opposed to simply eating equally out of each dish. While each pairing covered a similar nutritional area the crickets would have to eat different amounts of each food in the different pairs to reach the same intake target.

After the crickets were placed in the experimental arenas they were checked three times per day for signs of molting. When a molt was determined the cricket was weighed and each of the food dishes was changed for another pre-weighed dish containing the same diet. The original dishes were weighed to determine the consumption of the diet during each instar. The food dishes were also exchanged if one of them began to run low so that the cricket would always have the option to choose between the two dishes. All food dishes were allowed to sit at room humidity for at least 24 hours to allow them to equilibrate before each weighing to prevent errors from the diets absorbing or losing water in the arenas or storage. When the crickets molted to adulthood they were weighed and their wing morph was recorded as well as their final mass and development time. They were then frozen and their lipid content was measured following the protocols outlined in (Simpson et al. 2002). Wing morph was categorized by the length of the hindwings. We didn’t use the color of the wing muscles because the crickets weren’t fully sclerotized at the time of freezing.

No-choice experiment

In this experiment freshly molted penultimate instar crickets were weighed and placed in arenas containing a pre-weighed dish of one of the experimental diets that was previously described. As in the choice experiment the arenas were checked three times per day for signs of molting and when a cricket was found to have molted it was weighed and the food dish was replaced with another pre-weighed dish of the same diet. Final mass, development time, wing morph and lipid content were recorded as outlined in the choice experiment.

Experiments using non-selected lines

The protocols for both the choice and no-choice experiments that were performed using crickets from the selected lines were repeated using crickets from the unselected line. In addition, the final morph ratios of crickets maintained under stock rearing conditions were analyzed to allow comparison between crickets reared under stock conditions and experimental conditions.

Statistical Analysis

For each experiment we assessed whether the crickets survived to the adult stage, time to adulthood, adult mass and wing morph, and lipid composition. For each cricket I also analyzed the amount of food eaten, as well as its protein-carbohydrate intake. Survival rates and final wing morph were analyzed using Logistic Regression, developmental time was analyzed using Survival Analysis. MANCOVA, ANOVA and

ANCOVA were used to analyze mass, consumption, and growth rate. All analyses were carried out using JMP pro 11.2.1. When analyzing the data from the unselected line no-choice experiment, data from the stock and dilute diets were not included because they wouldn't provide a direct comparison with the other diets. Instead, these data are shown simply as a reference.

Results

Selected Lines

Choice experiment

In this experiment we examined the feeding behavior of the crickets in several ways. Firstly, we used t-tests to determine if they favored one diet over the other. Crickets from both lines, and on each diet pair, showed a preference for one dish of food (Table 1). Next, we used ANCOVA's to determine if the crickets from each line and diet pairing selected the same ratio of protein to carbohydrates. This analysis showed no significant difference in the self-selected P:C ratio between the treatments (Table 2, Figure 1a). We then analyzed the total amounts of protein and carbohydrates the crickets ate during their last two instars using MANCOVA. For each line there was no significant difference in protein-carbohydrate intake on the different diet pairs. The two lines did consume significantly different amounts of protein and carbohydrates, but there was no interaction between line and diet pair (Table 3, Figure 1b). Since we found significant differences in the total amounts of protein and carbohydrates that the crickets ate during the experiment, but no differences in the self-selected ratio of the two nutrients, the differences were likely a function of the two cricket lines eating different absolute amounts of food. We tested this using an ANCOVA and found that crickets from the LW line consumed significantly more food than those from the SW line (Table 4, Figure 1c). When averaged across the diet pairings crickets from the LW line consumed 981.84 ± 24.76 mg of food compared with those from the SW line that consumed 812.63 ± 36.25 mg. These differences were found despite the fact that crickets from both lines had similar starting mass (T-test T = -1.89, d.f. 161, P = 0.0598).

Table 1. Results from t-tests testing whether the crickets in the choice experiment fed randomly out of both food dishes or selectively ate one diet over the other.

Line	Diet pairing	Total food eaten from dish 1	Total food eaten from dish 2	T-value	P-value
LW	p7:c35 w/ p28:c14	583.689 ± 23.483	393.638 ± 19.095	6.7238	<0.0001
	p7:c35 w/ p35:c7	685.021 ± 35.742	301.523 ± 15.674	9.6806	<0.0001
SW	p7:c35 w/ p28:c14	465.566 ± 30.566	318.456 ± 26.792	5.6447	<0.0001
	p7:c35 w/ p35:c7	550.381 ± 41.460	297.683 ± 20.428	6.1585	<0.0001

Table 2. Results from an ANCOVA testing the effects of line and diet pairing on the log transformed P:C ratio consumed by the crickets.

Source of Variation	F-ratio	D.F.	P-Value
Line	2.9868	1	0.0872
Diet pair	2.4036	1	0.1243
Line by diet pair	2.5378	1	0.1144
Sex	0.0003	1	0.9857
Starting mass	1.5352	1	0.2184

Table 3. Results from a MANCOVA testing the effects on line and diet pairing on the protein and carbohydrates eaten by the crickets in the choice experiment.

Source of Variation	F-ratio	D.F.	P-value
Line	60.7034	2	0.0076
Diet pair	5.1455	2	0.3134
Line by diet pair	1.1747	2	0.5480
Sex	0.7253	2	0.4868
Starting mass	4.4824	2	0.0138

Table 4. Results from an ANCOVA testing the effects of line and diet pairing on the total amount of food consumed by the crickets.

Source of Variation	F-ratio	D.F.	P-value
Line	14.9115	1	0.0002
Diet pair	0.2138	1	0.6448
Line by diet pair	0.0700	1	0.7920
Sex	5.2746	1	0.0238
Starting mass	8.8515	1	0.0037

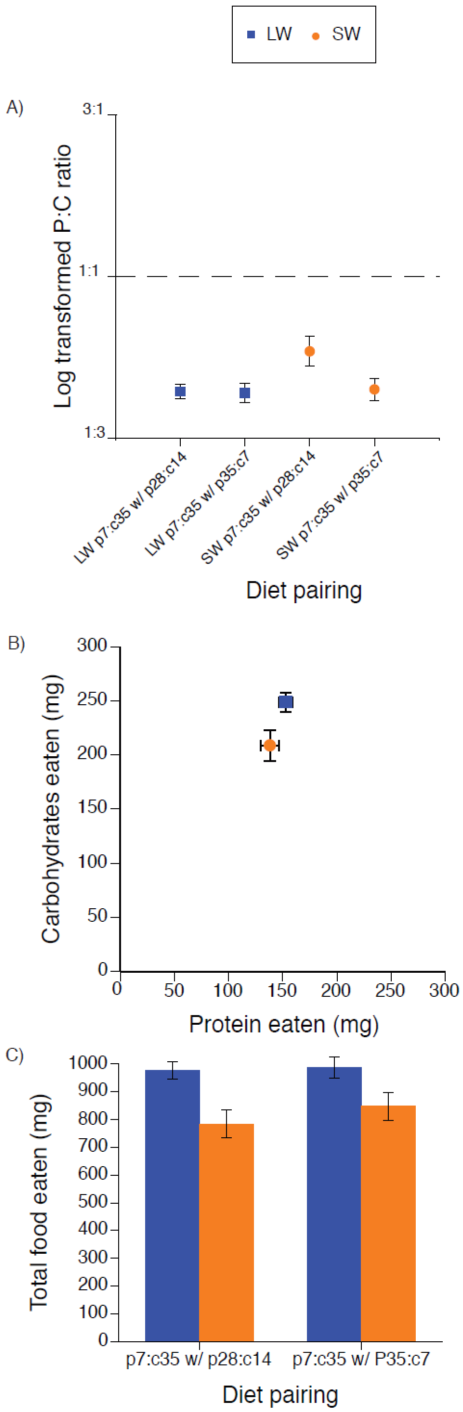

Figure 1. A) P:C ratios selected by the crickets in the choice experiment (mean ±SEM). B) Protein and carbohydrate intake targets found in the choice experiment with the selected lines of crickets. C) Total food eaten by each line on each diet pair (Mean ± SEM).

After analyzing the feeding behavior, we looked at how the crickets performed on the two different diet pairings. The crickets from the LW line gained more mass than those of the SW line and in both lines the crickets on the p7:c35 w/ p35:c7 diet pairing gained slightly more mass than those on the p7:c35 w/ p14:c28 diet pairing (Table 5, Figure 2a). The LW line crickets also had a higher lipid content than the SW crickets, however, there was no effect from diet pairing (Table 6, Figure 2b). There was no significant difference in development time between the two lines of crickets, but there was a significant difference between crickets on the different diet pairings (Table 7, Figure 2c). In both lines the crickets on the p7:c35 w/ p28:c14 diet pair took on average one day longer to grow to adulthood. Finally, while the cricket lines used in these experiments typically produce 80% or more of the selected morph of crickets (Zera and Cisper 2001) over half of the LW line crickets showed the SW phenotype, while all of the SW line crickets remained true to their line (Figure 2d). Logistic regression of the final morph of the crickets using diet pair and line as factors showed that there was a significant line effect but no difference between the diet pairs (Line: $\chi^2_{df=1} = 31.816$, $P < 0.001$; Diet: $\chi^2_{df=1} = 0.000$, $P = 1.000$).

Table 5. Results from an ANCOVA testing the effects of line and diet pairing on the mass gained by the crickets.

Source of Variation	F-ratio	D.F.	P-value
Line	24.3404	1	<0.0001
Diet pair	4.1596	1	0.0440
Line by diet pair	0.4653	1	0.4967
Sex	15.5479	1	0.0001
Starting mass	0.0062	1	0.9374

Table 6. Results from an ANCOVA testing the effects of line and diet pairing on the lipid content of the crickets.

Source of Variation	F-ratio	D.F.	P-value
Line	7.7175	1	0.0066
Diet pair	3.5928	1	0.0612
Line by diet pair	2.5179	1	0.1160
Sex	2.7424	1	0.1011
Starting mass	1.0881	1	0.2996
Carbohydrates eaten	38.0043	1	<0.0001

Table 7. Results from a survival analysis of the time spent by the crickets in their last two instars.

Source of Variation	Chi Squared value	D.F.	P-value
Line	0.0106	1	0.9179
Diet pair	15.0655	1	0.0001
Line by diet pair	0.09211	1	0.3372
Sex	11.3585	1	0.0008
Starting mass	28.3567	1	<0.0001

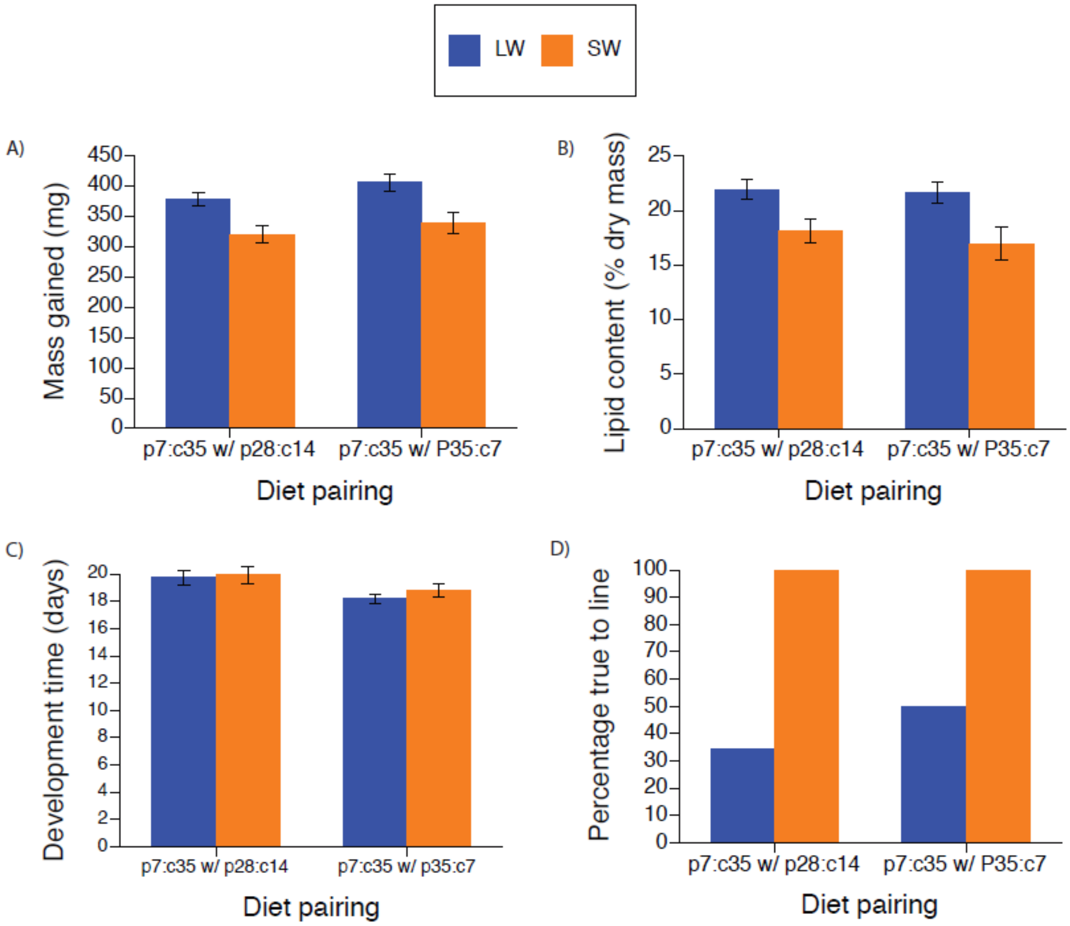

Figure 2. A) Mass gained by the crickets during their last two instars (Mean ± SEM). B) Lipid composition of the crickets as a percentage of dry mass (Mean ± SEM). C) Development time of the crickets during their last two instars (Mean ± SEM). D) Final morph distribution of the crickets in the choice experiment with the selected lines of crickets shown as percentage of crickets that matured into the adult morph their line was selected to produce.

No-choice experiment

Intake arrays were fitted to consumption data to analyze how food protein-carbohydrate content affected the nutrient regulation strategies of the different lines (Raubenheimer and Simpson 1999, Behmer 2009). The array for the LW crickets most closely approximated a quadratic fit while the SW crickets' array more closely approximated a linear fit (Figure 3a). We also plotted the protein:carbohydrate intakes for the dilute diet and intake target diet for comparison and they showed the same trend as those from the intake target diet with the LW line eating more overall than the SW line (Figure 3b). Following this we generated protein + carbohydrate (P+C) error plots (Raubenheimer and Simpson 1999). The error plot (Figure 3c) for the LW line more closely approximated a quadratic fit (quadratic contrast $F_1 = 15.39$, $P < 0.0001$), while the SW line more closely approximated a linear fit (linear contrast $F_1 = 3.78$, $P = 0.052$). Again when the dilute diet was plotted with the intake target diet for comparison the same trends were seen (Figure 3d). There were significant line and diet differences for the total amount of food eaten (Table 8). The average consumption for the LW crickets during their last two instars was 1050.90 ± 27.34 mg, but only 785.34 ± 19.37 mg for the SW line. The two lines showed similar patterns of consumption across the diets however (Figure 4). Consumption was the highest on the dilute diet (p8:c13), and equally low on the most protein based diet (p8:c34) and two most carbohydrate biased diets (p8:c34 and p12:c30). It was intermediate on the two remaining diets (p16:c26 and p20:c22).

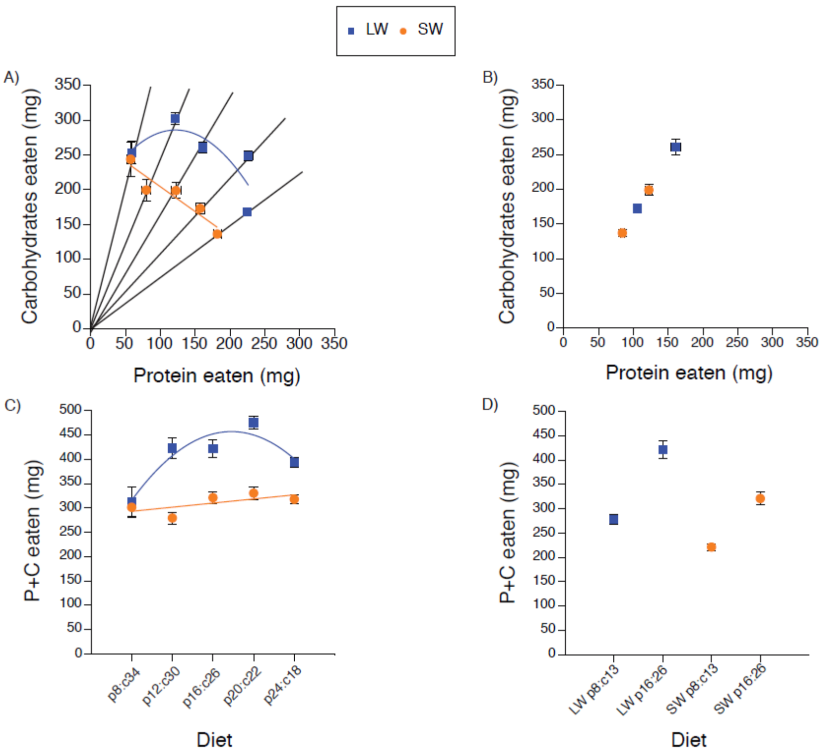

Figure 3. A) Intake arrays for each of the selected lines. The quadratic line is fitted to the LW array and the linear fit is the SW. B) Intake arrays showing how the crickets on the dilute diet compared with the 42% macronutrient diet with the same p:c ratio. C) P+C error plot of the crickets. D) P+C error plot comparing the dilute diet with the 42% macronutrient diet with the same p:c ratio.

Table 8. Results from an ANCOVA of total food eaten by the crickets from the no-choice experiment. The 8:13 diet was excluded for this analysis due to the fact that it contains a different amount of macronutrients from the rest of the diets and so wouldn't be a direct comparison.

Source of Variation	F-value	D.F.	P-value
Line	47.6733	1	<0.0001
Diet	5.7092	4	0.0004
Line by Diet	2.2216	4	0.0736
Sex	1.3514	1	0.2484
Starting Mass	8.0640	1	0.0057

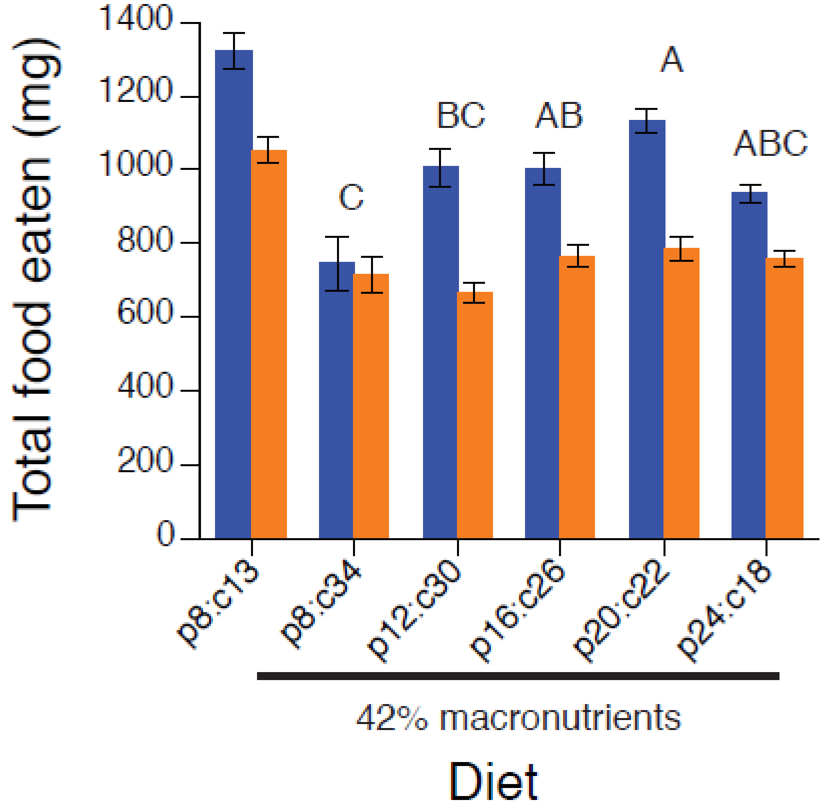

Figure 4. Total food eaten by the crickets from the selected lines during their last two instars in the no-choice experiment (Mean ±SEM).

Development time of the crickets was significantly affected by both the line of the crickets as well as their diet and there was a significant interaction between the two (Table 9). Post Hoc analysis showed that crickets on the two carbohydrate biased diets (p8:c34 and p12:c30), had significantly longer development time than those on the diet that matched the intake target, or were protein biased. The dilute diet also showed slower development than the most carbohydrate biased diet (Figure 4a). Cricket mass gain was significantly affected by both line and diet, with a significant interaction between both factors (Table 10). For both lines mass gain was highest on the protein biased diets and lower on the carbohydrate biased diets. Crickets on the dilute diet gained more mass than those on the most carbohydrate biased diet in both lines. Across all the diets crickets from the LW line gained more mass then those from the SW line (Figure 4b). Across all of the diets crickets from the LW line had a significantly higher final lipid content then those from the SW line. For both lines lipid content was lowest on the dilute diet (p8:c13), but was relatively similar across the higher macronutrient content diets (Table 11, Figure 4c). As was seen in the choice experiment a significant number of crickets from the LW line showed the SW phenotype as adults, while most of the SW crickets remained SW as adults (Figure 5d). A full model examining final morph, including a Line-by-Diet interaction term, indicated there was no significant line or diet effect, and no significant Line-by-Diet interaction (Table 12). However, when a simplified model was used (excluding the Line-by-Diet interaction term), Line, but not Diet, was found to be significant (Table 12). Survival of the crickets was significantly different across the different diets though there was no difference between the lines, and there was no significant interaction (Table 13, Figure 6). For both lines the p8:c34 diet showed significantly lower survival compared to the other diets.

Table 9. Results from a survival analysis of time spent by the crickets in their last two instars. The 8:13 diet was excluded due to it having a different total macronutrient content and therefore not being a direct comparison.

Source of Variation	Chi Square value	D.F.	P-value
Line	8.3255	1	0.0039
Diet	150.0477	5	<0.0001
Line by Diet	9.0685	5	0.0609
Sex	3.7832	1	0.0518
Starting Mass	0.9900	1	0.3197

Table 10. Results of an ANCOVA of mass gained by the crickets with diet and line. The 8:13 diet was excluded due to it having a different total macronutrient content and therefore not being a direct comparison.

Source of Variation	F-ratio	D.F.	P-value
Line	39.7494	1	<0.0001
Diet	47.2320	4	<0.0001
Line by diet	2.0175	4	0.0828
Sex	3.9013	1	0.0511
Starting mass	2.8977	1	0.0919

Table 11. Results from an ANCOVA of lipid content of the crickets as a percent of dry mass with line and diet. The 8:13 diet was excluded due to it having a different macronutrient content and therefore not being a direct comparison.

Source of Variation	F-ratio	D.F.	P-value
Line	25.3128	1	<0.0001
Diet	1.2554	4	0.2943
Line by diet	1.2614	4	0.2919
Sex	6.5078	1	0.0126
Starting mass	1.3165	1	0.2546
Carbohydrates eaten	45.7930	1	<0.0001

Table 12. Results from a nominal regression of final morph of the crickets from the no-choice experiment. First is the full model followed by the simplified model.

Full Model			
Source of Variation	Chi Square value	D.F.	P-value
Line	9.37523*10^-6	1	0.9976
Diet	5.9128679	5	0.3148
Line by Diet	2.62941093	5	0.7569
Simplified Model			
Source of Variation	Chi Square value	D.F.	P-value
Line	7.25621*10^-6	1	<0.0001
Diet	5.678681	5	0.0968

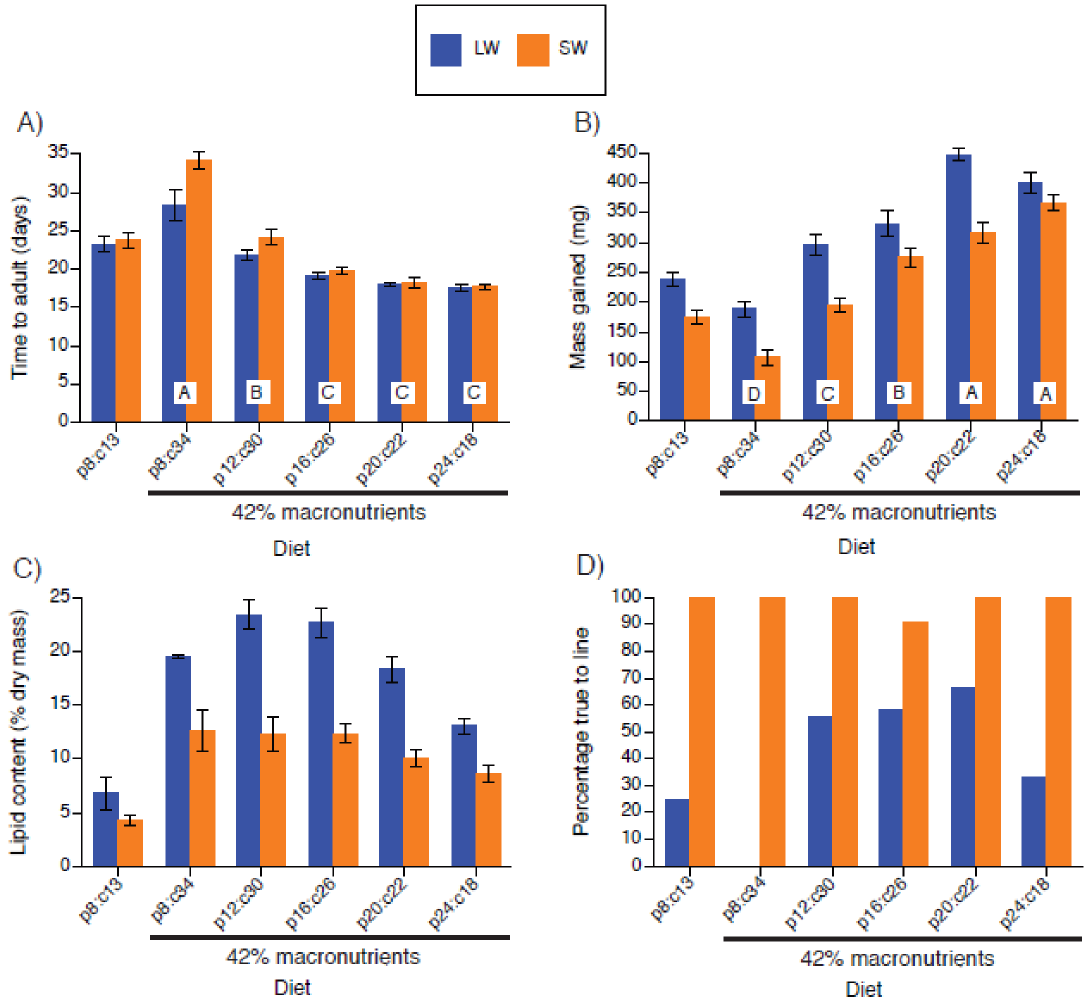

Figure 5. A) Development time of the crickets from the no-choice experiment during their last two instars (Mean ±SEM). B) Mass gained by the crickets during their last two instars (Mean ±SEM). C) Lipid content of the crickets as a percentage of dry mass (Mean ±SEM). D) Final morph distribution of the crickets in the choice experiment with the selected lines of crickets shown as percentage of crickets that matured into the adult morph their line was selected to produce.

Table 13. Results from a nominal logistic regression of survival to adulthood with line and diet.

Source of Variation	Chi Square value	D.F.	P-value
Line	3.3266*10^-7	1	0.9998
Diet	15.3648	5	0.0090
Line by Diet	7.7742	5	0.2249

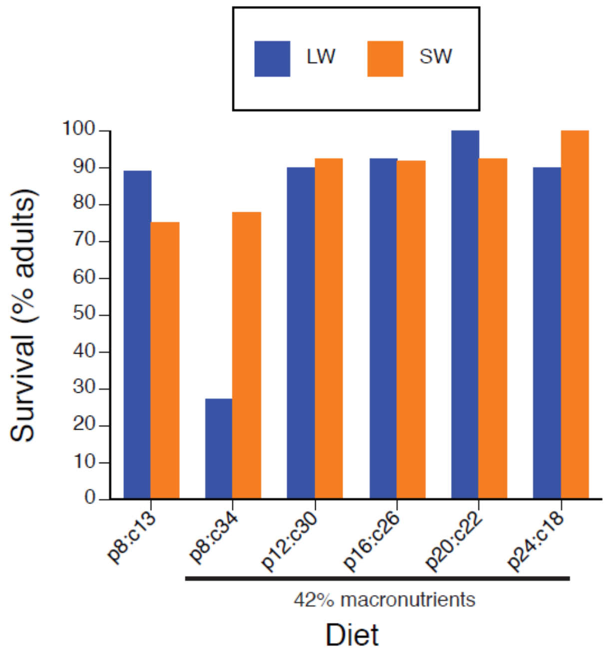

Figure 6. Survival of the crickets from the selected lines during their last two nymphal instars in the no choice experiment.

Non-selected Lines

Choice experiment

As with the selected lines we started by analyzing to see whether the crickets on the different diets ate randomly from both dishes, or preferred one dish over the other. The crickets on the diet pairing of p7:c35 with p28:c14 showed no preference, while crickets on the pairing of p7:c35 with p35:c7 showed a preference for the p7:c35 food (Table 14, Figure 7a). In this experiment the crickets on each of the two diet pairings did eat significantly different amounts of protein and carbohydrates (Table 15). When the amounts of protein and carbohydrates ingested by the crickets in each diet pair were analyzed separately it was shown that the differences were due to different amounts of protein being eaten while there were no significant differences in carbohydrates eaten between the two diet pairs (Table 16, Figure 7b). There were also no significant differences in the total food consumed by the crickets feeding on each diet pair (Table 17, Figure 7c), or in their starting mass (T-test: T = -1.14, df = 37, $P = 0.260$).

Table 14. Results from T-tests testing if the crickets from the wild type line fed randomly from the two offered food dishes or if they selected one diet over the other.

Diet Pair	Total food eaten from dish 1	Total food eaten from dish 2	T-value	P-value
p7:c35 w/ p28:c14	458.9168 ± 32.0896	421.592 ± 15.2236	1.2215	0.2337
p7:c35 w/ p35:c7	475.6130 ± 23.8256	379.0515 ± 19.8037	4.8845	0.0001

Table 15. Results from an ANCOVA testing the effect of diet pairing and sex on the log transformed p:c ratio eaten by the crickets.

Source of Variation	F-ratio	D.F.	P-value
Diet pair	17.1549	1	0.0002
Sex	4.7922	1	0.0353
Starting Mass	0.6031	1	0.4426

Table 16. Results from a MANCOVA of protein and carbohydrates eaten by crickets from the wild type lines on the two diet pairings.

Source of Variation	F-ratio	D.F.	P-value
Diet Pair	9.9555	2	0.0004
Sex	8.7038	2	0.0009
Penultimate Mass	1.9783	2	0.1539

Table 17. Results from an ANCOVA testing the effect of diet pairing and sex on the total amount of food eaten by the crickets.

Source of Variation	F-ratio	D.F.	P-value
Diet pair	0.0240	1	0.8778
Sex	15.3026	1	0.0004
Penultimate mass	3.9175	1	0.0557

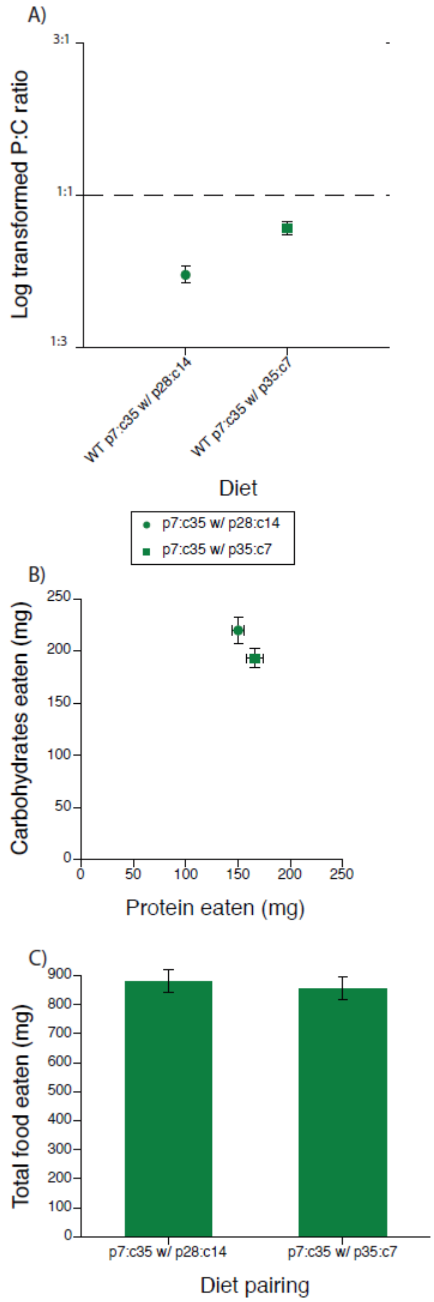

Figure 7. A) P:C ratios selected by the crickets from the unselected line in the choice experiment (mean ±SEM). B) Protein and carbohydrate intake targets found in the choice experiment with the unselected line of crickets. C) Total food eaten by the unselected line on each diet pair (Mean ± SEM).

There was no significant difference in mass gained between crickets on the two diet pairings, though there was a significant sex effect (Table 18, Figure 8a); generally, females gained more mass. There was a significant effect of both final morph and diet pairing on the lipid content of the crickets, as well as an interaction between them. The amount of lipid stored by the crickets was positively correlated with carbohydrate intake (Table 19, Figure 8b). There was a significant difference in development time between the crickets on each diet pairing, as well as a significant sex effect, though there was no interaction between the two (Table 20, Figure 8c). The crickets on the diet pair consisting of p7:c35 with p35:c7 took slightly longer to grow to adulthood than those on the pairing of p7:c35 with p28:c14. Crickets from this non-selected line typically show a morph ratio of 40-65% LW, but most of the experimental crickets became SW (Figure 8d). Logistic regression of the final morph of the crickets showed that there was no significant difference between the diet pairs (Table 21).

Table 18. Results from an ANCOVA testing the effect of diet pairing and sex on the mass gained by the crickets.

Source of Variation	F-ratio	D.F.	P-value
Diet pair	0.2625	1	0.6115
Sex	11.1560	1	0.0020
Penultimate mass	1.2427	1	0.2723

Table 19. Results from an ANCOVA of lipid content of the crickets as a percent of dry mass with diet pair and final morph

Source of Variation	F-ratio	D.F.	P-value
Diet Pair	2.5474	1	0.1195
Sex	33.4163	1	<0.0001
Penultimate Mass	1.1527	1	0.2903
Carbohydrates Eaten	0.3236	1	0.5731

Table 20. Results from a survival analysis testing the effects of diet pairing and sex on the development time of the crickets.

Source of Variation	Chi-Squared Value	D.F.	P-value
Diet Pair	0.038546	1	0.5347
Sex	14.3401	1	0.0002
Penultimate Mass	11.89737	1	0.0006

Table 21. Results from a logistic regression examining the effects of diet pair on the final morph of the crickets.

Source of Variation	Chi-squared Value	D.F.	P-value
Diet pair	0.1757113	1	0.6751

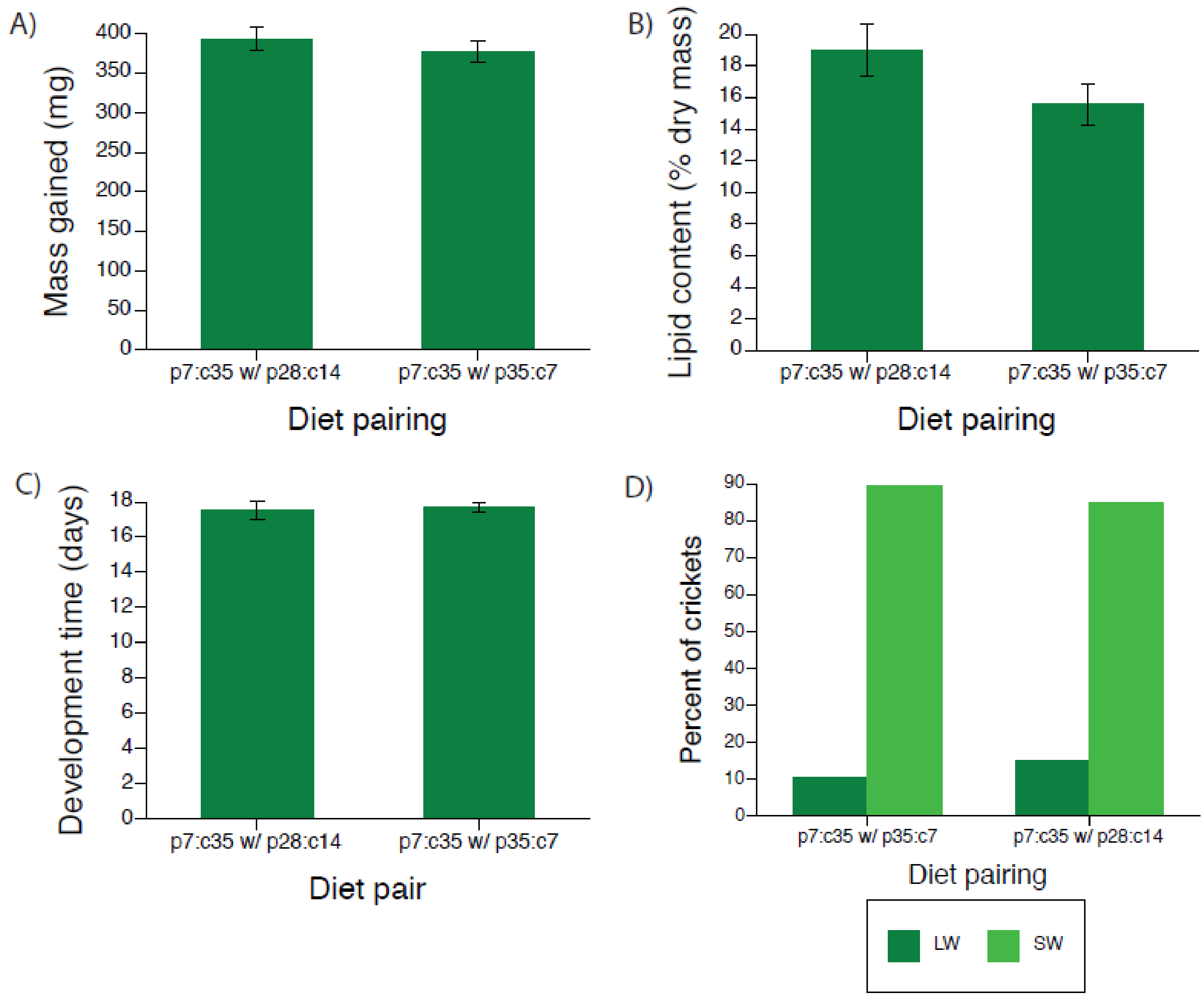

Figure 8. A) Mass gained by the crickets during their last two instars (Mean ± SEM). B) Lipid composition of the crickets as a percentage of dry mass (Mean ± SEM). C) Development time of the crickets during their last two instars (Mean ± SEM). D) Final morph distribution of the crickets in the choice experiment with the unselected line of crickets.

No-choice experiment

For this experiment we started by analyzing the feeding behavior of the crickets using intake arrays and error plots, as done with the selected lines. The unselected line's intake array approximated a quadratic fit (Fig 9a, 9b). The error plot also approximated a quadratic fit (quadratic contrast $F_1 = 18.9151$, $P = 0.001$; Figures 9c, 9d). Significant differences in consumption were observed between crickets on the different diets and between the sexes, but there was no significant effect of final morph (Table 22). Consumption was the highest on the dilute diet (p8:c34), and lowest on the most protein-based diet p8:c34 and the stock diet. It was intermediate on the remaining diets (Figure 10).

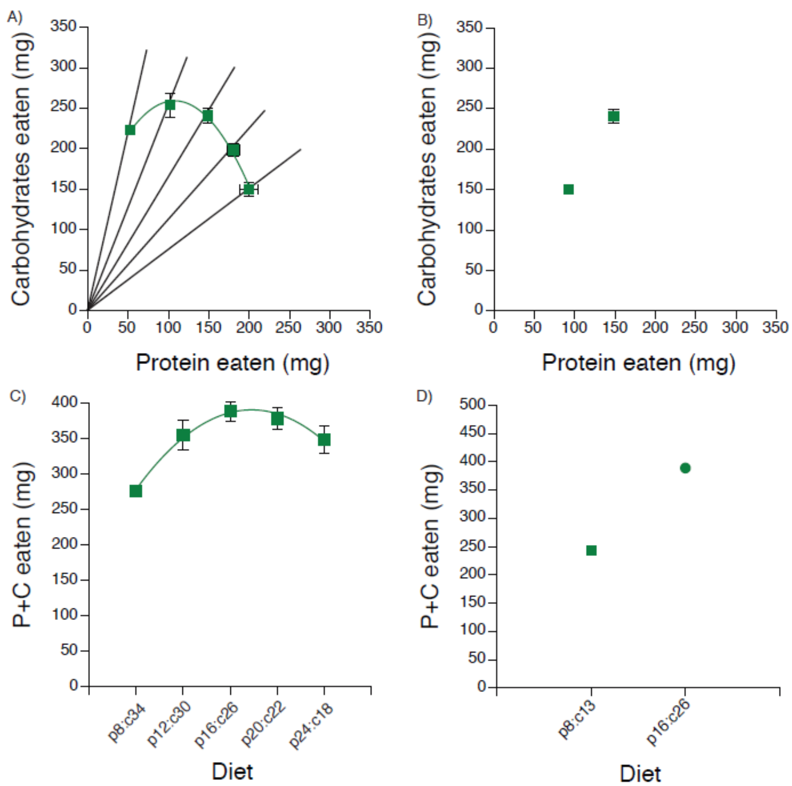

Figure 9. A) Intake arrays for the unselected line. B) Intake arrays showing how the crickets on the dilute diet compared with the 42% macronutrient diet with the same p:c ratio. C) P+C error plot of the crickets. D) P+C error plot comparing the dilute diet with the 42% macronutrient diet with the same p:c ratio.

Table 22. Results from an ANCOVA testing the effect of diet on the total amount of food eaten by the crickets. For this analysis the dilute and stock diets were excluded because they wouldn't provide a direct comparison.

Source of Variation	F-ratio	D.F.	P-value
Diet	8.9283	4	<0.0001
Sex	14.4607	1	0.0003
Penultimate Mass	1.0235	1	0.3158

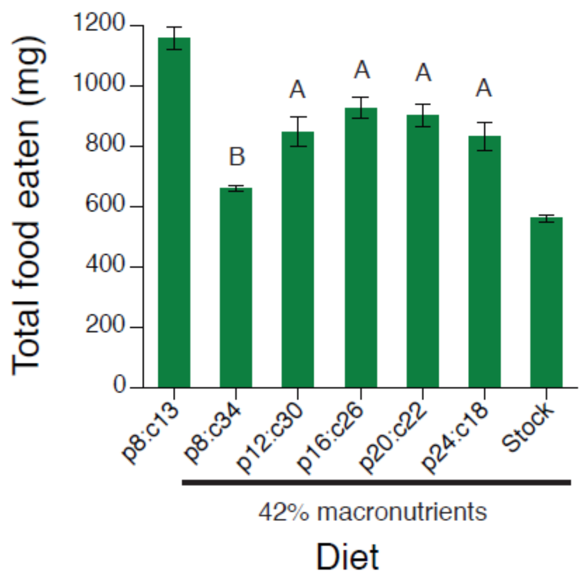

Figure 10. Total food eaten by the crickets from the unselected line during their last two instars in the no-choice experiment (Mean ±SEM).

Development time of crickets from the non-selected line was significantly affected by both Diet and Sex, (Table 23). Post hoc analysis showed that crickets on the most carbohydrate-biased diet had the longest development time, followed next by the p8:c13 and p12:c30 diet (Figure 11a); development time on the remaining diets was equally fast. Mass gain for the non-selected line crickets was significantly affected by the diet and sex, but the interaction between diet and sex was not significant (Table 24). It was equally high on the p16:c26, p20:c22 and p24:c16 diets, intermediate on the p12:c30, and lowest on the p8:c34 diet (Figure 11b). Lipid content showed significant differences as a function of diet, but there was no significant influence of sex, and the diet-by-sex interaction term was not significant (Table 25). The lipid content of crickets was lowest on the dilute diet (p8:c13) and in the higher nutrient diets the lipid content of the crickets increased as the diets became more balanced and it increased as the amount of carbohydrates eaten by the crickets increased (Figure 11c). As was seen in the choice experiment a significant number of crickets showed the SW phenotype as adults (Table 26). There was no significant effect of the different diets on the final wing morph of the crickets (Table 11d) despite the fact that the LW crickets on the most carbohydrate biased diet p8:c34 all became SW adults. Crickets on all the treatments had a high survival rate, unlike the selected lines where some diets caused high mortality (Figure 12).

Table 23. Results from a survival analysis testing the effect of diet on the development time of the crickets. For this analysis the stock diet was excluded because it wouldn't provide a direct comparison.

Source of Variation	Chi-squared Value	D.F.	P-value
Diet	64.1294	4	<0.0001
Sex	3.9836	1	0.0459
Penultimate mass	0.1159	1	0.7335

Table 24. Results from an ANCOVA testing the effect of diet on the mass gained by the crickets. For this analysis the dilute and stock diets were excluded because they wouldn't provide a direct comparison.

Source of Variation	F-ratio	D.F.	P-value
Diet	25.9139	4	<0.0001
Sex	6.1201	1	0.0162
Penultimate Mass	2.8627	1	0.0958

Table 25. Results from an ANCOVA of lipid content of the crickets as a percent of dry mass with diet and final morph. For this analysis the dilute and stock diets were excluded because they wouldn't provide a direct comparison.

Source of Variation	F-ratio	D.F.	P-value
Diet	11.1014	5	<0.0001
Sex	1.2645	1	0.2645
Penultimate Mass	1.9503	1	0.1668
Carbohydrates Eaten	81.0319	1	<0.0001

Table 26. Results from a logistic regression examining the effect of diet the final morph of the crickets. For this analysis the stock diet was excluded because it wouldn't provide a direct comparison

Source of Variation	Chi-squared Value	D.F.	P-value
Diet	13.75224	5	0.0173

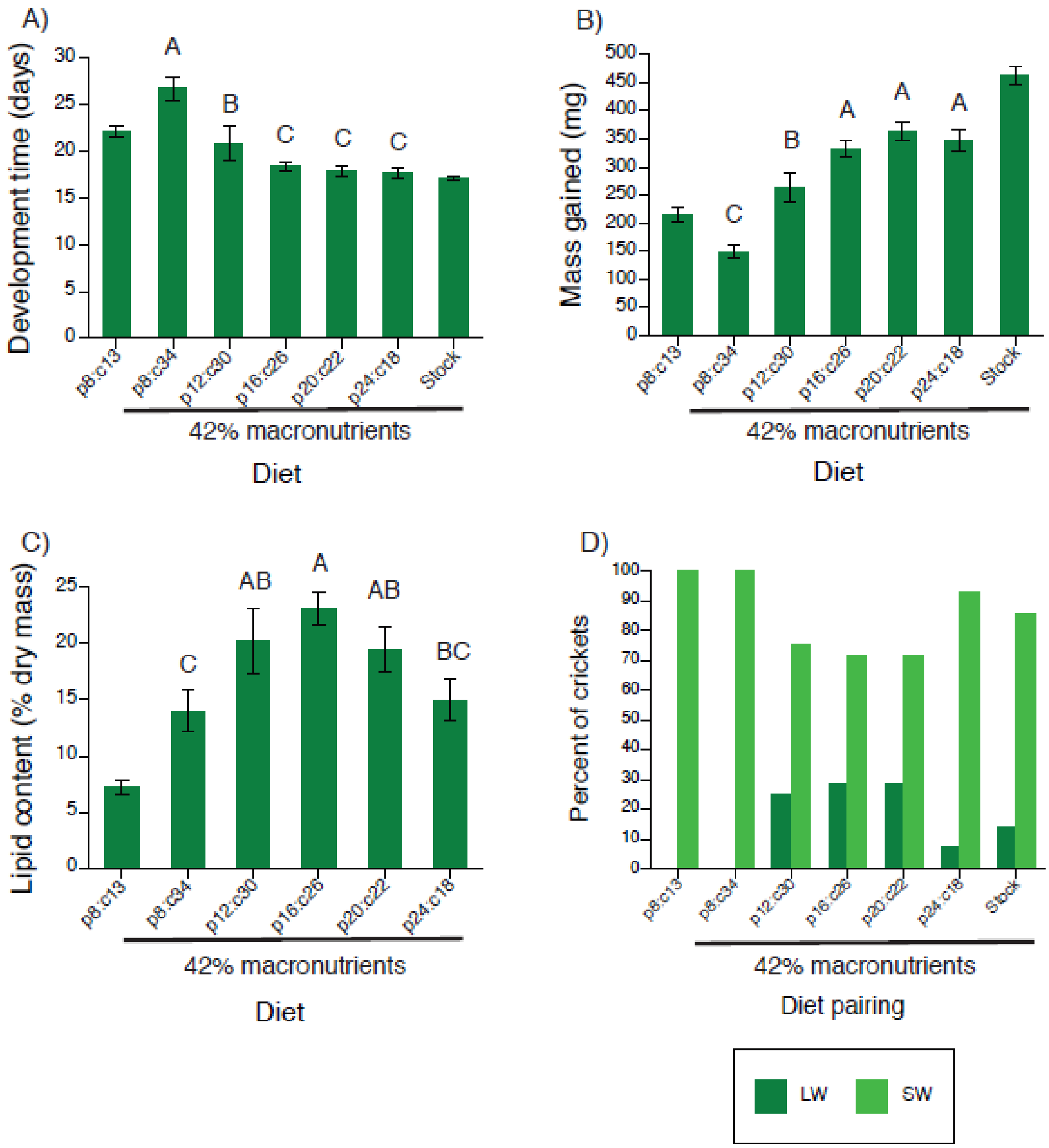

Figure 11. A) Development time of the crickets from the no-choice experiment during their last two instars (Mean ±SEM). B) Mass gained by the crickets during their last two instars (Mean ±SEM). C) Lipid content of the crickets as a percentage of dry mass (Mean ±SEM). D) Final morph distribution of the crickets in the choice experiment with the unselected lines of crickets.

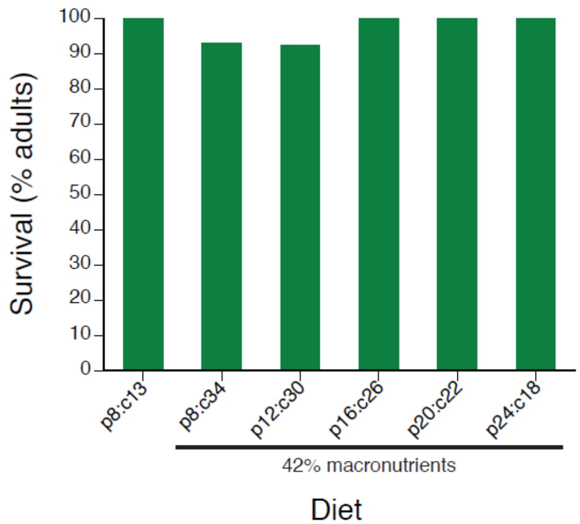

Figure 12. Survival of the crickets from the unselected line during their last two nymphal instars in the no-choice experiment.

Discussion

Wing morph polymorphism has been well studied in *Gryllus* crickets due to the fact that they are large, easily reared in the laboratory, and have easily distinguishable phenotypes (Zera and Harshman 2001). However, despite the fact that many researchers have investigated this trade-off it wasn't until recently that any studies looked at the nutrient regulation in these crickets and this study is the first to look at how they regulate their nutrition as nymphs and how it affects which wing morph they develop as adults. This is important because the differences that are found between adults of the different phenotypes may be linked to the nutritional state of the nymphs, and knowing how nymphs regulate their intake could be useful in understanding their trade-off between dispersal and reproduction as adults (Simpson et al. 2002, Lee et al. 2008, Boggs 2009).

Insects commonly regulate their nutrient intake by eating a mixture of foods that contain different ratios of the nutrients they are regulating and will self-select diets that contain ideal amounts of the various nutrients to allow them to have the highest performance possible (Behmer 2009). In these experiments the cricket nymphs from all three lines self-selected similar protein to carbohydrate ratios, though they did vary in the total amounts of both nutrients that they ate with the crickets from the LW line eating significantly more than those from the SW line. This was somewhat unexpected because adult females of the two different morphs self-select significantly different ratios with the LW crickets preferring a diet higher in carbohydrates and while the adults eat significantly different amounts of total food it is the SW females that eat more as adults (Clark et al. 2013). This difference could possibly be due to the fact that even though the LW nymphs are growing their large wing muscles they don't need a different ratio of nutrients to be able to do so and instead just need more nutrients overall.

The differences in the total food that the crickets eat as nymphs and as adults are likely to be because the nymphs that are going to be LW have to obtain enough nutrients, especially protein, to grow their large wing muscles (Clark et al. 2015). In contrast, SW crickets grow large ovaries, but only after they have become adults (Tanaka 1993, Clark et al. 2015). As such, they have a need to eat more as adults so that they can acquire sufficient quantities of protein and energy to build their ovaries. Despite nymphs showing a reversal in total consumption (compared to the adults), the nymphs from the selected lines did regulate their intake of nutrients in a fashion similar to their adult counterparts. An inspection of the P+C error plots revealed the LW line showed a quadratic fit, while the SW line showed a linear fit. This shows that even though the nymphs from the two lines don’t select significantly different protein:carbohydrate ratios they still apply the same rules for dealing with imbalanced diets as they will as adults.

In many studies, organisms have been shown to have better performance (e.g., growth or reproduction) as the nutrient ratios in their diets become more balanced toward their ideal ratio. Additionally, those on balanced but nutrient poor diets (i.e. total nutrient content is low) often have better performance compared to those on diets that have greater absolute total nutrient amounts of nutrients, but are extremely imbalanced relative to the optimal ratio (Raubenheimer and Simpson 1999, Le Gall and Behmer 2014, Roeder and Behmer 2014, Clark et al. 2015). This trend of having better performance on balanced diets than dilute diets is also shown by all three lines in regards to their growth and development. Insects from all three lines showed similar trends in their performance, those on the dilute diet gained more mass, developed faster, and had more lipid reserves at their adult molt than those on more imbalanced diets especially the highly carbohydrate biased diets. These results are likely due to the costs incurred from consuming excess amounts of nutrients, especially carbohydrates. Insects as well as other organisms can use multiple strategies to regulate the nutrients they absorb and utilize nutrients obtained from their food, but this has been shown to be costly and therefore will negatively impact the insect’s performance (Lee et al. 2008, Cease et al. 2012).

In both the choice and no-choice experiments significantly more crickets became SW than would have been expected. Interestingly, it seems that the LW may be particularly susceptible to stress, and when this occurs they expresses the SW morph (Zera and Tiebel 1988). The exact cause of the stress is unknown. It may be the arenas or some aspect of the experimental protocol. A group of crickets from the WT line that were maintained on the stock diet fed to the colony but in otherwise identical experimental conditions to the rest of the crickets also produced mostly SW adults. However, the non-selected line experiment suggests nutrition may be playing a contributing role, as significant diet effects were observed. In contrast, diet was not significant for the selected line no-choice experiment, but some trends were observed. In both experiments the diets that were less balanced compared to the intake target produce more SW adults while those closer to the target produce more LW. Also in the LW line the dilute diet produced more LW adults than the most carbohydrate biased diet even though the carbohydrate biased diet contained twice the total macronutrients of the dilute diet. These results fit well with other studies that have looked at nutrient balancing on insect performance. In these studies it is generally shown that the balance of nutrients in the diets in comparison to the ideal balance for the specific insect is more important to their performance than the total amount of nutrients in the diet (Le Gall and Behmer 2014, Roeder and Behmer 2014, Clark et al. 2015).

While these results have allowed for greater insight into the nutritional aspects of the wing length polymorphism of these crickets there is still further work that could be done. One important study that would provide highly useful information would be to look at what these crickets feed on in field conditions. At this point all that is known of their natural diet is that they are generalists that feed on a variety of plants, fungi, and insect remains (Capinera et al. 2004). With this information, the diets used in the laboratory and the diet they eat in the field could be compared which would allow for predictions of how nutrition affects the polymorphism in the wild. Other studies that would provide additional useful information would be ones that examine the effects of population density on the nutritional regulation of these crickets and how those two factors together influence their wing-morph polymorphism.

CHAPTER III

CONCLUSIONS

These experiments provide more information about nutrient regulation in Gryllus firmus and how it impacts their performance and final wing morph. By using the geometric framework to show how they selected their specific protein to carbohydrate ratio I was able to analyze how the patterns of regulation used by the nymphs compared with those used by the adults in other studies. In general, the nymphs followed similar regulation strategies to the adults with the LW line of crickets being more selective and sensitive to errors in overeating one nutrient to compensate for a lack of the other while the SW line ate similar amounts of nutrients across the range of different diets even though there were no differences in the p:c ratio that the two lines were regulating towards. However, there were differences in that the LW line of crickets ate more as nymphs than those from the SW line when SW adults eat more than the LW adults. These differences are interesting, however, they aren't entirely unexpected. As adults the LW crickets prefer a diet that is high in carbohydrates compared with SW adults because they will use those carbohydrates to make lipids for fueling their flights. Since nymphs don't fly those from the LW line don't yet need to eat as many carbohydrates as they will as adults so the two lines will therefore be more likely to select the same ratio of protein to carbohydrates. The differences in total amount of food the crickets eat are also likely due to the different developmental stages requiring different amounts of nutrients. The cricket nymphs are producing their wing muscles during their last few nymphal instars and so since the crickets from the LW line will need to produce much larger wing muscles they are likely to need to eat more food overall to acquire the protein and energy they need. Unlike the wing muscles, ovary development happens mostly during early adulthood in these crickets and so since SW crickets are developing much larger ovaries during early adulthood than the LW crickets are it makes sense that they would also need larger amounts of food to support the growth.

These crickets also showed a similar trend as many other studies looking at how nutrients affect performance. All three lines consistently performed better on a diet that was balanced relative to their intake target but had been diluted to half the normal nutrient content than on diets that weren't diluted but were highly imbalanced. Since crickets like many other organisms are capable of using post-ingestive methods to regulate their nutrient uptake they can still perform fairly well on diets that imbalanced but are relatively close to their ideal balance. However, as the diets became more imbalanced the crickets weren't able to perform as well because post-ingestive methods of nutrient regulation also carry costs and the harder the crickets have to work to compensate for imbalanced diets the higher those costs become.

These trends were also seen in the final wing morph of the crickets in these experiments. Across all three lines there were significantly more SW adults produced than would have been expected. Since SW crickets are thought to be more stress resistant these results are likely due to the crickets being stressed somehow during the experiments either by the experimental conditions or the p:c ratios of the diets being imbalanced. Since there were no significant differences between the ratios of LW and

SW adults produced by the LW selected line and the SW selected line it is likely that diet doesn't play a large role in determining what morph these crickets become as adults.

Future work is still needed to thoroughly understand exactly what environmental conditions are important in determining the wing morph of these crickets and how the different factors interact to determine the final morph. More work is also needed on the nutrition of these crickets. There is very little known about what they actually eat in the field other than that they are generalists that eat a wide variety of plants, insect remains, and fungi. Field studies are needed to determine what they are eating in the field so that the diets they are fed in the laboratories can be compared to the natural diet. If the lab diets are too different from the field diets than the results from many of the lab studies could be shown to be simply artifacts of the laboratory conditions and not relevant to how these crickets behave in the field.

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