Manipulation of carbohydrate diet and ensuing changes in weight and glycogen storage in bumble bee (*Bombus impatiens*) queens

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Introduction

Carbohydrates, commonly known as sugars, are one of the most prevalent organic compounds used by living organisms for energy. The smallest unit of carbohydrate is called a monosaccharide, and the most common of these molecules are glucose, fructose and galactose, which are all isomers of one another. These monosaccharides are rich in high energy carbon and hydrogen bonds that can be oxidized to produce large amounts of adenosine triphosphate (ATP) to power rapid cellular processes. Carbohydrates can also serve as a temporary energy storage that facilitates future cellular processes.

Glycogen is a storage form of carbohydrate, which is formed when excess glucose in the cells undergo condensation reactions to form alpha and beta glycosidic linkages. These condensation reactions are catalyzed by glycosyltransferases, which link individual glucose molecules together in different orientations to produce large storage polysaccharides known as glycogen (Arrese, 2010). The structure of glycogen is a highly branched, multichained polymer of glucose residues surrounding a core protein called glycogenin. The chain structure of glucose around glycogenin consists of 1,4 alpha glycosidic bonds with 1,6 glycosidic bonds as the branch points of the polymer. Through various sedimentation and electron micrograph methods, the average molecular weight of glycogen is estimated to be 10-500 x 10^6 Da (Calder, 1991).

Glycogen is an excellent energy storage molecule due to its branching structure. The branched structure of glycogen allows for multiple reaction sites, which accounts for the breakdown of carbohydrate at rapid rates so that energy can be easily accessed when needed (Gilbert, 2011). As a large, multi-branched sphere of glucose, glycogen can be broken down into glucose by glycoside hydrolases that cleave the glycosidic bonds between different glucose monomers. Once in its monomeric form, glucose can be metabolized into pyruvates, acetyl CoA, and eventually into ATP by the glycolytic enzymes, pyruvate dehydrogenase complex and oxidative phosphorylation of the electron transport chain, respectively (Arrese, 2010).

Glycogen is a particularly important form of energy storage in animals (Gilbert, 2011). Insects store glycogen in an organ called the fat body, which is located inside the lining of the abdomen. The fat body is analogous to the vertebrate liver because they both play important roles in storage of carbohydrates and fat (Liu et. al., 2009). For instance, glycogen provides energy for developing insect embryos, for extended periods of flight, for cuticle formation and for the preservation during periods of cold and drought (Suarez, 2005; Arrese, 2010.)

Bumble bees (genus *Bombus*) are the most economically important native pollinator in the U.S. (National Research Council 2007) and also play an important role in healthy ecosystem function. Bumble bees have highly developed social system with stratified castes of males, queens and workers. They obtain carbohydrates primarily from ingesting floral nectar, which is highly rich in glucose. In queen bumble bees, glycogen storage is essential for various life stages, including during larval development and also for survival over a winter diapause period (Alford, 1969). Queen bumble bees spend approximately two months during the winter in a diapause state, before emerging to find new colonies of their own the following spring. During diapause, queens do not forage or eat, but instead utilize copious amounts of stored glycogen in their fat

bodies to survive (Alford, 1969). Previous studies have shown that glycogen levels in queens increase prior to diapause, then plummet post diapause (Alford, 1969; Arrese, 2010). This strongly suggests that queen bumble bees rely on glycogen as an energy source for surviving through the winter (Alford, 1969).

Although glycogen storage in bumble bee queens has been studied fairly extensively (Arrese, 2010; Inagaki, 1986; Liu, 2009; Roseler, 1986), it remains unknown if differing carbohydrate intake affects carbohydrate storage, and thereby the survival of queen bumblebees during and after diapause. This information is important because elucidating the relationship between carbohydrate intake and glycogen storage in bumble bees can contribute to our understanding of the basic biology and nutritional needs of bumble bees. This information may ultimately contribute to conservation and management strategies, because more informed conservational efforts can be made available to enhance food availability for bumble bees. Here, we explored the relationship between carbohydrate intake and glycogen storage in bumble bee queens, by experimentally manipulating carbohydrate intake in bumble bee queens and observing how this influences weight gain and glycogen storage. We hypothesized that bumble bee queens with little or no carbohydrate in their diet would fail to gain weight or sequester glycogen during their first 12 days post-emergence from diapause.

Methods

Bees. 29 colonies of the bumble bee *Bombus impatiens* were obtained from Koppert Biological Systems (Romulus, MI) and allowed to mature until queen production. The colonies were housed in a dark room at 25˚ C and 50-70% relative humidity, which simulated their natural underground colony environment (Biobees, 2011.) Emergent young queen bees were drawn from

each colony (age 1 day) for each treatment. The approximate age of the queens was visually detected by their silvery color (Bugguide, 2014). These queens were pulled and individually labeled by small number tags on their thorax to individually distinguish each queen.

Diet manipulations. Newly emerged queens were randomly assigned to one of four treatment groups (below) and were isolated in small, circular plastic Tupperware cups cages (approximately 1-cup size). Queens were fed *ad libitum* every day.

(*i*) Nectar Starvation group (hereafter, "NS"): Queens were fed deionized water (0% sucrose) and pollen (multisource, mixed with deionized water) every day.

(*ii*) Low Quality Nectar (hereafter, "LQN"): Queens were fed a solution of 25% w/v sucrose solution ("nectar") and pollen (multisource, mixed with 25% w/v sucrose solution) every day.

(*iii*) Control group (hereafter, "CTL"): Queens were fed a solution of 50% w/v sucrose solution ("nectar") and pollen (multisource, mixed with 50% w/v sucrose solution) every day.

(*iv*) High Quality Nectar (hereafter: "HQN"): Queens were fed a solution of 75% w/v sucrose solution ("nectar") and pollen (multisource, mixed with 75% w/v sucrose solution) every day.

Experiment 1: Weight gain. For this experiment, the queens in all groups (N = 111) were weighed approximately every 24 hr for twelve days, and their weights documented to determine any daily changes in weight. To estimate total weight gain (total Δ weight), the mass of bees on day 1 was subtracted from their mass on day 12. Across the four groups, mean Δ weight values were compared using comparative, pairwise two-tailed *t*-tests.

Experiment 2: Glycogen Storage. To compare glycogen levels across the four groups, total body glucose levels were compared. Because glycogen primarily consists of glucose (Arrese, 2010), glucose levels were used as a proxy for measurement of glycogen levels. The bodies of a subset of bees (N = 40, 10 per group) of the four treatment groups were pulverized using a bead beater and carbohydrate levels were quantified using a protocol modified from Judd et. al. (2010). Six glucose standards were prepared each containing 0, 12.5, 25, 50, 100, 200 µg. Using 50 µL subsets of samples, 18% NaSO4 solution, followed by anthrone reagent, were added. Heat was applied at 100 °C for 12 min and samples were cooled to room temperature away from light. Total body glucose levels were measured using absorbance of standards and samples at 625 nm on spectrophotometer. Each sample was run in triplicate and the average was taken across the separate assays to ensure replicability. Glucose concentrations were estimated using standard curve extrapolation of the absorbance using Beer Lambert's law. Differences in carbohydrate levels across the groups were compared using an ANOVA and post-hoc HSD Tukey Tests.

Results

Experiment 1: Weight Queens. Queens in all treatment groups gained the most weight in the first 24 hr post-emergence, then only continued to gain additional weight if they obtained enough carbohydrates through their diet (Figure 1). Relative to the other treatment groups, NS queens failed to gain any weight in a 12-day period post-emergence (mean Δ weight = 0.0373g). All other groups (LQN, CTL, HQN) gained more weight than the NS group (Δ weights = 0.1142g, 0.1778g, 0.1605g, *P*-values of 0.0036, 0.0014, 0.0006, and respectively; Figure 2). Similarly, LQN queens' Δ weight (mean Δ weight = 0.1142) were significantly lower relative to that of the

CTL (P = 0.0484). The HQN queens gained weight (mean Δ weight = 0.1605g) but not significantly more than that of the CTL group (P >0.05; Table 1; Figure 2).

Experiment 2: Glycogen Storage. NS queen carbohydrate levels were significantly lower than all the other groups (P < 0.0001, comparison to CTL; P < 0.0001, comparison to HQN; and P= 0.0020, comparison to LQN; Table 2 and Figure 3). LQN queens' total body carbohydrate level (4.6753 ug) was not significantly lower than that of the CTL group (P > 0.05). The HQN queens' total body carbohydrate level (7.0700 ug) was not significantly higher than that of the CTL group (P > 0.05, Tukey HSD). (Table 2, Figure 3)

Analysis of Variance (ANOVA) was used to analyze intrinsic variations within each treatment group's glycogen levels. NS queens had lower mean glycogen levels than all other groups (t < 0.0001; Figure 4). The results of this ANOVA analysis confirmed the analysis of Tukey HSD test shown previously (Table 3). Similar differences among different treatment groups can also be observed with whiskers-boxplot distribution of the data (Figure 4).

Discussion:

Carbohydrates, especially in the form of glycogen, serve as important form of energy storage in animals and insects (Gilbert, 2011). Carbohydrates are crucial for development of insect embryos, cuticle formation and particularly for sustenance in the form of glycogen during diapause (a state in which bumble bee queens spend during the winter) (Arrese, 2010). Therefore, without sufficient nutrition such as carbohydrates, we hypothesized that glycogen storage in bumble bee queens might be jeopardized when they are fed low-carbohydrate diets.

The study of the relationship between carbohydrate intake and glycogen storage can contribute to our understanding of the nutritional needs of bumble bee queens.

Consistent with our hypothesis, we found that queens with no carbohydrates in their diet (NS group) failed to gain weight, whereas queens in other treatment groups did gain weight in the first 12 days of adult life. These weight data suggest that when queens cannot get enough sugar in their diet, they are not able to build up much (or any, as in the case of NS queens) stored glycogen. As predicted, the NS group also had lower glycogen levels compared to all the other groups, because their diet treatment did not contain any carbohydrate to make glycogen.

Additionally, LQN (25% sucrose) queens gained significantly more weight than the NS queens and their glycogen levels were significantly higher than those of the NS queens, equaling to that of CTL queens. These findings suggest that the young emergent queens probably increase their food intake to compensate for their sugar deficient diet. It has been shown that mice fed nutrient poor food eat more because Gcn2, a transcription factor, is activated upon starvation, inducing the starved mouse to feed more (Hao, 2005). However, despite any increase in nectar consumption to make LQN gained as much glycogen as CTL queens, LQN queens are still hindered by their diet treatment as their weight gain was minimal (not significant) relative to the CTL and HQN queens and their glycogen levels remained significantly lower than that of HQN (75% sucrose). These findings mean that LQN queens were not able to fully compensate for their low quality nectar by consuming enough extra nectar to equal the advantages of the CTL and HQN queens. Additionally, the LQN group's minimal weight gain could also be due to the fact that the largest contributor of weight is the insect exoskeleton. Exoskeleton cannot fluctuate in size and weight due to fluctuations in diet, which is infinitesimal compare to the large weight of the exoskeleton, therefore little weight change was observed.

Interestingly, HQN queens did not gain more weight than CTL queens and there was no statistical difference between CTL and HQN carbohydrate levels. These findings suggest that these queens might have reached a saturation point that did not permit more cellular absorption of carbohydrate despite copious extra intake of carbohydrate. Alternatively, queens may be able to sense highly concentrated nectar, and this might cause queens of the HQN group alter their feeding behavior to not consume as much as the CTL queens, thereby equalizing the two groups' weight differences and carbohydrate levels.

Together, we found that our manipulations of feeding treatments altered weight gain and glycogen storage in newly emergent bumble bees. The HQN group shows that extra nourishment does little to affect the weight and glycogen of young queen bumble bees. LQN and NS groups together show that without adequate carbohydrate intake, young queens are unable to gain weight or sequester abundant glycogen. With these data, we call to attention the decline of bumble bees and assert that nutritional deficiencies, particularly carbohydrate deficiencies, may play a role in lowering survival likelihood of bumble bee queens through the diapause period (Goulson, 2008). Lowering survival likelihood of a young queen has serious overarching implications because a death of a young queen means no opportunity for an entire bee colony to be created. Given the importance of queen bumble bees and their strong need for stored glycogen to survive diapause, carbohydrate deficiencies may be one of the many contributing factors leading to the bumble bee population decline.

Bumble bee population decline is very important because it could in turn lead to fewer pollination events, a lower crop yield and therefore our own poor nutrition (Goulson, 2008). Studying their varying feeding rates in reaction to different levels of carbohydrate facilitates an understanding that carbohydrate is essential to their survival. With this information, we can

target their essential food sources in the wild and focus conservation efforts on maintaining this food source.

Figures and Tables.

Table 1. Comparison of queen weight gain across treatment groups. P values are for t-tests of comparison of different treatment groups for experiment 1 (Weight Queens). Significant differences at a 95% confidence interval are denoted in red.

Weight Difference (End Weight - Start Weight)				
	CTL	**HQN**	**LQN**	**NS**
Group mean starting Weight	0.5519	0.5615	0.5677	0.5367
Group mean ending Weight	0.7342	0.7312	0.6920	0.5740
Group mean Weight difference	0.1778	0.1605	0.1142	0.0373
Standard error	0.0255	0.0158	0.0124	0.0200
P value: comparison to CTL		0.6576	0.0484	0.0014
P value: comparison to HQN			0.0376	0.0006
P value: comparison to LQN				0.0036

Table 2: Comparison of queen carbohydrate levels across treatment groups. *P*-values are for pos-hoc HSD Tukey test of comparisons of means glycogen concentration of different treatment group for experiment 2 (Glycogen storage). Significant differences at a 95% confidence interval are denoted in red.

Glycogen Concentration Comparisions				
	CTL	**HQN**	**LQN**	**NS**
Group mean glycogen levels	5.7606	7.0699	4.6753	1.2914
St. Error	0.7331	0.6589	0.6723	0.3409
Mean difference: comparison to CTL		1.2945	1.1001	4.4840
Mean difference: comparison to HQN			2.3946	5.778
Mean difference: comparison to LQN				3.3838
P value: comparison to CTL		0.4670	0.5803	<0.0001
P value: comparison to HQN			0.0479	<0.0001
P value: comparison to LQN				0.0020

Table 3: Comparison of queen carbohydrate levels across treatment groups. *P*-values are for analysis of variance of means glycogen concentration of different treatment group for experiment 2 (Glycogen storage). Significant intrinsic variations within each group are denoted in red.

Glucose level comparisons				
Group	**CTL**	**HQN**	**LQN**	**NS**
Mean difference	5.7754	1.2945	-1.1001	4.4840
Standard Error	0.6068	0.8817	0.8582	-0.8582
t-value	9.5170	1.4680	-1.2820	-5.2250
P	3.04e-11	0.1510	0.2080	8.12e-06

Figure 1: Daily changes in queen weight across the 12 day experiment period, separated by treatment group. X axis represents days in the experiment; Y axis represents the mean weight of queens in grams. Bars represent standard errors.

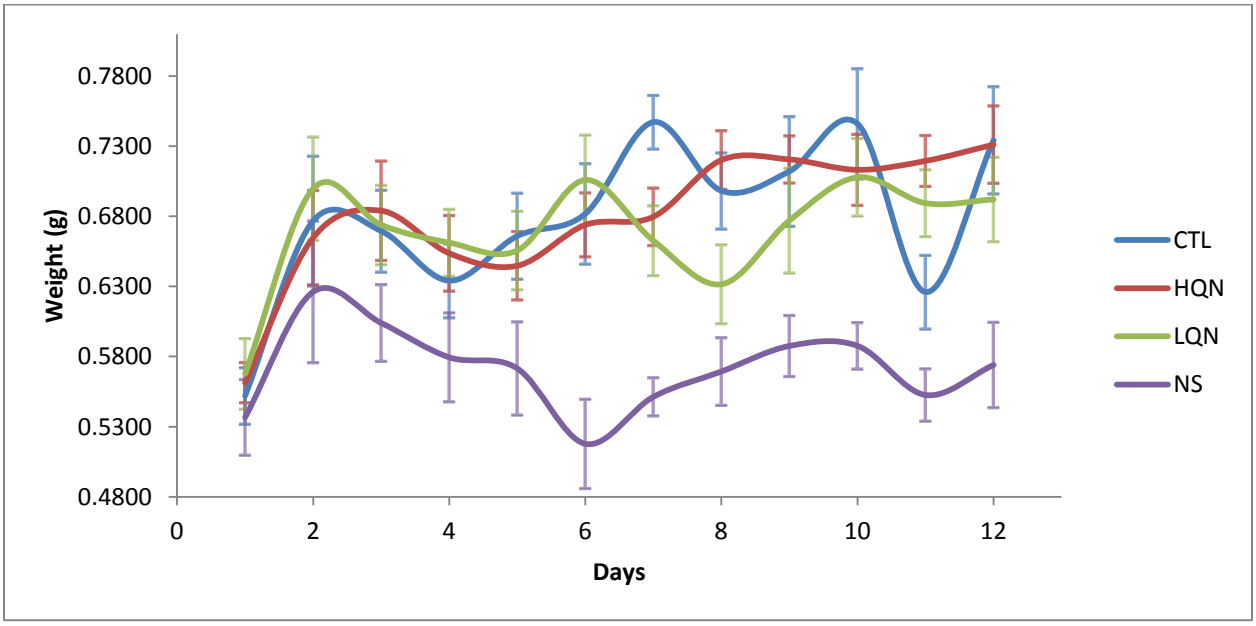


Figure 2. Changes in queen weight after the 12 day experiment period, separated by treatment group. X axis represents different treatment groups, Y axis represents mean weight changes (from day 1 to 12) of queens in grams. Different letters denotes there is a significant difference between weight changes of these treatment groups. Bars on each column represent standard errors.

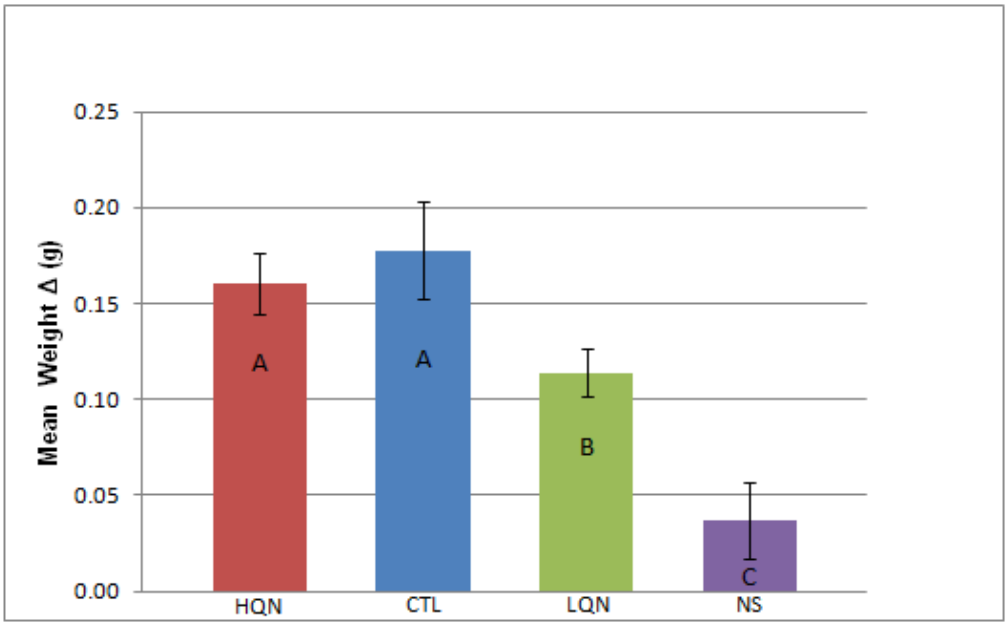


Figure 3. Glycogen levels of queens across different treatment groups of experiment 2 (Glycogen storage). X axis represents different groups, Y axis represents mean glycogen concentrations of queens in µg. Different letters denotes there is a significant difference between weight changes of these treatment groups. Bars on each column represent standard errors.

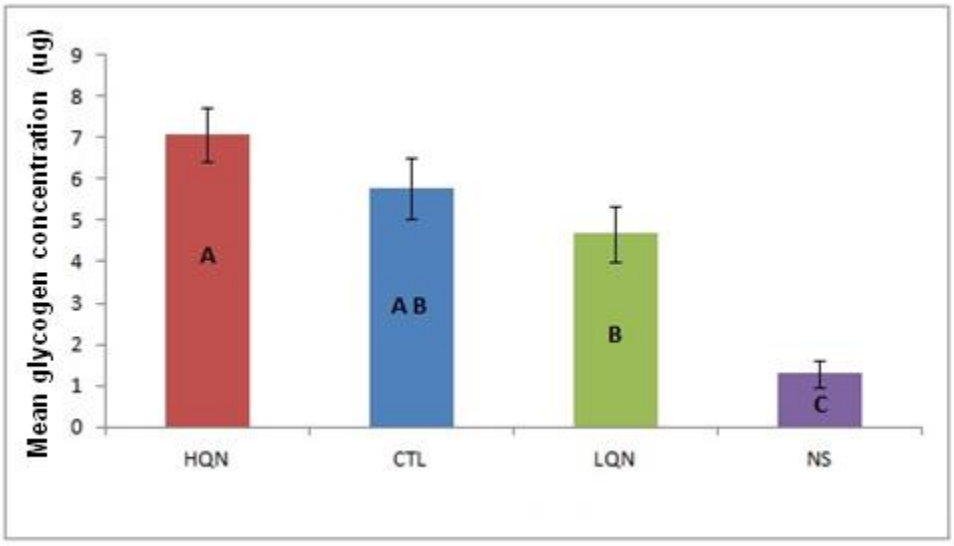
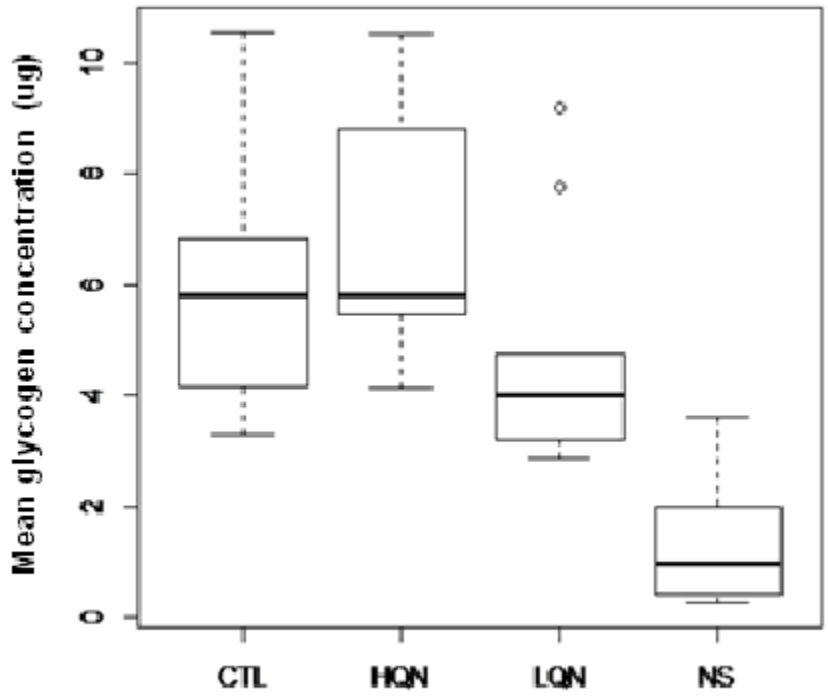


Figure 4. Distribution of carbohydrate levels across different treatment groups of experiment 2 (Glycogen storage). X axis represents different group, Y axis represents mean glycogen concentrations of queens in µg. Bars on each day represent standard errors. Overlapping whiskers and median show no significant differences between groups.



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