THESIS

DOES INTERINDIVIDUAL VARIATION IN ENERGETIC DEMAND INFLUENCE FOOD SHARING IN THE HONEYBEE?

Submitted by

Abbie J. Reade

Department of Biology

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Master's Committee:

Advisor: Dhruba Naug

Gregory Florant
Paul Ode

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ABSTRACT

DOES INTERINDIVIDUAL VARIATION IN ENERGETIC DEMAND INFLUENCE FOOD SHARING IN THE HONEYBEE?

A central benefit of group living is the cooperative acquisition and sharing of resources but the costs associated with these processes set up a potential conflict between individual and group level fitness. This means that all individuals do not get an equal share of the benefits or pay an equal share of the costs, which also results in an overall decrease in the average fitness of all group members. In contrast to group living animals in which behavior is driven by considerations of individual fitness, in eusocial groups such as the honeybee colony, it is generally considered that all group members contribute equally toward group efforts with selection primarily acting at the colony level. However, one can hypothesize that if individuals differ in their intrinsic energetic requirements, this difference in the cost of self-maintenance would lead to differences in the amount of resources they can contribute to the colony pool. Using the honeybee colony as a model, I investigated this idea regarding whether differences in individual energetic requirements among eusocial group members influence the amount of food that an individual shares with the group. First I investigated whether there was interindividual variation in carbohydrate demand among foragers using a capillary feeder assay. Next I asked whether the carbohydrate demands of individual foragers were a function of their metabolic rates. Then I used a series of sharing experiments in the field and in the lab to determine whether food sharing by an individual forager was influenced by her own energetic demand. The results of my research show that even though there is substantial variation in energetic demand among

the members of a honeybee colony, it does not influence the amount of food an individual shares with the colony. This suggests that either honeybee colony members indeed work in a truly "altruistic" fashion or that there are other possible implications of such differences.

ACKNOWLEDGEMENTS

First and foremost I want to thank my advisor and mentor Dhruba Naug for his guidance, persistence, and patience. Keziah Katz has played a significant role in helping me to develop a strong set of statistical analysis tools and techniques, encouraging me with logical prodding and hugs along the way. Thanks to my committee members Greg Florant and Paul Ode for their insight, encouragement and helpful suggestions. Additional thanks are due to Amanda Lark, Aja Mattise-Lorenzen, and Duncan Ziegler for assistance with data collection and processing. Carrie Tait has kept me going with insightful conversations and whimsical banter. I could not have accomplished this without the supportive community that is the Biology Department here at Colorado State University! Thank you.

DEDICATION

I dedicate this work to my family. To my mother, who always told me that I could be anything I wanted through hard work and dedication. My father, who has stood by me and supported me through seemingly endless hours of work, and to my daughter, in demonstration that indeed, hard work pays off. Never give up!

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CHAPTER 1

Inter-individual variation in nutrient balancing in the honeybee (*Apis mellifera*)[1]

INTRODUCTION

All animals must obtain a specific combination of different nutrients to optimize different life history traits. For instance, an animal that is maximizing growth or reproduction may require a larger proportion of protein in its diet, while an animal that is more concerned about survival is likely to maximize the intake of carbohydrates as a quickly available fuel source. This is in contrast to what is predicted by optimal foraging theory (Charnov, 1976), which has traditionally considered energetic gain as the primary currency driving the foraging decisions of animals. Nutritional geometry, a bottom-up, state-space modeling approach specifically developed to address this issue, explains foraging behavior in terms of satisfying a ratio among different nutrients that maximizes fitness (Simpson and Raubenheimer, 1993, 2012). The level of a nutrient that provides the maximum contribution to a given life history trait is defined as the intake target for that nutrient, thus requiring an animal to satisfy a multidimensional intake target. Faced with different food items that vary in their nutritional compositions, an animal is therefore confronted with the complex problem of how to reach or approach this multidimensional target in a way that achieves a nutritional balance for maximum fitness.

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[1] Reade, A. J., & Naug, D. (2016). Inter-individual variation in nutrient balancing in the honeybee (Apis mellifera). *Journal of Insect Physiology*, *95*, 17-22. http://dx.doi.org/10.1016/j.jinsphys.2016.09.002

Nutritional geometry has been shown to be a robust model for explaining how animals regulate their foraging to balance the intake of different nutrients. While the Geometric Framework has been used to explain nutrient balancing with respect to different fitness parameters in a variety of species (Simpson and Raubenheimer, 2012), whether such nutritional regulation plays any role in social dynamics is only beginning to be considered (Behmer, 2009; Cook et al., 2010; Lihoreau et al., 2014). Social insect colonies of honeybees and ants have been shown to behave in a manner consistent with the Geometric Framework of nutrient balancing, regulating their nutrient intake at a collective level (Dussutour and Simpson, 2008, 2009; Hendriksma and Shafir, 2016). In an interesting contrast to what might be expected from optimal foraging theory, ant colonies were found to switch from consuming a concentrated sugar solution to a more dilute solution with time, which suggests that they were balancing their diet with respect to the nutrients sugar and water, rather than simply maximizing their energetic intake (Dussutour and Simpson, 2008).

Within any group such as a social insect colony, one can expect a substantial amount of inter-individual variability in intake targets not only between members of different behavioral groups (Paoli et al., 2014), but also within a behavioral group with each individual possessing different physiological dispositions. For example, it has been shown that pollen foragers have higher metabolic rates than non-pollen foragers (Feuerbacher et al., 2003) and these foragers with higher metabolic demands could exhibit a higher carbohydrate intake target than other foragers. Individuals varying in their overall foraging efforts might also be driven in part by how closely they monitor their own intake target, which has been referred to as an individual's 'nutritional latitude' (Senior et al., 2015). It is therefore important to understand the nature of

such variation within a colony and how it might impact the nutritional intake at the colony level, which in turn might have played a role in the evolution of social behavior itself.

The capillary feeder (CAFE) assay, originally developed to examine the prandiology of the fruit fly, Drosophila melanogaster (Ja et al., 2007), is a technique that allows precise measurement of liquid food consumption by individual animals and can be applied to both short- and long-term feeding experiments (Deshpande et al., 2014). Because the diet of adult workers in social insect colonies consists primarily of carbohydrates (Altaye et al., 2010; Ihle et al., 2014; Paoli et al., 2014), we used a modified CAFE assay to investigate the variation in carbohydrate intake target and nutrient balancing strategy with respect to sucrose and water among individual honeybee foragers. In the absence of any substantial fat reserves, these foragers critically rely on their nectar based carbohydrate diet, consisting mainly of water and sucrose, to meet their large energetic requirement for flight and foraging performance (Sacktor, 1970; Candy et al., 1997), subjecting them to strong selection for managing their carbohydrate budgets. By removing a forager from the colony and allowing her to choose between two different concentrations of sucrose solutions, we were able to examine the variation in how an individual bee regulates her nutritional requirements, independent of the nutritional state of the colony.

METHODS

Gustatory responsiveness assay

We collected returning honeybee (*Apis mellifera*) foragers from five different colonies, noting whether or not they were carrying pollen, and chilled them on ice just enough to allow them to be harnessed into plastic straws. The gustatory sucrose sensitivity of each bee was assessed by stimulating its antennae first with water and then with an ascending series of sucrose

concentrations up to 60% (0, 0.1, 0.3, 1, 3, 10, 30, 45 and 60%) and testing for the extension of its proboscis, the Proboscis Extension Response (PER). All bees were stimulated with water between the presentations of two successive sucrose concentrations in order to reduce the effects of any potential sensitization to sucrose. The concentrations of all sucrose solutions in this study were prepared and reported as w/w sucrose solution: weight (g) sucrose/(weight (g) sucrose + (g) water). A Gustatory Responsiveness Score (GRS) was calculated for each bee as the sum of the PERs elicited to the initial presentation of water and the eight sucrose concentrations (Scheiner et al., 2001). The gustatory responsiveness scores (GRS) in this experiment therefore have a range of 0–9, a score of 0 indicating that the bee did not respond to any of the stimuli, including the first presentation of water, while a score of 9 indicates that the bee responded to the initial water presentation and all the sucrose concentrations.

CAFE assay

Immediately following the GRS assay, each bee was fed until satiation with a 30% sucrose solution (to equalize their energetic states) and subjected to a 16-h CAFE assay to determine its individual intake target with respect to sucrose and water. Each bee was placed in a clear acrylic chamber (3 cm ID and 3 cm tall) with ventilation holes and two glass capillary feeding tubes (152 mm long, 1.12mm ID; World Precision Instruments, item number: TW150-6), each filled with 110 µl of sucrose solution of a different concentration, representing two alternative food choices. The two solutions were enhanced with either blue or yellow food coloring to enable their discrimination during analysis and the two colors were alternated between the two concentrations and the two sides of the chamber in different replicates to correct for any potential color or side bias. The chambers were placed in an incubator set at 25° C and 60% Relative Humidity (RH) and a camera with an automatic timer was used to record the level

of the solution in each capillary at hourly intervals. We conducted two series of CAFE assays, one in which the two sucrose solutions provided were 45% and 5%, and another in which the two solutions were 45% and 1%. Each replicate of the assay also included a control chamber identical to the others, but without a bee in it, to account for any evaporative loss of the solutions.

Statistical analysis

The hourly consumption of each solution by each bee was calculated after subtracting the average hourly rate of evaporation from the control chambers, and from this the total amounts of sucrose and water consumed were used to calculate the hourly intake and the final intake target for each bee, expressed as sucrose concentrations. A one-sample t-test was used to compare the aver- age final intake target across all bees to an intake target equivalent to the mean concentration of the two solutions. A two-sample t-test was used to compare the intake targets in the two treatments. An F-test of variance was used to compare the variation in the amount of water consumed to the variation in the amount of sucrose consumed. The nutritional latitude of a bee was calculated as the mean absolute difference between its final intake tar- get and its intake target at each hour, given by $(\Sigma\ |IT_{final} - IT_{hour}|)/\ n$, where n is the number of hourly observations for the bee. Kolmogorov-Smirnov goodness of fit tests were used to compare the distributions of individual intake targets and nutritional latitudes with expected normal distributions. Pearson's correlations were used to investigate the relationships between gustatory responsiveness, forager type, and the final intake target of each bee. All statistical analyses were conducted using R (version 3.1.1).

RESULTS

Intake target

A total of 200 bees, which completed the CAFE assay without exhausting either of the sucrose solutions, were used in the analysis to ensure that all of them had a choice between the two foods during the entire assay. At the end of the 16-h assay, the bees in the two CAFE assays, consisting of different pairs of sucrose concentrations, converged on the same, statistically indistinguishable intake target (Welch Two-sample t-test: $t_{188} = 0.15$, $p = 0.88$, Figure 1.1A). The intake target for the experiment in which the bees had a choice between 1% and 45% solutions was 0.33 ± 0.009 and the intake target observed in the experiment with 5% and 45% solutions was 0.33 ± 0.01, both equivalent to a 33% sucrose solution. The two intake targets were significantly different from the mean of the two concentrations in both treatments (1% vs. 45%: $t_{109} = 2485.51$, $p < 0.0001$; 5% vs. 45%: $t_{89} = 2351.48$, $p < 0.0001$), demonstrating that the bees were not simply feeding randomly. In both treatments, the pattern of hourly intake indicates an initial bias followed by a decline in the consumption of the high concentration solution, such that by the end of the assay the two solutions were being consumed in a specific ratio (Figure 1.1B). This pattern of consumption suggests that the bees were actively regulating their intake target.

Although the intake targets realized by the two groups of bees in the two treatments were the same, there was considerable inter-individual variation with a significantly higher variation in the amount of water consumed than in the amount of sucrose consumed by the bees (1% vs. 45%: $F_{109,109} = 1.95$, $p < 0.001$; 5% vs. 45%: $F_{89,89} = 2.5$, $p < 0.001$, Figure 1.2A). This resulted in

individual intake targets showing a significant departure from a normal distribution (Kolmogorov-Smirnov Test: $D = 0.16$, $n = 200$, $p < 0.001$, Figure 1. 2B), with a larger number of bees demonstrating high intake targets.

Nutritional latitude

Individuals can reach similar intake targets but remain true to the target or stray away from it by different extents throughout the assay, the magnitude of which is defined as one's nutritional latitude. A low nutritional latitude is seen as a relatively straight trajectory to the intake target while a high latitude is seen as a more meandering trajectory to the target, punctuated by large changes in the slope of the line (Figure 1.3A). Nutritional latitudes were normally distributed (Kolmogorov-Smirnov test, $D = 0.07$, $n = 200$, $p = 0.28$, Figure 1.3B), and the nutritional latitude of a bee did not show any correlation with her intake target (Pearson's correlation: $t_{198} = 0.75$, $p = 0.45$, Figure 1.3C).

Gustatory responsiveness

Pollen foragers had higher gustatory responsiveness scores (GRS) than non-pollen foragers ($t_{158} = 3.07$, $p < 0.01$) and the GRS of an individual bee was positively correlated with its intake target (Pearson's correlation: $t_{192} = 2.86$, $p < 0.01$, Figure 1.4).

DISCUSSION

In this study we used the principles of nutritional geometry to examine the nutritional intake and nutrient balancing strategies of individual honeybee foragers with respect to sucrose and water. This is the first time, to the best of our knowledge, that a CAFE assay has been used to investigate the variation in the fine-scale feeding behavior among individual honeybees. Our

results show that individual bees balance their nutritional intake independent of the colony context and that there is a substantial amount of inter-individual variation in both intake targets and how they reach these targets.

Honeybee foragers vary in terms of their gustatory responsive- ness to sucrose (Page et al., 1998; Scheiner et al., 2001) and individual bees have been shown to use gustatory information to discriminate between different diets (Hendriksma and Shafir, 2016). Gustatory information is known to play a key role in nutrient balancing, helping an animal to assess the suitability of a food resource with respect to its intake target and thereby inform its foraging decisions (Simpson and Raubenheimer, 1993, 1996). The positive correlation between GRS and intake target observed in this study could indicate that a higher sensitivity to sucrose may be key to obtaining a higher intake target with respect to the carbohydrate requirements of individual honeybees. Since individuals with higher GRS are known to be pollen foragers (Pankiw and Page, 2000), who also have higher metabolic rates (Feuerbacher et al., 2003), it seems likely that the higher intake targets observed in these bees could be associated with their task-related metabolic needs. In both the treatments in our study, the bees were required to choose between two alternative food resources, and remarkably the bees consistently combined the available foods in a way that allowed them to achieve an intake target equivalent to a 33% sucrose solution. That the bees were not feeding randomly is demonstrated both by a final intake target greater than the average of the two solutions offered and by the change in relative consumption of the two solutions over time. The fact that there was greater variation in the amount of water consumed in comparison to that of sucrose, suggests that the bees were prioritizing the sucrose component of the available food resources. However, they were also not merely trying to maximize their net energetic gain, as would have been indicated had they consumed all of the

high concentration sucrose solution before consuming any of the low concentration solution, in fact only a single bee exhibited this behavior. Rather, the bees combined the two different foods in a way which indicates that a 33% sucrose solution represents an ideal homeostatic target (Simpson and Raubenheimer, 1993; Köhler et al., 2012) for honeybee foragers. This is in contrast to what might be predicted by energy-maximizing optimal foraging models and shows that honeybees also actively regulate their water intake and even when considering a simple carbohydrate and water diet, nutritional geometry provides a more comprehensive description of the feeding behavior of animals. Few studies on Geometric Framework have considered water as a nutrient, but those which do, find that water has direct effects on fitness traits (Raubenheimer and Gäde, 1994; Fanson et al., 2012; Köhler et al., 2012).

The variation in nutrient balancing observed among individual bees is of particular interest because behavioral variation is considered to be adaptive in most biological systems, providing social groups with the flexibility to respond to environmental challenges (Mattila and Seeley, 2007; Pruitt and Riechert, 2011). The foraging behavior of each member in a social insect colony is limited by her own energetic and nutritional demands (Wolf et al., 1989; Feuerbacher et al., 2003), which suggests that the difference among individual foragers in terms of their contribution to the colony (Pankiw and Page, 2000) could be a reflection of differences in their intake targets. As the subjects in our CAFE assays were kept at a constant and ideal temperature and allowed little movement, our results represent a close approximation of their carbohydrate requirement for maintaining a basal metabolic rate and the observed variation in intake target probably reflects the intrinsic difference among individuals with respect to it. The variation in intake target can be expected to be substantially higher within the context of natural colonies due to differences in activity level and task dependent metabolic demand among

individuals and our future work aims to address such differences. Individuals with higher intake targets with respect to sucrose could also have higher survival as indicated by bees surviving longer on diets with a higher carbohydrate bias (Altaye et al., 2010; Paoli et al., 2014).

We defined nutritional latitude as the mean deviation of an individual bee from its own intake target, which translates to the degree to which that individual can tolerate a nutrient excess or deficit associated with ingesting a nutritionally imbalanced food (Senior et al., 2015). This latitude may arise from the action of several independent mechanisms such as the physiological cost of sustenance on an imbalanced diet, the travel cost between different food resources, or the level of competition for each of these resources. Each of these factors differs in terms of how it is expected to act on behavior, for example a high physiological cost of imbalance should induce an individual to switch more frequently between resources, leading to a lower latitude, while a high travel cost between resources should discourage such a switch, leading to a higher latitude. The nutritional latitude of an individual is therefore an outcome of the relative magnitudes of these different forces. Since in our experiment there is no competition for food and the magnitude of travel cost can be considered negligible, the observed nutritional latitude of an individual is a likely outcome of its physiological tolerance to unbalanced food. Bees with a higher latitude are therefore likely to be individuals with a higher tolerance for physiological imbalance than bees with a lower nutritional latitude. It would be interesting to ask if bees with different levels of physiological tolerance to unbalanced diets have different levels of resilience during times of resource scarcity or have different amounts of flexibility in terms of taking advantage of a wider variety of food resources in a natural ecological setting.

An unbalanced diet is costly to animals as any excess nutrient must be stored or excreted, both of which might require energetic expenditure. In insects, an excess amount of sugars can be toxic, especially as the insect ages (Garrido et al., 2015). This may be a relevant factor in the transition of a honeybee into the role of for- ager, which is accompanied by a shrinkage of the fat bodies (Toth et al., 2005) and a possible decrease in the ability to store excess sugars. However, an ability to unload any such excess sugars into a communal food cache for group level benefits may have contributed to the evolution of cooperative living in the honeybee. It has been shown that the nutritional needs of adult bees shift toward a carbohydrate biased diet when they make the transition from within-hive duties to foraging (Paoli et al., 2014). However, it would be interesting to determine if there is also a decrease in nutritional latitude with age that would suggest a decrease in physiological tolerance to high concentrations of sugar and if this is related to the propensity of an individual to share food with its nestmates.

Nutritional interactions are a central component of all social groups. A social insect colony, generally comprised of a single reproductive queen and thousands of her offspring who forego their own reproduction in order to work for the benefit of the group, is often referred to as a superorganism (Wilson, 1971; Moritz and Southwick, 1992). The traditional viewpoint regarding cooperative foraging and food sharing in social insects is centered on the idea that the behavior of individual foragers is primarily regulated by the nutritional state of the colony (Seeley, 1995). However, recent research show that foraging decisions are also regulated at the level of the nutritional state of the individual (Toth et al., 2005; Mayack and Naug, 2013), leading to the idea that altruistic foraging in eusocial insects is driven by regulatory mechanisms that have been evolutionarily co-opted from solitary insects (Toth and Robinson, 2007). The current study supports these previous findings by demonstrating that individual honey- bees are

sensitive to their own nutritional requirements independent of the colony, especially in terms of carbohydrate which serves as a primary component of the adult honeybee diet and directly affects their performance and survival. We therefore pro- pose that any inter-individual variation in the intake target among foragers should reflect in their performance and contribution toward the intake target of the colony and that understanding how a collection of individuals with different intake targets might drive social dynamics can contribute to our understanding regarding the evolution of social behavior.

FIGURES

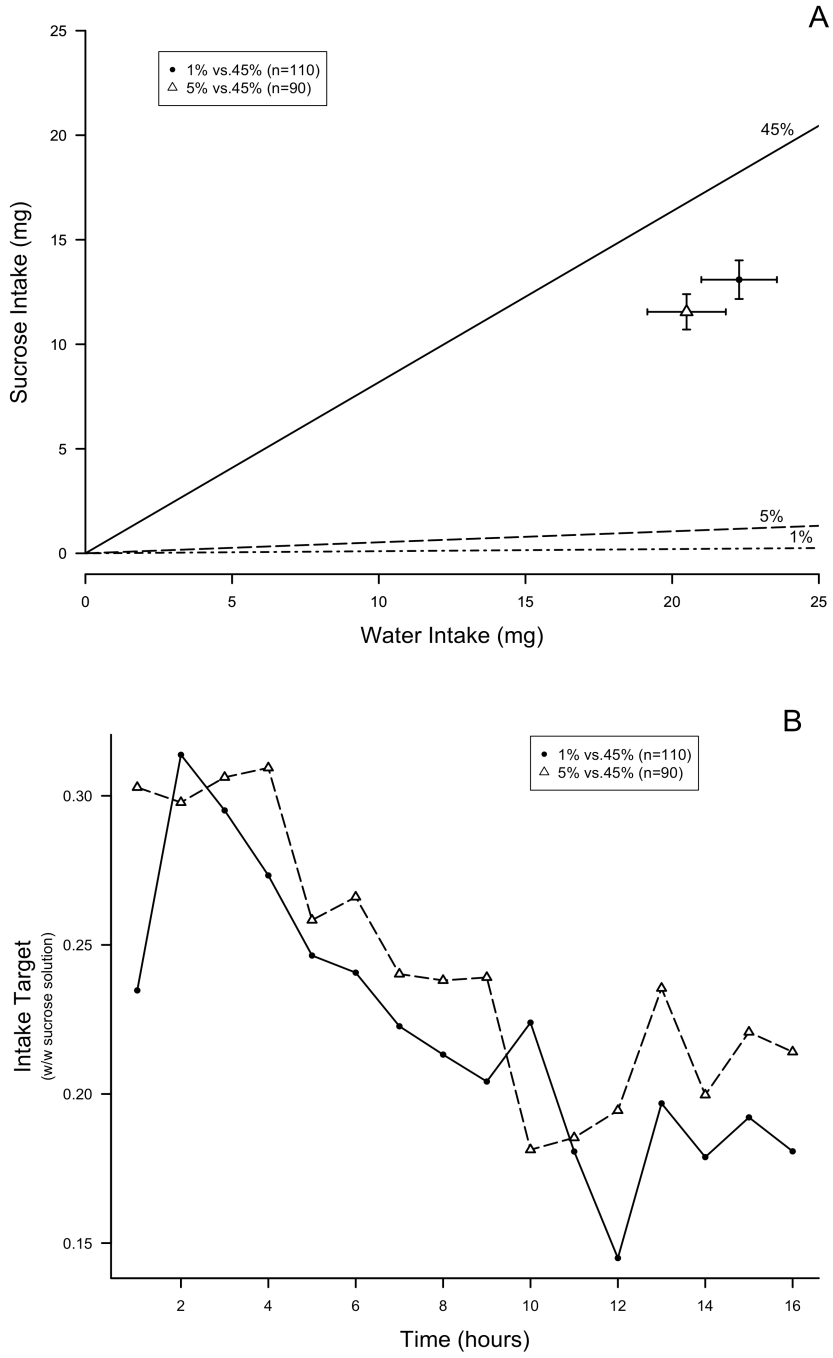


Figure 1.1: Intake target of bees (mean ± SE) with respect to sucrose and water determined by a 16-h CAFE assay, with data represented as (A) final amount of sucrose and water consumed, the three different lines representing the concentrations of the different sucrose solutions in the two experiments, and (B) hourly intake target of bees, calculated as consumption within each hourly interval, in the two treatments.

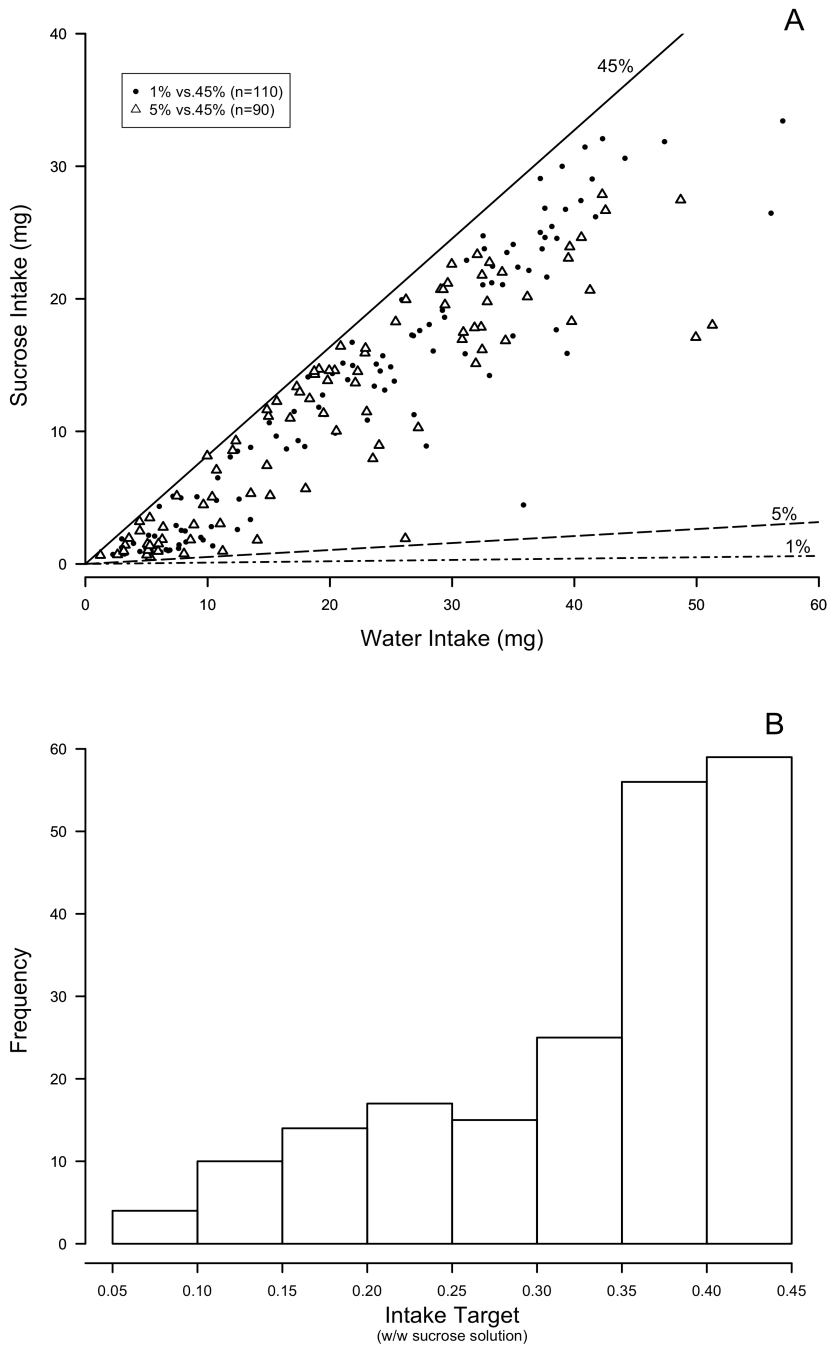
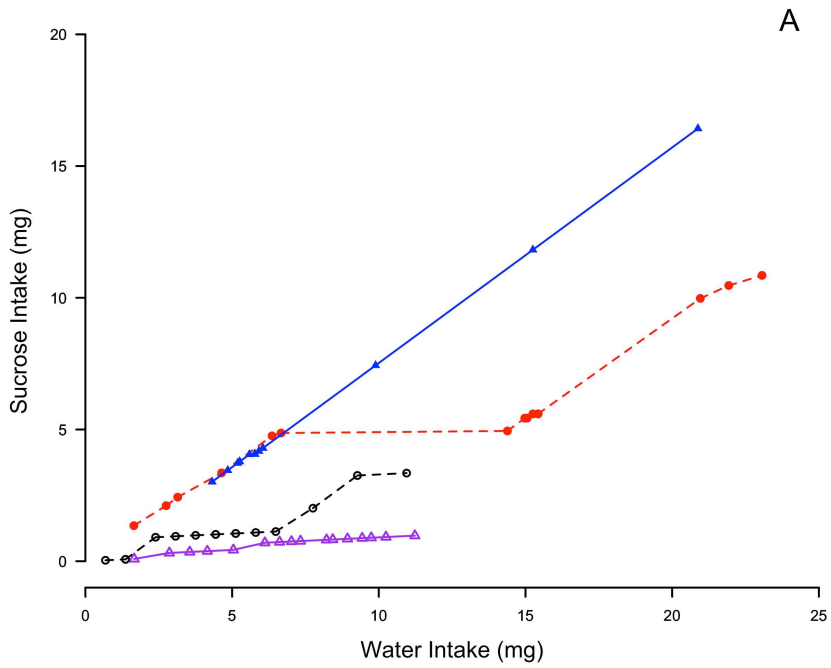
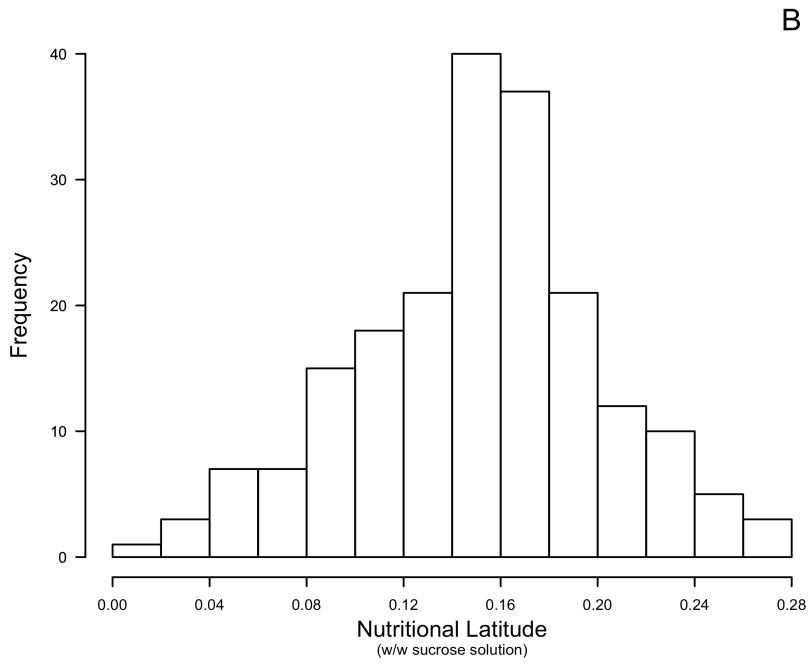


Figure 1.2: Inter-individual variation in the final intake target among bees in the two treatments showing (A) a higher variation on the water than on the sugar axis, and (B) a significant departure from an expected normal distribution.


A
Sucrose Intake (mg)
Water Intake (mg)
0
5
10
15
20
25


B
Frequency
Nutritional Latitude
(w/w sucrose solution)
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20
30
40
0.00
0.04
0.08
0.12
0.16
0.20
0.24
0.28

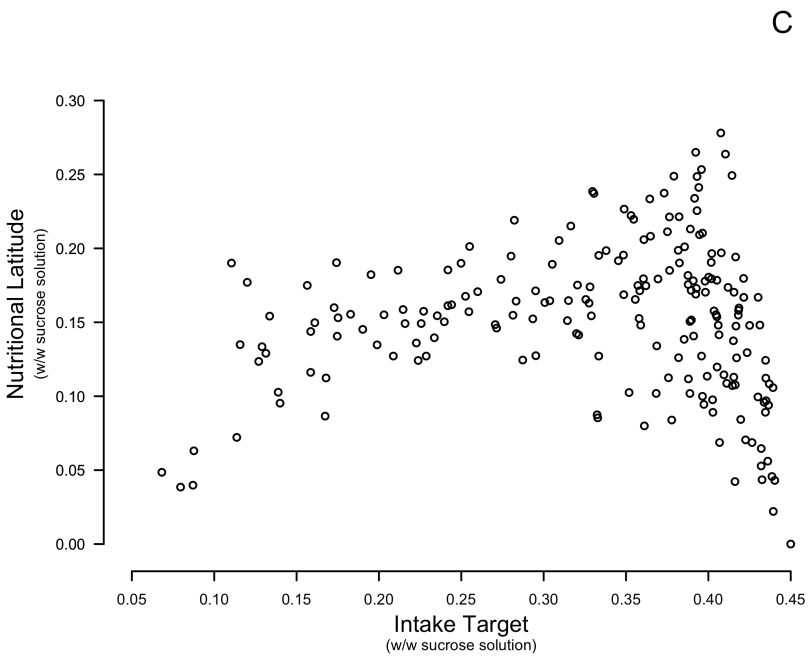

Figure 1.3: Nutritional latitudes of bees illustrated by (A) cumulative consumption of four representative individuals with high or low intake targets and high or low latitudes. Each point represents the cumulative intake at a given time point; circles represent a bee with high latitude and triangles represent a bee with low latitude, filled shapes indicate a bee with high intake target and open shapes represent a bee with low intake target, the points overlap during hours that a bee did not consume either of the two solutions, (B) their normal distribution among individuals, and (C) their non-significant correlation with intake target.

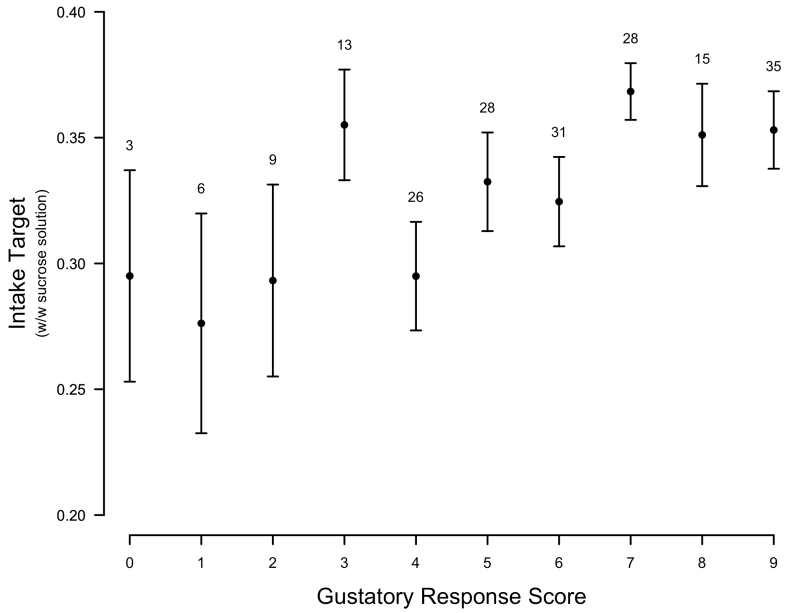

Figure 1.4: Intake target (mean ± SE) and Gustatory Responsiveness Score (n = 194) of individual bees are significantly correlated. The number of bees with a given GRS is indicated above each data point.

REFERENCES

Altaye, S.Z., Pirk, C.W., Crewe, R.M., Nicolson, S.W., 2010. Convergence of carbohydrate-biased intake targets in caged worker honeybees fed different protein sources. J. Exp. Biol. 213, 3311–3318.

Behmer, S.T., 2009. Animal behaviour: feeding the superorganism. Curr. Biol. 19, R366–R368.

Candy, D.J., Becker, A., Wegener, G., 1997. Coordination and integration of metabolism in insect flight. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 117, 497–512.

Charnov, E.L., 1976. Optimal foraging, the marginal value theorem. Theor. Popul. Biol. 9, 129–136.

Cook, S.C., Eubanks, M.D., Gold, R.E., Behmer, S.T., 2010. Colony-level macronutrient regulation in ants: mechanisms, hoarding and associated costs. Anim. Behav. 79, 429–437.

Deshpande, S.A., Carvalho, G.B., Amador, A., Phillips, A.M., Hoxha, S., Lizotte, K.J., Ja, W.W., 2014. Quantifying Drosophila food intake: comparative analysis of current methodology. Nat. Methods 11, 535–540.

Dussutour, A., Simpson, S.J., 2008. Carbohydrate regulation in relation to colony growth in ants. J. Exp. Biol. 211, 2224–2232.

Dussutour, A., Simpson, S.J., 2009. Communal nutrition in ants. Curr. Biol. 19, 740– 744.

Fanson, B.G., Yap, S., Taylor, P.W., 2012. Geometry of compensatory feeding and water consumption in Drosophila melanogaster. J. Exp. Biol. 215, 766–773.

Feuerbacher, E., Fewell, J.H., Roberts, S.P., Smith, E.F., Harrison, J.F., 2003. Effects of load type (pollen or nectar) and load mass on hovering metabolic rate and mechanical power output in the honey bee Apis mellifera. J. Exp. Biol. 206, 1855– 1865.

Garrido, D., Rubin, T., Poidevin, M., Maroni, B., Le Rouzic, A., Parvy, J.P., Montagne, J., 2015. Fatty acid synthase cooperates with glyoxalase 1 to protect against sugar toxicity. PLoS Genet. 11, e1004995.

Hendriksma, H.P., Shafir, S., 2016. Honey bee foragers balance colony nutritional deficiencies. Behav. Ecol. Sociobiol. 70, 509–517.

Ihle, K.E., Baker, N.A., Amdam, G.V., 2014. Insulin-like peptide response to nutritional input in honey bee workers. J. Insect Physiol. 69, 49–55.

Ja, W.W., Carvalho, G.B., Mak, E.M., de la Rosa, N.N., Fang, A.Y., Liong, J.C., Brummel, T., Benzer, S., 2007. Prandiology of Drosophila and the CAFE assay. Proc. Natl. Acad. Sci. 104, 8253–8256.

Köhler, A., Raubenheimer, D., Nicolson, S.W., 2012. Regulation of nutrient intake in nectar-feeding birds: insights from the geometric framework. J. Comp. Physiol. B. 182, 603–611.

Lihoreau, M., Buhl, J., Charleston, M.A., Sword, G.A., Raubenheimer, D., Simpson, S.J., 2014. Modelling nutrition across organizational levels: from individuals to superorganisms. J. Insect Physiol. 69, 2–11.

Mattila, H.R., Seeley, T.D., 2007. Genetic diversity in honey bee colonies enhances productivity and fitness. Science 317, 362–364.

Mayack, C., Naug, D., 2013. Individual energetic state can prevail over social regulation of foraging in honeybees. Behav. Ecol. Sociobiol. 67, 929–936.

Moritz, R.F.A., Southwick, E.E., 1992. Bees as Superorganisms: An Evolutionary Reality. Springer-Verlag, Berlin Heidelberg, Germany.

Page Jr., R.E., Erber, J., Fondrk, M.K., 1998. The effect of genotype on response thresholds to sucrose and foraging behavior of honey bees (Apis mellifera L.). J. Comp. Physiol. A. 182, 489–500.

Pankiw, T., Page Jr., R.E., 2000. Response thresholds to sucrose predict foraging division of labor in honeybees. Behav. Ecol. Sociobiol. 47, 265–267.

Paoli, P.P., Donley, D., Stabler, D., Saseendranath, A., Nicolson, S.W., Simpson, S.J., Wright, G.A., 2014. Nutritional balance of essential amino acids and carbohydrates of the adult worker honeybee depends on age. Amino Acids 46, 1449–1458.

Pruitt, J.N., Riechert, S.E., 2011. How within-group behavioural variation and task efficiency enhance fitness in a social group. Proc. R. Soc. B-Biol. Sci. 278, 1209– 1215.

Raubenheimer, D., Gäde, G., 1994. Hunger-thirst interactions in the Locust, Locusta migratoria. J. Insect Physiol. 40, 631–639.

Sacktor, B., 1970. Regulation of intermediary metabolism, with special reference to the control mechanisms in insect flight muscle. Adv. Insect Physiol. 7, 267–347.

Scheiner, R., Page, R.E., Erber, J., 2001. Responsiveness to sucrose affects tactile and olfactory learning in preforaging honey bees of two genetic strains. Behav. Brain Res. 120, 67–73.

Seeley, T.D., 1995. The Wisdom of the Hive: The Social Physiology of Honey Bee Colonies. Harvard University Press, Cambridge, Massachusettes.

Senior, A.M., Charleston, M.A., Lihoreau, M., Buhl, J., Raubenheimer, D., Simpson, S.J., 2015. Evolving nutritional strategies in the presence of competition: a geometric agent-based model. PLoS Comput. Biol. 11, e1004111.

Simpson, S.J., Raubenheimer, D., 1993. A multi-level analysis of feeding behaviour: the geometry of nutritional decisions. Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci. 342, 381–402.

Simpson, S.J., Raubenheimer, D., 1996. Feeding behaviour, sensory physiology and nutrient feedback: a unifying model. Entomol. Exp. Appl. 80, 55–64.

Simpson, S.J., Raubenheimer, D., 2012. The Nature of Nutrition: A Unifying Framework From Animal Adaptation to Human Obesity. Princeton University Press, Princeton.

Toth, A.L., Robinson, G.E., 2007. Evo-devo and the evolution of social behavior. Trends Genet. 23, 334–341.

Toth, A.L., Kantarovich, S., Meisel, A.F., Robinson, G.E., 2005. Nutritional status influences socially regulated foraging ontogeny in honey bees. J. Exp. Biol. 208, 4641–4649.

Wilson, E.O., 1971. The Insect Societies. Harvard University Press, Cambridge, MA.

Wolf, T.H.J., Schmid-Hempel, P., Ellington, C.P., Stevenson, R.D., 1989. Physiological correlates of foraging efforts in honey-bees: oxygen consumption and nectar load. Funct. Ecol. 3, 417–424.

CHAPTER 2

A capillary feeder (CAFE) assay to measure food consumption and diet choice of individual honeybees[2]

Honey bees are a widely used model system in the con- text of fundamental questions about social behavior and evolution, and their critical role as pollinators makes them an important model system for understanding the effects of nutrition and pesticides. As nutrition is considered to play a significant role in both the evolution of social behavior (Ament, Wang, & Robinson, 2010; Mayack & Naug, 2013) and the health of honey bee populations (Alaux, Ducloz, Crauser, & Le Conte, 2010; Naug, 2009), studies regarding honey bee nutrition and feeding behavior have become increasingly important (Brodschneider & Crailsheim, 2010).

Studies on the nutritional regulation of social behavior have generally relied on nutritional manipulations at the colony level (Schulz, Huang, & Robinson, 1998; Seeley, 1996; Toth, Kantarovich, Meisel, & Robinson, 2005), or with small groups of caged bees (Altaye, Pirk, Crewe, & Nicolson, 2010). However, such studies cannot capture the details of individual feeding behavior or any inter-individual variation in nutritional requirements, details necessary for more sophisticated understanding of honey bee behavior and health. For example, acute oral toxicity or disease susceptibility is usually assessed using groups of caged bees and estimates are based on group level consumption of an inoculum or toxin (Chaimanee et al., 2013; Cresswell,

[2] Reade, A., Katz, K., & Naug, D. (2016). A capillary feeder (CAFE) assay to measure food consumption and diet choice of individual honey bees. *Journal of Apicultural Research*, *55*(4), 353-355. DOI: 10.1080/00218839.2016.1243293

Robert, Florance, & Smirnoff, 2014; Doublet, Labarussias, Miranda, Moritz, & Paxton, 2015), even though the effects of toxins and pathogens are obviously better assessed using assays on individual animals.

The Capillary Feeder (CAFE) assay, originally developed for the fruit fly, Drosophila (Ja et al., 2007), has been demonstrated to be a highly sensitive and robust method for making precise measurements of food intake at the individual level (Deshpande et al., 2014). It has been applied in a variety of contexts that include studies to understand nutrient balancing, taste discrimination, metabolism and addiction behavior (Lee et al., 2008; Masek & Scott, 2010; Shohat-Ophir, Kaun, Azanchi, Mohammed, & Heberlein, 2012; Xu, Zheng, & Sehgal, 2008). However, to the best of our knowledge, this informative assay has been limited in its use to Drosophila, probably due to the incompatibility of the standard CAFE assay for larger insects such as honey bees. The small diameter of the capillary tubes used in the standard CAFE assay does not provide easy access to the larger proboscis of a honey bee and also does not pro- vide the volume of food necessary to sustain a bee for a reasonably long period.

Here, we introduce a modified CAFE assay that can be used for accurate measurements of food consumption and diet choice in individual bees and can provide a more precise method for dosing and inoculation that will produce more robust data regarding the effects of nutrition, toxins, and pathogens in honey bees. Several modifications to the standard CAFE assay were required with the primary difference in our method being the use of longer capillary tubes with a larger diameter (152 mm long, 1.12 mm ID; World Precision Instruments, item number: TW150-6). This modification allows the provision of a larger volume of food that a bee can feed on for over 15 h without requiring replenishment. However, capillary tubes of larger diameters cannot hold a substantial volume of liquid by capillary action. We therefore added a U-shaped-bend to

the capillaries using a butane torch: two bends, each at a 90˚angle, were made at 18 and 24 mm from the feeding end of the tube. A third bend was added 18 mm above the bottom of the "U", at a 120˚angle in the opposite direction (Figure 2.1). This design prevented the capillaries from dripping while allowing the liquid to gravity feed toward the feeding end as the solution was consumed by the bee.

A CAFE chamber was constructed from a plastic vial (8.25 cm tall, 3 cm ID; Thorton Plastic Company, item number: 55–15). Ventilation holes (3 mm diameter) were drilled into the top and the sides of the chamber and vertical 1 cm slits were made on opposite sides of the chamber to allow for insertion of the capillary feeding tubes. The volume of the chamber was adjusted using a plastic disc inserted from the top to restrict the movement of the subject. Additional feeding tubes can be placed in a chamber to increase the volume of food or to introduce more feeding options.

In our experiment, two feeding tubes were each filled with 110 µl of sucrose solution, using a micropipette. Food coloring was added to the solution to enhance its visibility. A drop of mineral oil was added to the distal, non-feeding end of the capillary to reduce evaporation. The presence of air bubbles within the feeding tube inhibits the flow of the solution and can distort the meniscus and must be avoided to allow precise measurements. The initial level of the solution at the beginning of the experiment was marked on the outside of each capillary. Each feeding tube was inserted through the vertical slits on opposite sides of the CAFE chamber and held in place with non-toxic, non-drying modeling clay.

A set of CAFE chambers, each containing a single bee (Figure 2.2), was secured to a tray with adhesive putty. A control CAFE chamber, without a bee, was included in each set to account for any evaporative loss from the capillaries. The chambers were placed in an incubator set at 25℃ and 60% RH. A camera with a timer was used to automatically photograph the chambers every 15 min, an interval that can be adjusted according to the desired resolution. The photographs were analyzed using a freely available on-screen measuring tool (MB-Ruler 5.3, http://www.markus-bader.de/MB- Ruler/index.php). The amount of food consumed was calculated by measuring the distance between the initial level of the feeding solution, and the level of the meniscus in the capillary at each time interval. The distance was converted into volume using a reference capillary which was created by placing a known volume of solution into a capillary tube and marking the corresponding distance on the outside of the tube. The reference capillary was included with each set of CAFE chambers to define the conversion scale in each photograph.

We obtained precise feeding choice data for individual honey bees, including the hourly and cumulative consumption of two sucrose solutions of different concentrations (Figure 2.3). This modified CAFE assay allows precise measurement of the ingestion of liquid foods by individual honey bees and can be used to investigate the dietary requirements, food consumption and nutritional decisions of individual honey bees and other large insects.

FIGURES

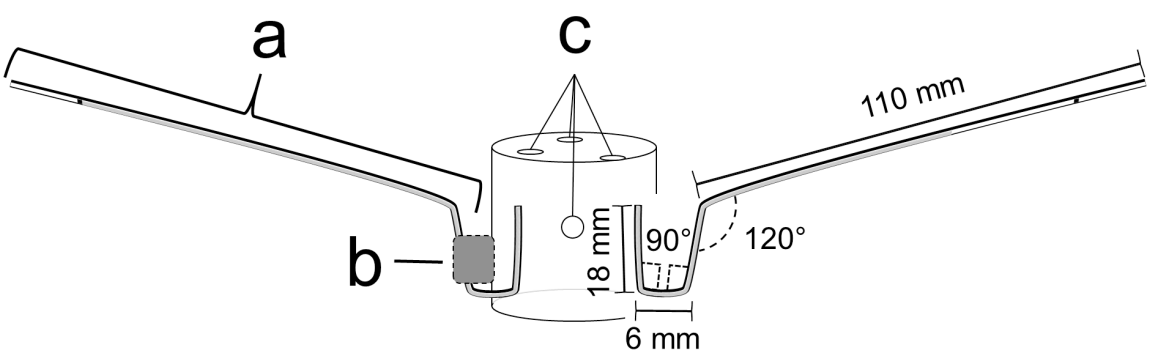

Figure 2.1: A diagram of the CAFE chamber consisting of (a) two capillary feeding tubes held in place by (b) modeling clay (excluded from right side of diagram to indicate feeding tube angles) and (c) ventilation holes.

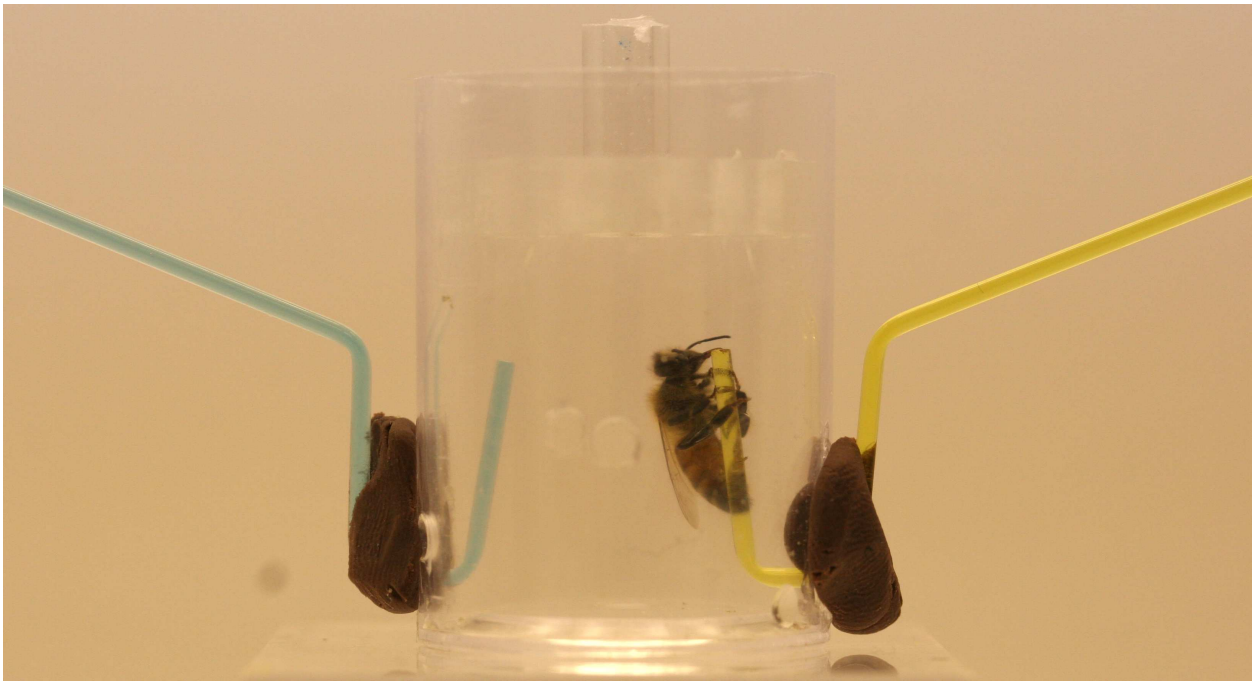

Figure 2.2: An individual honey bee feeding in the CAFE chamber. The bee is allowed restricted movement in the chamber and can choose between the available feeding solutions.

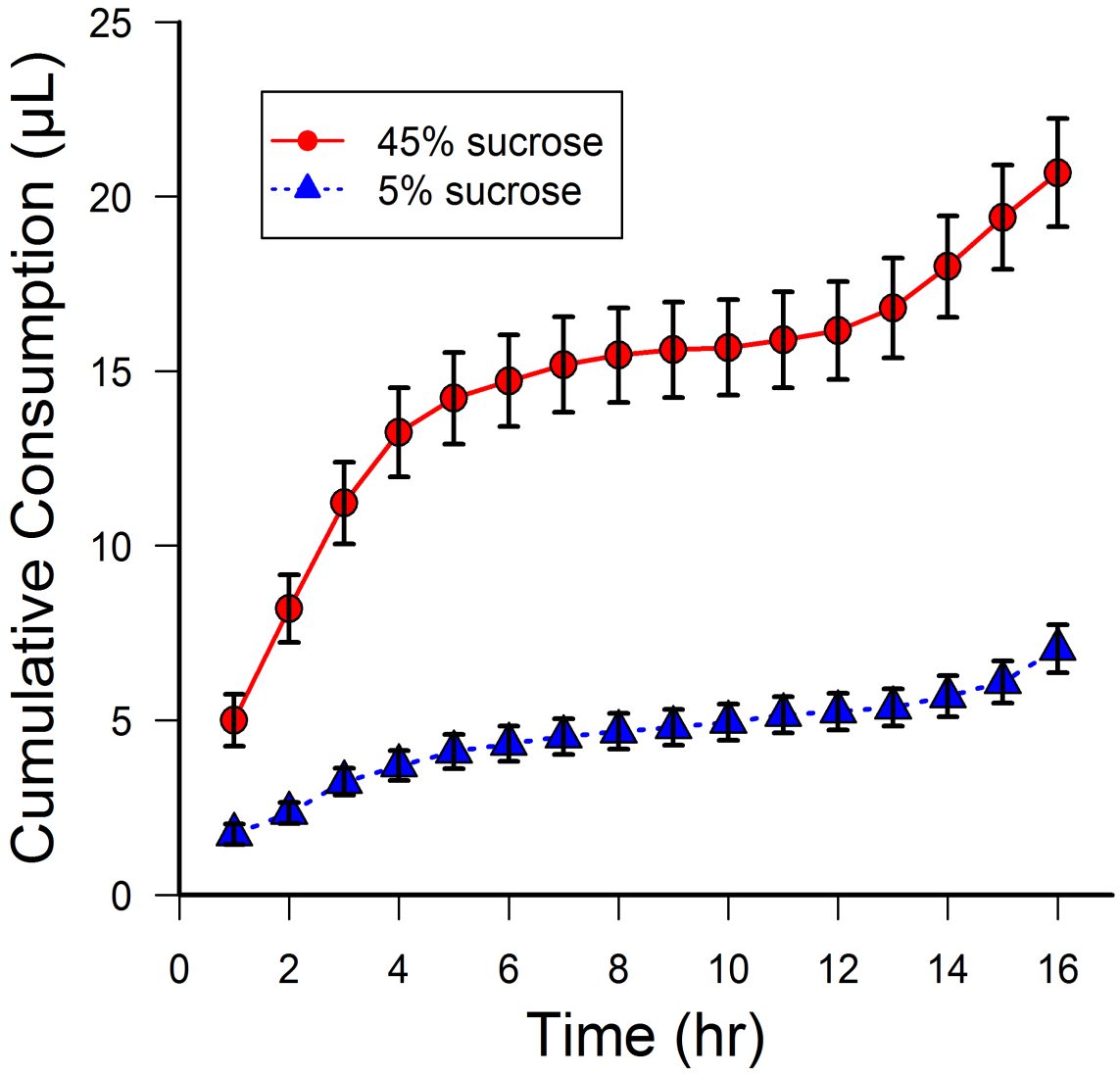

Figure 2.3: Cumulative consumption of 5% and 45% sucrose solutions by individual honey bees in a choice assay conducted with the modified CAFE assay for a period of 16 h. Data consist of means with standard error bars (N = 90) and show that the cumulative consumption of bees was significantly affected by time (Wald $\chi 2 = 355$, df = 1, $p < 0.00001$, repeated measures linear regression), the concentration of the solution (Wald $\chi 2 = 126$, df = 1, $p < 0.00001$) and their interaction (Wald $\chi 2 = 72$, df = 1, $p < 0.00001$), indicating that bees fed at a higher rate from the high concentration than the low concentration solutions.

REFERENCES

Alaux, C., Ducloz, F., Crauser, D., & Le Conte, Y. (2010). Diet effects on honey bee immunocompetence. Biology Letters, rsbl20090986. doi:10.1098/rsbl.2009.0986

Altaye, S. Z., Pirk, C. W., Crewe, R. M., & Nicolson, S. W. (2010). Convergence of carbohydrate-biased intake targets in caged worker honey bees fed different protein sources. Journal of Experimental Biology, 213, 3311–3318. doi:10.1242/jeb.046953

Ament, S. A., Wang, Y., & Robinson, G. E. (2010). Nutritional regulation of division of labor in honey bees: Toward a systems biology perspective. Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 2, 566–576. doi:10.1002/ws- bm.73

Brodschneider, R., & Crailsheim, K. (2010). Nutrition and health in honey bees. Apidologie, 41, 278–294. doi:10.1051/ apido/2010012

Chaimanee, V., Pettis, J. S., Chen, Y.-P., Evans, J. D., Khongphinitbunjong, K., & Chantawannakul, P. (2013). Susceptibility of four different honey bee species to Nosema ceranae. Veterinary parasitology, 193, 260–265. doi:10.1016/j.vetpar.2012.12.004

Cresswell, J. E., Robert, F. X. L., Florance, H., & Smirnoff, N. (2014). Clearance of ingested neonicotinoid pesticide (imi- dacloprid) in honey bees (Apis mellifera) and bumble bees (Bombus terrestris). Pest Management Science, 70, 332–337. doi:10.1002/ps.3569

Deshpande, S. A., Carvalho, G. B., Amador, A., Phillips, A. M., Hoxha, S., Lizotte, K. J., & William, W. J. (2014). Quantify- ing Drosophila food intake: Comparative analysis of current methodology. Nature Methods, 11, 535–540. doi:10.1038/ nmeth.2899

Doublet, V., Labarussias, M., Miranda, J. R., Moritz, R. F., & Paxton, R. J. (2015). Bees under stress: Sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. Environmental Microbiology, 17, 969–983. doi:10.1111/1462-2920.12426

Ja, W. W., Carvalho, G. B., Mak, E. M., Noelle, N., Fang, A. Y., Liong, J. C., ... Benzer, S. (2007). Prandiology of Drosophila and the CAFE assay. Proceedings of the National Academy of Sciences, 104, 8253–8256. doi:10.1073/pnas.0702726104

Lee, K. P., Simpson, S. J., Clissold, F. J., Brooks, R., Ballard, J. W. O., Taylor, P. W., ... Raubenheimer, D. (2008). Lifes- pan and reproduction in Drosophila: New insights from nutritional geometry. Proceedings of the National Academy of Sciences, 105, 2498–2503. doi:10.1073/pnas.0710787105

Masek, P., & Scott, K. (2010). Limited taste discrimination in Drosophila. Proceedings of the National Academy of Sciences, 107, 14833–14838. doi:10.1073/pnas.1009318107

Mayack, C., & Naug, D. (2013). Individual energetic state can prevail over social regulation of foraging in honey bees. Behavioral Ecology and Sociobiology, 67, 929–936. doi:10.1007/s00265-013-1517-6

Naug, D. (2009). Nutritional stress due to habitat loss may explain recent honey bee colony collapses. Biological Con- servation, 142, 2369–2372. doi:10.1016/j.bio-con.2009.04.007

Schulz, D. J., Huang, Z. Y., & Robinson, G. E. (1998). Effects of colony food shortage on behavioral development in honey bees. Behavioral Ecology and Sociobiology, 42, 295–303. doi:10.1007/s002650050442

Seeley, T. D. (1996). The wisdom of the hive: The social physiology of honey bee colonies. Cambridge, MA: Harvard University Press.

Shohat-Ophir, G., Kaun, K. R., Azanchi, R., Mohammed, H., & Heberlein, U. (2012). Sexual deprivation increases ethanol intake in Drosophila. Science, 335, 1351–1355. doi:10.1126/science.1215932

Toth, A. L., Kantarovich, S., Meisel, A. F., & Robinson, G. E. (2005). Nutritional status influences socially regulated for- aging ontogeny in honey bees. Journal of Experimental Biology, 208, 4641–4649. doi:10.1242/jeb.01956

Xu, K., Zheng, X., & Sehgal, A. (2008). Regulation of feeding and metabolism by neuronal and peripheral clocks in Drosophila. Cell metabolism, 8, 289–300. doi:10.1016/j.cmet.2008.09.006

CHAPTER 3

Does interindividual variation in metabolic rate and energetic requirement influence food sharing in the honeybee?

INTRODUCTION

A central benefit of group living is often considered to be an ability for higher resource acquisition (Krause and Ruxton 2002), but what is often overlooked is that both the costs and the benefits associated with the process may be unequally distributed among group members. This inequitable distribution of efforts and rewards is taken into account in the producer-scrounger framework, whereby certain individuals within a group – the producers – are more responsible for locating resources while others – the scroungers – take advantage of these discoveries (Barnard and Sibly, 1981). While scroungers decrease the overall performance and fitness of the group, their presence is an inevitable consequence of group living and the relative frequencies of the two phenotypes are maintained by negative frequency dependent selection.

Unlike groups in which behavior is driven by considerations of individual fitness, eusocial groups such as honeybees are assumed to be guided by colony level selection, whereby all group members work toward maximizing the reproductive output of the colony. In almost all analyses of work performance in these eusocial groups, the implicit underlying assumption is that all members disregard their own interests and contribute maximally and equally to colony performance. However, there is evidence that this may not necessarily be true and individuals with different physiological dispositions might differ in terms of the amount of work they contribute to the colony (Wolf et al., 1989; Feurbacher et al., 2003). While individuals are known

to qualitatively differ with regard to the task they perform, whether they differ quantitatively in terms of their work efforts as a result of physiological and energetic constraints remains mostly underappreciated.

Metabolic rate, the biological rate of energy processing, has been considered to be the fundamental driver of activity and performance at all levels of biological organization (Brown et al., 2004). It therefore follows that any interindividual variation in metabolic rate should translate to differences in energetic requirement and performance (Careau et al., 2008; Burton et al., 2011). It has been suggested that although a higher metabolic rate may allow a higher level of performance, the maintenance of a high metabolic rate is also energetically expensive (Biro and Stamps, 2010). It is therefore not entirely clear how such intraspecific differences in metabolic rate translate to differences in net performance, although it has been shown that individuals with high metabolic rates might have an advantage only in environments with high resource abundance (Burton et al., 2011; Auer et al., 2015a). In the context of a eusocial group such as the honeybee colony, it also means that individuals with higher metabolic rates may need a higher share of the food they bring back to the colony to meet their own energetic needs, thereby possibly contributing a lower fraction of their returns to the colony stores.

There is considerable interindividual variation in metabolic rate within a honeybee colony that is known to be correlated with the genotype (Harrison et al., 1996), behavioral phenotype (Harrison, 1986; Stabentheiner et al., 2003; Hrassnigg and Crailsheim, 2005), forager type (Feuerbacher et al., 2003) and activity level (Rothe and Nachtigall, 1989; Wolf et al., 1993), We have previously shown that there is considerable variation within a honeybee colony with respect to individual carbohydrate demand (Reade and Naug, 2016). Our earlier studies have also shown that the foraging rate of honeybee individuals is significantly influenced by their

individual energetic demands, independent of the colony energetic state (Mayack and Naug, 2013; Katz and Naug, 2015 and 2016). Based on these findings, in this study we test the hypotheses that interindividual differences in energetic demand are correlated to differences in metabolic rate and that these individual energetic demands pose a constraint in terms of the foraging return an individual honeybee forager can share with the colony.

METHODS

Experiment 1: *The influence of metabolic rate on individual carbohydrate demand*

We collected returning foragers from a colony of honeybees (*Apis mellifera*) at around 1 pm each afternoon for four days. The captured foragers were transported back to the lab in a flight cage, within 30 minutes of which each bee was chilled on ice until immobile and harnessed into a plastic straw. Bees were allowed to acclimate for 20 minutes and then tested for gustatory responsiveness by presenting each bee with an ascending series of sucrose concentrations (0.1%, 0.3%, 1%, 3%, 10%, 30%, 45%, and 60%) and using the sum of her responses to these concentrations as her gustatory responsiveness score (GRS). All bees were then fed to satiation with a 30% sucrose solution to equalize their energetic states and placed in an incubator (~25° C and 60% RH) for 18 hours.

After 18 hours, each bee was placed in a glass respirometry chamber (47 X 17 mm) within an insulated box (~25° C) where they were allowed to acclimate for 5 minutes before their carbon dioxide production (VCO_2, ml/hr) was measured for 10 minutes. Bees were oriented horizontally within the chamber, facing toward the incoming air and their movement was minimal due to the harness. VCO_2 was quantified using a Sable Systems flow-through gas analysis system (LI-COR LI-7000 CO_2 and H_2O analyzer). Room air was drawn through a 25-

liter carboy, scrubbed with two Drierite anhydrous $CaSO_4$ columns and then pushed through the chamber at a flow rate of 150 ml/minute. Baseline CO_2 data was collected immediately before and after each recording from an identical but empty chamber to correct for any CO_2 drift and lags.

Following the respirometry measurement, each bee was weighed (defined as its body weight), fed to satiation with a 30% sucrose solution and then weighed again to calculate the amount of sucrose she consumed. This amount was then divided by the number of hours the bee was starved (18 hours) and this was defined as its energetic demand (mg sucrose/hr).

Experiment 2: *The influence of individual energetic demand on food sharing*

a) Field Experiment: A three-frame observation hive with approximately 3500 bees was set up and foragers were trained to a feeder containing a 40% w/w sucrose solution located 50 meters away. Only a single bee was allowed to access the feeder at a time. Bees at the feeder were individually marked and the duration for which an individual collected sugar water was recorded for three trips. The end of a collection trip was communicated to an observer seated by the observation hive and the total time spent by a marked forager engaged in trophallaxis on her return was recorded.

All marked foragers were captured on their fourth visit to the feeder, transported to the lab, chilled on ice, harnessed into a straw, fed to satiation with a 30% sucrose solution to equalize their energetic states and placed in an incubator maintained at 25° C and 60% RH. After 16 hours each bee was again fed to satiation and placed into a feeding chamber equipped with two feeding capillaries (a CAFE assay, Reade et al., 2016) filled with sucrose solution and her sucrose consumption was measured for 12 hours to measure her carbohydrate demand.

b) *Lab Experiment:* Returning foragers were collected at the hive entrance from one of five colonies each day, chilled on ice and fed to satiation. Half of the bees were placed in a CAFE assay to determine their carbohydrate demand while the other half were marked with a small dot of paint for later identification. All bees were placed into an incubator set at 25° C and 60% RH for 16 hours. Each bee that participated in the CAFE assay was designated as a donor and paired with a receiver bee for a trophallaxis experiment, which consisted of weighing the recipient bee to the nearest 0.1 mg, feeding the donor bee 30 µl of a 30% sucrose solution and placing both the bees in a chamber (5cm x 5cm x 1.75cm) for 10 minutes, after which the recipient bee was re-weighed to calculate the amount of food she received from the donor.

Data Analysis

The 10-minute VCO_2 data for each bee was scanned to obtain a period of 2 minutes with the least variance and the mean value over this period was considered as the resting metabolic rate of the individual. In order to ensure the inclusion of only resting bees in the data, all bees with a variance beyond one standard deviation of the mean variance, across all bees, during the 2-minute observation were excluded from the analysis. The variation in metabolic rates was compared to a normal distribution using a Kolmogorov-Smirnov test. A linear model using metabolic rate, bee weight and gustatory responsiveness (GRS) was used to predict the energetic demand (mg sucrose/hr) of a bee.

In the food sharing experiment conducted in the field, the time that a marked forager spent engaged in trophallaxis was divided by the time she spent collecting sugar water at the feeder to calculate the proportion of food shared by her from each foraging trip. In the lab experiment, the difference between a recipient's pre and post interaction weight was used to

calculate the total volume of food transferred from the donor to the receiver. The energetic demand of each forager and donor in the two experiments was calculated from the hourly rate of sucrose consumption in the CAFE assays. Pearson's correlations were used to examine the relationship between individual energetic demand of a forager and the amount of food that she shared with her nestmates, using an arcsine transformation on the proportion data. All statistical analyses were performed using R (version 3.1.1).

RESULTS

Experiment 1

A total of 44 bees were included in the respirometry data, which showed an asymmetric variation in metabolic rate, with most bees exhibiting a relatively low metabolic rate (Kolmogorov-Smirnov test: $D = 0.4$, $p < 0.01$, Figure 3.1). The model that best explained energetic demand included main effects and interactions for gustatory responsiveness (GRS), metabolic rate (MR), and bee weight (Table 3.1). Both metabolic rate and GRS of an individual had a significantly positive influence on its energetic demand and there was a significant negative interaction between these two factors (Figure 3.2).

Experiment 2

In both the field and the lab experiments, the amount of food shared by an individual was not correlated with its own energetic demand (Pearson's correlation, Field: $t_{91} = 0.21$, $p = 0.83$, $r = - 0.02$; Lab: $t_{158} = 0.8$, $p = 0.42$, $r = 0.06$; Figure 3.3).

DISCUSSION

Our results demonstrate that the variation in metabolic rate among foragers in a honeybee colony translates to differences in individual energetic demands. The diet of honeybee foragers is largely composed of carbohydrates (Paoli et al., 2014) and they have been shown to have a respiratory quotient (RQ) of one (Rothe and Nachtigall, 1989), indicating that they rely almost exclusively on carbohydrates to meet their individual energetic needs. Honeybee foragers are known to either self-feed or be fed by other colony members with a supply of carbohydrate nectar before embarking on a foraging trip (von Frisch, 1967; Harano et al., 2013; Harano and Nakamura, 2016). While these studies show that the amount of feeding depends on several factors such as the distance to the food source, the type of load the forager is expecting to return with, etc., it is not known whether foragers with higher metabolic rates and therefore higher energetic demands need to be fed more to fuel their flights. If the latter is true, it would mean that foragers with higher metabolic rates have a higher maintenance cost that would require them to either draw a larger quantity of carbohydrates from colony food stores or share a lower fraction of the food they bring back to the colony.

However, contrary to our predictions, individual energetic demand did not translate to differences in the amount of food an individual shared with the colony. The fact that we find similar results from two different experiments, one under controlled laboratory conditions and another in a more natural field context, offers persuasive evidence that individuals with higher energetic demands are not imposing a higher maintenance cost on the colony. It is possible that our measurement of resting rather than flight metabolic rate underestimates the energetic demand of these foragers and the subsequent maintenance cost the colony may incur as a result. While one may assume that there is likely a positive correlation between these two metabolic rates,

studies show that the relationship is likely much more complex and the difference between these two rates, defined as aerobic scope, may be more indicative on a individual's feeding capacity (Auer et al., 2015b).

It is also possible that variation in metabolic rate and energetic demand influence some other aspect of how individuals may differ in terms of their contribution to the colony. Differences in metabolic rate have been proposed lead to distinctive personality types (Careau et al., 2008) for instance, individuals with a higher metabolic rate are often bolder, more risk prone, and more active in general (Mathot et al., 2015). In a honeybee colony, such differences could lead to differences in the exploration-exploitation tradeoff displayed by an individual, which in fact has been shown to be a function of her own energetic demand (Katz and Naug, 2015 and 2016). There was a small percentage of bees (~10%) in the field sharing study that, rather than sharing food with nestmates, shared information about the food source by dancing. Individuals with higher metabolic rates may be better able to travel farther or faster and thereby allow a colony to more effectively respond to periods of resource abundance by maximizing information and resource collection, while those with lower metabolic rates may allow the colony to reduce its overall maintenance costs during times of resource scarcity.

Behavioral diversity and individual specialization have been considered as an asset to any group of animals (Bolnick et al., 2002), and this may be especially important in eusocial insect colonies (Jeanson and Weidenmüller, 2014). Several studies have shown the positive contributions of such behavioral diversity in honeybee colonies (Page et al., 1995; Jones et al., 2004; Matilla and Seeley, 2007). However, whether metabolic diversity can benefit a group of animals in a similar capacity is an idea that needs to be tested, given that metabolic rate and energy processing are considered the fundamental drivers of life history traits that set the pace of

life (Reale et al., 2010). Studies have shown that individuals with different metabolic rates are suited to different environmental conditions and flexibility in metabolic capacity can provide advantages under changing conditions (Auer et al., 2015b). Even though individuals with different metabolic rates may incur different costs on a eusocial group such as the honeybee colony, maintaining such diversity may allow the colony to display a distributed metabolic capacity which imparts additional flexibility to respond to a variety of environmental challenges.

TABLES

Table 3.1: Parameter estimates from the regression model predicting the effects of metabolic rate, bee weight, and gustatory responsiveness score on the energetic demand of individual honeybee foragers.

	Estimate	*t*	P(>|*t*|)
Metabolic Rate (MR)	4.066	1.876	0.037 *
GRS	0.625	0.223	0.008 **
Bee Weight	9.193	7.850	0.249
MR × GRS	-1.095	0.414	0.012 *
MR × Bee Weight	-38.793	18.019	0.038 *
GRS × Bee Weight	-5.485	2.157	0.015 *
MR × GRS × Bee Weight	10.332	4.033	0.015 *

FIGURES

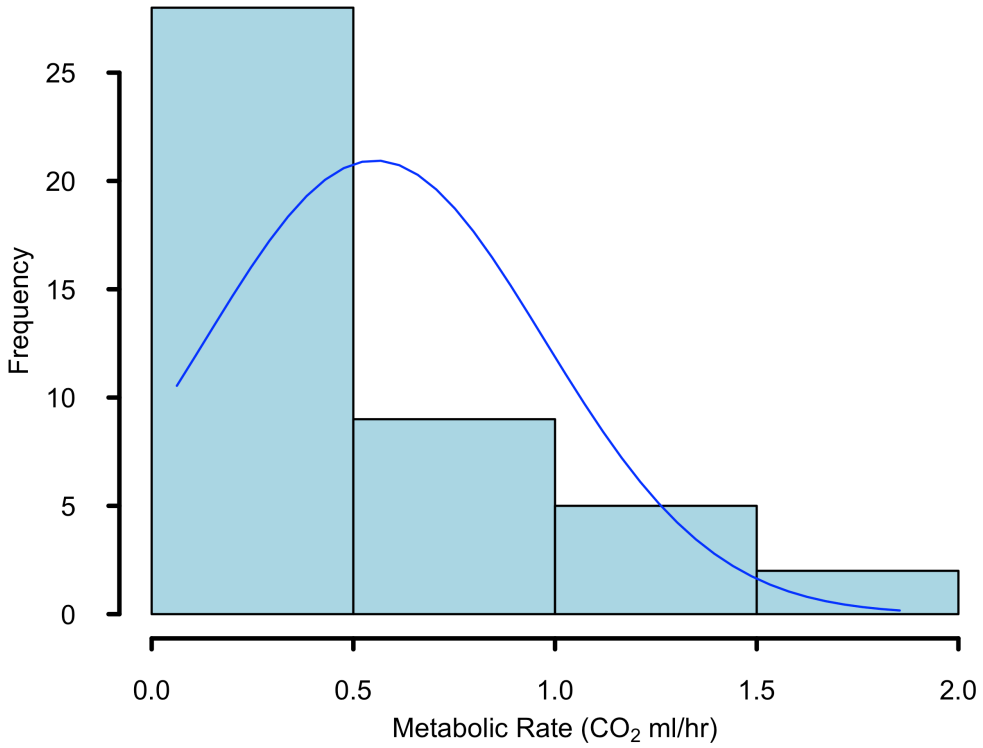

Figure 3.1: Inter-individual variation in resting metabolic rates with the solid line showing the fitted distribution.

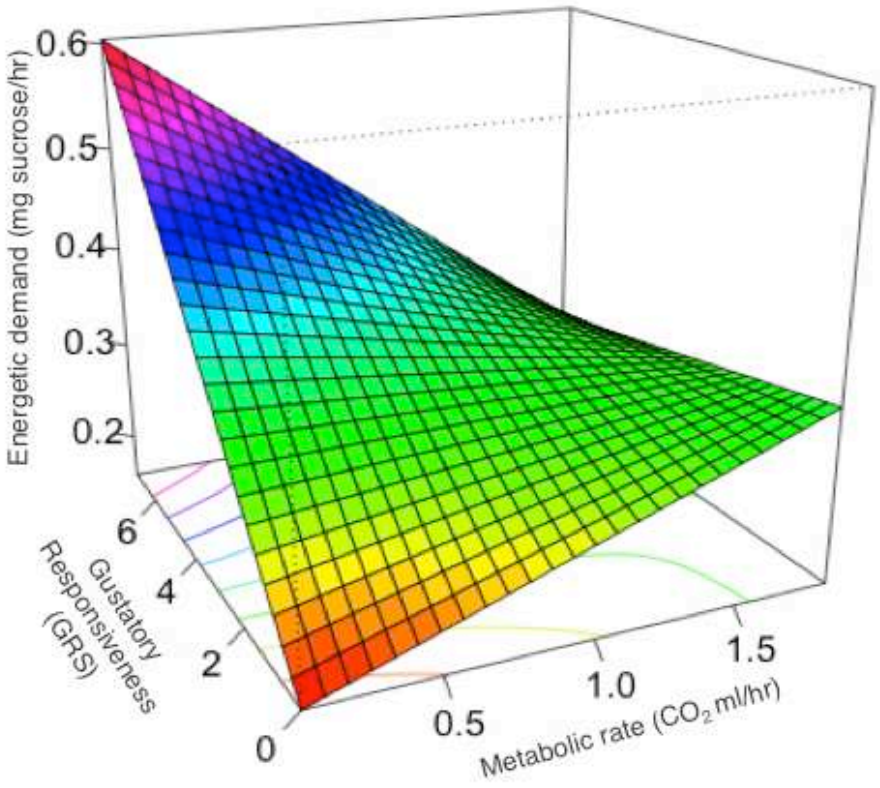

Figure 3.2: Energetic demand of an individual as predicted by metabolic rate and GRS, holding bee weight constant at the mean bee weight (0.11g).

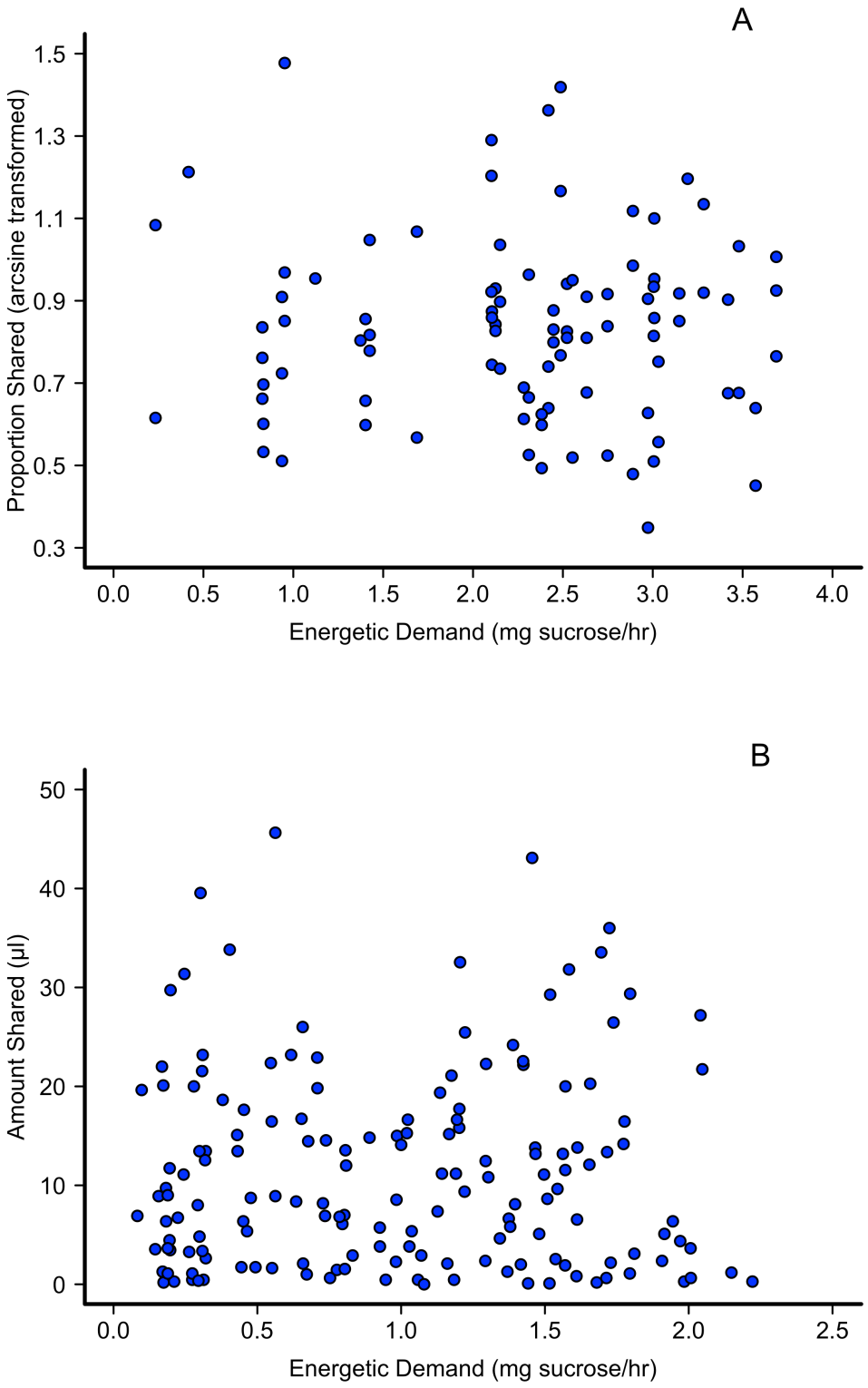

Figure 3.3: Magnitude of food sharing by an individual honeybee as a function of her individual energetic demand in the A) field experiment, and B) lab experiment.

REFERENCES

Auer, S. K., Salin, K., Rudolf, A. M., Anderson, G. J., & Metcalfe, N. B. (2015a). The optimal combination of standard metabolic rate and aerobic scope for somatic growth depends on food availability. *Functional Ecology*, *29*(4), 479-486.

Auer, S. K., Salin, K., Anderson, G. J., & Metcalfe, N. B. (2015b). Aerobic scope explains individual variation in feeding capacity. *Biology letters*, *11*(11), 20150793.

Barnard, C. J., & Sibly, R. M. (1981). Producers and scroungers: a general model and its application to captive flocks of house sparrows. *Animal behaviour*, *29*(2), 543-550.

Biro, P. A., & Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior?. *Trends in Ecology & Evolution*, *25*(11), 653-659.

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2002). The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist*, *161*(1), 1-28.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*(7), 1771-1789.

Burton, T., Killen, S. S., Armstrong, J. D., & Metcalfe, N. B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences?. *Proceedings of the Royal Society of London B: Biological Sciences*, *278*(1724), 3465-3473.

Careau, V., Thomas, D., Humphries, M. M., & Réale, D. (2008). Energy metabolism and animal personality. *Oikos*, *117*(5), 641-653.

Feuerbacher, E., Fewell, J. H., Roberts, S. P., Smith, E. F., & Harrison, J. F. (2003). Effects of load type (pollen or nectar) and load mass on hovering metabolic rate and mechanical power output in the honey bee Apis mellifera. *Journal of Experimental Biology*, *206*(11), 1855-1865.

Harano, K. I., Mitsuhata-Asai, A., Konishi, T., Suzuki, T., & Sasaki, M. (2013). Honeybee foragers adjust crop contents before leaving the hive. *Behavioral Ecology and Sociobiology*, *67*(7), 1169-1178.

Harano, K. I., & Nakamura, J. (2016). Nectar loads as fuel for collecting nectar and pollen in honeybees: adjustment by sugar concentration. *Journal of Comparative Physiology A*, *202*(6), 435-443.

Harrison, J. M. (1986). Caste-specific changes in honeybee flight capacity. *Physiological zoology*, *59*(2), 175-187.

Harrison, J. F., Nielsen, D. I., & Page Jr, R. E. (1996). Malate dehydrogenase phenotype, temperature and colony effects on flight metabolic rate in the honey-bee, Apis mellifera. *Functional Ecology*, 81-88.

Hrassnigg, N., & Crailsheim, K. (2005). Differences in drone and worker physiology in honeybees (Apis mellifera). *Apidologie*, *36*(2), 255-277.

Jeanson, R., & Weidenmüller, A. (2014). Interindividual variability in social insects–proximate causes and ultimate consequences. *Biological Reviews*, *89*(3), 671-687.

Jones, J. C., Myerscough, M. R., Graham, S., & Oldroyd, B. P. (2004). Honey bee nest thermoregulation: diversity promotes stability. *Science*, *305*(5682), 402-404.

Katz, K., & Naug, D. (2015). Energetic state regulates the exploration–exploitation trade-off in honeybees. *Behavioral Ecology*, *26*(4), 1045-1050.

Katz, K., & Naug, D. (2016). Dancers and followers in a honeybee colony differently prioritize individual and colony nutritional needs. *Animal Behaviour*, *119*, 69-74.

Krause, J, & Ruxton, G (2002) Living in groups. Oxford University Press.

Mathot, K. J., Nicolaus, M., Araya-Ajoy, Y. G., Dingemanse, N. J., & Kempenaers, B. (2015). Does metabolic rate predict risk-taking behaviour? A field experiment in a wild passerine bird. *Functional Ecology*, *29*(2), 239-249.

Mattila, H. R., & Seeley, T. D. (2007). Genetic diversity in honey bee colonies enhances productivity and fitness. *Science*, *317*(5836), 362-364.

Mayack, C., & Naug, D. (2013). Individual energetic state can prevail over social regulation of foraging in honeybees. *Behavioral Ecology and Sociobiology*, *67*(6), 929-936.

Page, R. E., Robinson, G. E., Fondrk, M. K., & Nasr, M. E. (1995). Effects of worker genotypic diversity on honey bee colony development and behavior (Apis mellifera L.). *Behavioral Ecology and Sociobiology*, *36*(6), 387-396.

Paoli, P. P., Donley, D., Stabler, D., Saseendranath, A., Nicolson, S. W., Simpson, S. J., & Wright, G. A. (2014). Nutritional balance of essential amino acids and carbohydrates of the adult worker honeybee depends on age. *Amino Acids*, *46*(6), 1449-1458.

Reade, A., Katz, K., & Naug, D. (2016). A capillary feeder (CAFE) assay to measure food consumption and diet choice of individual honey bees. *Journal of Apicultural Research*, *55*(4), 353-355.

Reade, A. J., & Naug, D. (2016). Inter-individual variation in nutrient balancing in the honeybee (Apis mellifera). *Journal of Insect Physiology*, *95*, 17-22.

Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., & Montiglio, P. O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *365*(1560), 4051-4063.

Rothe, U., & Nachtigall, W. (1989). Flight of the honey bee. *Journal of Comparative Physiology B*, *158*(6), 739-749.

Stabentheiner, A., Vollmann, J., Kovac, H., & Crailsheim, K. (2003). Oxygen consumption and body temperature of active and resting honeybees. *Journal of Insect Physiology*, *49*(9), 881-889.

Von Frisch, K. (1967). The dance language and orientation of bees.

Wolf, T. J., Schmid-Hempel, P., Ellington, C. P., & Stevenson, R. D. (1989). Physiological correlates of foraging efforts in honey-bees: oxygen consumption and nectar load. *Functional Ecology*, 417-424.