The perils of forcing a generalist to be a specialist: lack of dietary essential amino acids impacts honey bee pollen foraging and colony growth

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Commercial honey bee colonies pollinate large monocultures, which contain one type of food and thus, one unbalanced source of nutrients. We examined how a lack of dietary essential amino acids (EAAs) affects honey bee foraging behavior and colony growth. Using pollen traps and semi-synthetic diets, we raised bees on three treatments in the field: no diet manipulation, a diet with all 10 honey bee EAAs, or a diet with only 6 EAAs. In 2016, during a drought, bees raised on the diet lacking EAAs collected more pollen than bees raised with all EAAs, suggesting compensatory foraging. This was not found in 2017, when natural resources were likely more abundant. As such, honey bees lacking EAAs worked harder to fill the gap in the nutrient poor environment. In 2017, colonies raised on all 10 EAAs expanded slower than control colonies, and colonies raised on 6/10 EAAs did not expand at all. This suggests that EAA diversity, and likely other nutrients found in pollen, are essential for colony growth. Forcing this generalist pollinator to be a specialist drastically reduces colony growth and likely, honey bee fitness.

Keywords: Apis mellifera; macronutrients; nutritional ecology; pollen supplement; pollinator health; protein

Introduction

Animal-driven pollination is required for the production of one-third of globally important crops (Klein et al., 2007). Insect pollinators play a large role in pollination services (Rader et al., 2016), with the main players being bees: both managed honey bees (Apis mellifera L.) and wild native bees are economically important pollinators (Klatt et al., 2014; Winfree, Gross, & Kremen, 2011). Alarmingly, insect pollinator populations are declining across the globe (Potts, Biesmeijer, et al., 2010). In recent decades, 3 of the 25 bumble bee species in the United Kingdom have gone extinct, and 8 have experienced range declines (reviewed in Goulson, Lye, & Darvill, 2008). The number of managed honey bee colonies declined 59% between 1947 and 2005 in the United States (van Engelsdorp, Hayes, Underwood, & Pettis, 2010). While many of the losses in the United States in the early 2000s were attributed to colony collapse disorder (CCD), CCD has not been reported since 2012 (Millius, 2017; Seitz et al., 2016). Even without the threat of CCD, beekeepers continue to experience higher than acceptable annual losses of managed honey bee colonies (Seitz et al., 2016). Even without the threat of CCD, beekeepers continue to experience higher than acceptable annual losses of managed honey bee colonies (Seitz et al., 2016).

While such losses are likely due to synergistic effects of pesticides, pathogens, parasites, habitat loss, and others (Potts, Biesmeijer, et al., 2010), nutrition could be the key to improving pollinator health and bolstering pollinator populations. Bees obtain most of the nutrients they need from pollen (protein, lipids, vitamins and minerals) and nectar (carbohydrates) (Haydak, 1970). Specifically, honey bees need to acquire 10 essential amino acids (EAAs) from pollen for proper larval development: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (DeGroot, 1953). In honey bees, protein is important for the development of hypopharyngeal glands, ovaries, fat bodies (Herbert & Hill, 2015), and immune cells (Szymas & Jedruszuk, 2003). More specifically, dietary protein diversity has been linked to both individual and social immune responses; honey bees fed a more diverse protein diet exhibited increased phenoloxidase and glucose oxidase activity (Alaux, Ducloz, Crauser, & Le Conte, 2010). To our knowledge, there is no one plant that provides all 10 honey bee EAAs in the required amounts (Somerville, 2001). As such, monocultures (such as almond and sunflower) may be problematic for bee health (Table 1), both at the individual and the colony level.

Currently in the United States, managed honey bees used for commercial pollination are tasked with pollinating large monocultures, which only have one type of food: the crop of interest. With the use of herbicides, there are often not even weeds (e.g., dandelion and clover) present to supplement pollinator diet. Since honey bee workers take only about three weeks to develop (Winston, 1987), and pollen is not stored in the colony in large amounts (Rosov, 1944) and is collected on an “as needed” basis (Robinson & Oertel, 1975), a colony
pollinating a large monoculture raises bees on only one type of food. This one food item likely does not have all the required nutrients (Nicolson & Human, 2013; Standifer, Mccaughey, Dixon, Gilliam, & Loper, 1980). Furthermore, even though honey bees can forage up to a 10 km radius from the colony (Beekman & Ratnieks, 2000; Steffan-Dewenter & Kuhn, 2003; Visscher & Seeley, 1982), monocultures can be much larger than that. Thus, honey bee colonies located in the middle of the monoculture are forced to feed on only one type of food for the duration of pollination services, and likely the duration of larval development.

Over two field seasons, we tested how a diet lacking in EAAs affected honey bee foraging behavior and colony growth. Hendriksma and Shafir (2016) showed that when given the choice in a closed experiment, foragers from a colony lacking EAAs seek complementary EAAs. Similarly, we predicted that bees raised on a diet lacking EAAs in the field will compensate by increasing their pollen foraging effort. Regarding colony growth, in pollen shortages, colonies tend to terminate brood rearing (Imdorf, Rickli, Kilchenmann, Bogdanov, & Wille, 1998). Thus, we predicted that colonies raised on all 10 EAAs will expand at a similar rate as control colonies while colonies raised lacking EAAs will expand at a slower rate.

Materials and methods
Study system and field site
In June 2016, nine 5-frame nucleus colonies were installed in 10-frame Langstroth hives. Each Langstroth hive had one standard sized box and was equipped with a Sundance pollen trap (New England Beekeeping, Tyngsboro, MA, USA) (Todd & Bishop, 1940). All hives were located at the northern edge of an unmanaged wildflower field (site A) at the Cummings School of Veterinary Medicine at Tufts University in Grafton, MA, USA (Online Supplementary Data Figure S1). In May 2017, nine new 5-frame nucleus colonies were installed in nine new 10-frame Langstroth hives at the same edge of the same wildflower field (site A), and six additional 5-frame nucleus colonies were installed in 10-frame Langstroth hives at the northern edge of a second unmanaged wildflower field on the campus (site B) (Online Supplementary Data Figure S1). Both sites were surrounded by forested habitat. Upon installation, all colonies were queen-right, and had three frames of mixed brood and two frames of food.

In 2016, all nine colonies were randomly divided into three treatment groups (N=3 colonies/treatment): (1) control, which had the pollen traps at the off position for the duration of the experiment, (2) a semi-synthetic diet with all 10/10 honey bee EAAs, representing a polyfloral diet (DeGroot, 1953), and (3) a semi-synthetic diet treatment with only 6/10 EAAs, representing the EAAs honey bees would receive from a monofloral dandelion diet (Auclair & Jamieson, 1948; Loper & Cohen, 1987) (Table 1). The semi-synthetic “dandelion” diet is ecologically relevant as dandelions are one of the few floral resources available to bees in early spring in the North-eastern United States (Loper & Cohen, 1987). To control for hive location and allow for year-to-year comparison, the order of diet treatments at site A was kept the same in 2017 as 2016. In 2017, however, we installed two more colonies of each diet treatment (N=5 total colonies/treatment) at site B. Since there were two feral colonies near site B, the treatments were assigned so that the control and 10/10 colonies, which were expected to be the strongest, were closest to the feral colonies to prevent robbing.

Experimental diets and feeding
All amino acids used in this experiment were purchased in powdered form from either Sigma Aldrich (Natick, MA, USA) or US Biological (Salem, MA, USA)
For each diet, the appropriate EAAs were added to a powdered cellulose (Sigma Aldrich, Natick, MA, USA) base in the proportion that honey bees need to get from their food as determined by DeGroot (1953) (Online Supplementary Data Table S1). Both diets had equal amounts of added vitamins and minerals (Vitamin B Healthy, Brushy Mountain Bee Farm, New Columbia, PA, USA), and plant-derived cholesterol (Sigma Aldrich, Natick, MA, USA) to mimic natural pollen diets as closely as possible. Each powdered diet was prepared in a 5-gallon (approximately 19 L) bucket. Diets were homogenized by rolling each bucket back and forth for 3 min and then inverting the bucket for 1 min. We then divided 90 g of each diet, which was enough to fill one side of a standard-sized drawn Langstroth frame, into smaller containers for transport to the field.

In the field, the portioned-out diet was sprinkled into one side of an empty, sterilized (via freezing followed by a sodium bicarbonate soak; National Bee Unit, 2018), drawn frame (Herbert & Hill, 2015) using a sieve. The prepared frame was then lightly sprayed with a 50% honey solution to attract the bees to their new food source and keep the diet in the frame upon installation. This method of feeding the bees mimics the honey bee's natural method for storing and using pollen in the hive (Robinson & Oertel, 1975) (Figure 1a and b). A sterilized drawn frame (see above) with no diet treatment, and only the honey solution sprayed on it, was installed in each control colony. Diet frames were checked on a weekly basis and if needed, colonies were fed again. Since bees do store pollen (Figure 1b), bees were fed our diet treatments only after the pollen traps were switched "on" for 1 week. In that week, bees acclimated to their modified entrance (Beekeeping, 2019), and were unable to bring in new pollen and thus, consumed all their stored pollen (Hendriksma & Shafir, 2016). We verified the lack of stored pollen through regular beekeeping checks before and during feeding. Following feeding, the pollen traps on the experimental colonies remained on for the duration of the experiment and data collection, while the pollen traps on the control colonies were switched to the "off" position. Based on our colony growth data (see Results section), we are confident that our pollen traps excluded enough natural pollen so that our semi-synthetic diets were the primary source of protein brood were raised on.

**Pollen foraging success**

Starting one week after the diet and control frames were installed, we estimated weekly pollen foraging success by collecting two data points from each colony. First, since the experimental colonies had pollen traps, corbicular pollen was collected in a drawer that could be accessed from the back of the hive (Figure 1c and d). We emptied the contents of each drawer into a 1-gallon (approximately 3.75 L) Ziploc bag and took the pollen back to the lab to weigh. Since control colonies had the pollen traps "off," corbicular pollen was only collected from experimental (6/10 EAAs, 10/10 EAAs) colonies. Corbicular pollen was collected for 4 weeks in 2016 and 8 weeks in 2017; both collection periods are long enough for the complete development of worker brood on our semi-synthetic diets (Winston, 1987).

To estimate behavioral pollen foraging effort of each colony, two observers sat to the side of the hive and counted the number of bees exiting for 10 min (Figure 1e) (Delaplane, van der Steen, & Novoa-Guzman, 2013). Then, at the same hive, the observers counted the number of bees returning with visible corbicular pollen (Figure 1f) for an additional 10 min (Delaplane et al., 2000).
The proportion of successful pollen foraging trips was calculated as the average number of bees returning with corbicular pollen divided by the average number of bees exiting the hive during the 10-min observation periods. Data were collected once per week, weather permitting, during active foraging hours (0900–1400 h) (Abou-Shaara, 2014) for 4 weeks. To account for natural variation between hives, the order in which data were collected was randomized each week (Delaplane et al., 2013). In 2017, foraging behavior data was only collected from the nine colonies that were installed in the same site as 2016 (Online Supplementary Data Figure S1). Corbicular pollen weight was collected from all experimental colonies.

Colony growth
In 2017, during weekly beekeeping checks, we counted the number of frames covered by adult bees (Figure 1g) (Delaplane et al., 2013). Some colonies expanded so quickly that we had to add a second standard box on top of the first box during the experiment. While we did keep general colony health data in 2016 (brood pattern, signs of disease, etc.), we do not have quantitative data for the number of frames covered in bees. Data were collected over 15 weeks.

Statistical analysis
To analyze the effect of treatment on weight of pollen collected in the pollen trap, and the proportion of bees returning with corbicular pollen, we ran Gaussian (normal) linear mixed models (LMMs) with treatment as a fixed factor, and colony and date as crossed random factors. The random factors accounted for our repeated-measures design and natural variation between colonies. To determine significance, we used marginal hypothesis tests, implemented with the Anova() function. Analysis for each response variable and each year were run separately for a total of four models.

To analyze the effect of treatment on the number of frames of bees over time (colony growth), we ran a saturated Gaussian LMM with independent and interaction effects of treatment and date, and colony added as a random factor. To determine significance, we used marginal hypothesis tests, implemented with the Anova() function. To examine colony growth (number of frames covered in adult bees) over time, we paid attention to the interaction effect of treatment and date and examined the coefficients (intercept and slope) and 95% confidence intervals of the model.

Data were analyzed using car, MASS, and lme4 in R version 3.3.2 (31 October 2016) (R Development Core Team, 2008).

Results

Pollen foraging success
Bees brought more pollen back to the colony in 2017 (269.50 ± 37.65 g) than in 2016 (111.49 ± 21.48 g) (Figure 2a and b). In 2016, colonies raised on 6/10 EAAs collected near-significantly more pollen than bees raised on all 10 EAAs (LMM $X^2 = 3.54$, $df = 1$, $P = 0.059$). Mean proportion of honey bees returning with corbicular pollen in (c) 2016 and (d) 2017; there was no significant effect of treatment. A larger proportion of foragers returned with corbicular pollen in 2017 than in 2016 and overall, experimental colonies tended to forage for pollen more than control colonies. Differences in sample sizes were due to inclement weather.

Figure 2. The weight of pollen (g) collected by honey bees from each experimental treatment in (a) 2016 (b) and 2017. Overall, bees in 2017 collected more pollen than bees in 2016. In 2017 there was no effect of treatment however, in 2016, bees raised on 6/10 EAAs collected near-significantly more pollen than bees raised on all 10 EAAs (LMM $X^2 = 3.54$, $df = 1$, $P = 0.059$). Mean proportion of honey bees returning with corbicular pollen in (c) 2016 and (d) 2017; there was no significant effect of treatment. A larger proportion of foragers returned with corbicular pollen in 2017 than in 2016 and overall, experimental colonies tended to forage for pollen more than control colonies. Differences in sample sizes were due to inclement weather.
Figure 3. The mean number of frames covered in honey bees (see Figure 1e) ± 1 SE over time. There was a significant effect of treatment on colony growth (LMM $X^2 = 6.96; df = 2; P = 0.031$) and a significant interaction of treatment and date (LMM $X^2 = 25.26; df = 12; P = 0.014$). Control colonies (slope = 0.548) expand about three times faster than colonies raised on 10/10 EAAs (slope = 0.192). Colonies raised on 6/10 EAAs did not expand at all (slope = −0.009). Each point represents $N = 5$ colonies/treatment except in week 3 where $N = 1$ colony/treatment. Smaller sample size and gaps in data collection were due to inclement weather.

Colony growth

There was a significant effect of treatment on the number of frames covered in bees over time (i.e., colony growth) (interaction effect LMM $X^2 = 25.26; df = 12; P = 0.014$). Colonies in all three treatments started with about 3 frames of brood (Figure 3). By mid-July, the control colonies had expanded to a mean of 8.4 ± 2.9 frames of bees while colonies raised on 10/10 EAAs only had 4.3 ± 1.9 frames and colonies raised on 6/10 EAAs had 4.2 ± 1.0 frames (Figure 3). The colonies raised on 10/10 EAAs started to expand in August, however, they were already too far behind the control colonies to catch up. Colonies raised on 10/10 EAAs grew at about one-third the rate of control colonies (slopes = 0.192 and 0.548, respectively) while colonies raised on only 6/10 EAAs did not grow at all (slope = −0.009).

Discussion

Overall, pollen foraging success was likely affected by diet treatment in 2016 but not in 2017, and colonies were unable to grow when raised on a diet lacking EAAs.

Pollen foraging success

By weight, bees brought less pollen back to the colony in 2016 than in 2017 (Figure 2a and b). Accordingly, a smaller proportion of bees returned to the colony with visible corbicular pollen in 2016 than in 2017 (Figure 2c and d). This most likely due to the drought conditions experienced in 2016 (Figure 4). During pollen foraging observations in 2016, 100% of the county where our apiary was located experienced abnormally dry to severe drought conditions. In contrast, during 2017 observations, there was no drought across the entire county. During the drought, pollen foragers may have left the colony to find pollen only to return unsuccessful, without corbicular pollen. Indeed, drought was recently shown to reduce floral abundance and thus, resources for pollinators (Phillips et al., 2018). Additionally, during the drought year, there may have been more foragers allocated to water collection (Abou-Shaara, 2012) than food collection. These results are consistent with Bordier et al. (2017); when exposed to simulated heat waves, the colony’s proportion of water foragers doubled.

Regarding pollen weight, as food scarcity likely increased with drought (Phillips et al., 2018), so did the difference between the two diet treatments (Figure 2). In 2016, bees raised on 6/10 EAAs collected near-significantly more pollen than bees raised on 10/10 EAAs (Figure 2a). This suggests that bees raised on a diet lacking EAAs attempt to compensate by collecting more pollen, which is the bee’s main source of protein (DeGroot, 1953). These results are consistent with past studies done by Pernal and Currie (2001): when the overall pollen quantity in the colony is decreased, honey bees bring heavier pollen loads back to the colony. Beyond simple compensatory feeding, honey bees raised on 6/10 EAAs could also be attempting complimentary feeding in which they supplement their semi-synthetic diet with pollen sources that contain the specific EAAs they are lacking (Hendriksma & Shafir, 2016). While answering this question is beyond the scope of this study, investigating the EAA contents of the bee-collected pollen would be an interesting future direction.

In 2017, there is a general trend for both experimental treatments to have a higher proportion of foragers returning to the colony than the control colonies. This trend may be a result of something other than EAAs lacking in our semi-synthetic diets. Other than EAAs (DeGroot, 1953), pollen is a source of lipids (Roulston & Cane, 2000), vitamins, minerals (Brodischneider & Crailsheim, 2010), and microbes (Anderson et al., 2014;
Maxfield-Taylor, Mujic, & Rao, 2015). While we did add plant-derived cholesterol and Vitamin-B-Healthy to our semi-synthetic diets, we may not have added them in large enough quantities. Since foraging effort for all three treatments was higher in 2017 than in 2016, control colonies and experimental colonies were likely similarly affected by drought conditions (Figure 2).

Alternatively, our findings on the proportion of pollen foragers may be explained by the regulation of pollen foraging in honey bees (Camazine, 1993; Dreller, Page, & Fondrk, 1999). Honey bees regulate pollen foraging based on quantity of stored pollen: when there is enough pollen stored in the hive, pollen foraging is reduced (Camazine, 1993). We did not directly manipulate quantity of our semi-synthetic diets. Accordingly, we did not find a difference in pollen foraging rates between experimental diet treatments. Pollen foraging rates also directly correlate with the amount of young brood (Dreller et al., 1999) but our data suggest that pollen quantity may be a stronger regulation factor. We did not see a difference in pollen foraging rate between control and experimental colonies even though, on average, control colonies expanded faster than experimental colonies, and likely had a greater quantity of young brood.

**Colony growth**

Over the 15 weeks, colonies raised on only 6/10 EAAs did not expand at all (Figure 3). Colonies raised on all 10/10 EAAs expanded beyond the original 5 frames of bees, but they did so at a slower rate than control colonies. We are confident that this difference is not due to drifting.
between experimental hives with pollen traps and control hives with a more “accessible” entrance. Honey bees tend to only take three days to acclimate to their modified entrance (Beekeeping, 2019) and with our randomized design, experimental hives were not always within drifting distance of control hives. Differences in sample sizes were due to inclement weather.

Variation in the mean number of frames covered by bees tended to be larger in the control colonies than the experimental colonies suggesting that experimental colonies were constricted by the semi-synthetic diet. This constriction and the inability for the 6/10 EAA colonies to expand could be for various reasons: (1) the bees may be attempting to raise a new queen. Honey bees will raise a new queen for a number of reasons, including an unsatisfactory laying rate (Fefferman & Starks, 2006). In the case of the colonies raised on 6/10 EAAs, honey bees might attribute the lack of expansion to an inferior queen and attempt replacement. This would lead to allocation of resources in raising a new queen rather than to raising brood, thus preventing expansion. (2) The workers could be partaking in cannibalism of larvae as a method of compensatory feeding (Schmickl & Crailsheim, 2001). (3) There may have been high rate of larval death due to lack of EAAs. (4) General availability of food may have been affected by our experimental diets. Based on weekly beekeeping checks, the bees did eat our experimental diets and never ran out before we refilled the diet, however, bees in experimental colonies may not have eaten as much as those allowed to forage freely. Since there was no effect of treatment on the number of queen cells built (Figure 5), we can rule out the first explanation, but the others remain to be tested.

Taken together, our results suggest that while dietary EAA diversity is important, there is likely something in addition to EAAs, such as lipids (Li, Huang, & Xue, 2013), fatty acids (Arien, Dag, Zarchin, Masci, & Shafir, 2015), or trace minerals (Herbert & Shimanuki, 1978), that bees need to get from pollen and/or supplemental diets.

While honey bees may attempt to compensate for lack of EAAs by collecting more pollen in 2016 (as shown Figure 4), there is likely more to pollen foraging and honey bee health than EAAs alone. Bumble bees, for example, choose pollen sources based on the protein to lipid ratio (Vaudo, Patch, Mortensen, Tooker, & Grozinger, 2016) but when parasitized, they choose floral sources based on secondary metabolite content (Richardson, Bowers, & Irwin, 2016). Our results add yet another example of the importance of diet diversity on pollinator health. If honey bees are searching for complimentary EAAs, lipids, or micronutrients, they are likely not going to find them in one type of food. Thus, monocultures lacking EAAs and other nutrients should be supplemented with wildflower strips (Haaland, Naisbit, & Bersier, 2011) which will provide a suite of nutrients for honey bees and other insect pollinators (Donkersley et al., 2017; Feltham, Park, Minderman, & Goulson, 2015) to choose from, promote pollinator health (Alaux et al., 2010), and bolster crop yields (Pywell et al., 2015).

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Author contributions
REB and PTS conceived and designed this research. REB performed experiments, analyzed data, and wrote the paper; JG helped collect data, and wrote methods sections; PTS aided in data interpretation, and participated in revisions. All authors read and approved the submitted manuscript.

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