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Honey bee (*Apis mellifera*) intracolony genetic diversity influences worker nutritional status

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Abstract – Honey bee queens mate with multiple males resulting in high intracolony genetic diversity among nestmates; a reproductive strategy known as extreme polyandry. Several studies have demonstrated the adaptive significance of extreme polyandry for overall colony performance and colony growth. Colonies that are more genetically diverse collect more pollen than colonies with less diversity. However, the effects of intracolony genetic diversity on worker nutritional status are unknown. We created colonies headed by queens instrumentally inseminated with sperm from either 1 or 20 drones, then compared protein consumption, digestion, and uptake among nestmates. We found that nurse bees from colonies with multiple-drone-inseminated (MDI) queens consumed more pollen, had lower amounts of midgut tissue protease, and invested more protein into larvae than nurse bees from single-drone-inseminated (SDI) queens. Pollen foragers from MDI colonies had significantly higher hemolymph protein concentration than pollen foragers from SDI colonies. Differences in hemolymph protein concentration between nurses and pollen foragers were significantly smaller among MDI colonies than among SDI colonies. While intracolony genetic diversity is correlated with increased foraging, our results suggest that this relationship may be driven in part by the elevated resource demands of nurse bees in genetically diverse colonies that consume and distribute more protein in response to the social context within the hive.

intracolony genetic diversity / extreme polyandry / honey bee nutrition

1. INTRODUCTION

Multiple mating by honey bee (*Apis mellifera*) queens is a reproductive behavioral trait known as extreme polyandry. While relatively uncommon, extreme polyandry (i.e., ≥ 2 mates/queen) has been documented in 13 genera of the Hymenoptera (Hughes et al. 2008). There are inherent risks to the queen of mating in the open with multiple males, such as disease transmission (de Miranda and Fries 2008; da Cruz-Landim et al. 2012) or simply not returning to the colony (Tarpy and

Page 2000; Schlüns et al. 2005). However, extreme polyandry appears to offer a selective advantage at the colony level, largely as a consequence of the increased genetic diversity among the queen's offspring (Keller and Reeve 1994; Oldroyd et al. 1998; Cole and Wiernasz 1999).

A honey bee queen mates in flight with many males (Tarpy et al. 2004) after which she begins to lay eggs. The majority of fertilized eggs develop into female workers, forming the colony workforce. Worker offspring sired by any particular male with whom the queen mated comprise a "patriline" within the colony and, as a consequence of the queen's multiple mates, the colony is comprised of many patrilines. On average, workers within any patriline will be more similar to each other than to their half-sisters from different patrilines (Page and Laidlaw 1988). Genetic

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variability among patrines contributes to the overall genetic diversity of the colony and is a key factor in colony health and productivity for honey bees (Oldroyd et al. 1992b, Fuchs and Schade, 1994; Mattila and Seeley, 2007) as well as other social Hymenoptera (e.g. Wiernasz et al. 2004, 2008; Goodisman et al., 2007). In particular, colonies comprised of a genetically diverse workforce show increased disease resistance (Baer and Schmid-Hempel 1999; Palmer and Oldroyd 2003; Tarry 2003; Hughes and Boomsma 2004; Tarry and Seeley 2006; Seeley and Tarry 2007) and also exhibit improved efficiency in their division of labor [reviewed by Oldroyd and Fewell (2007)]. Robinson and Page (1989) proposed a genetically based response threshold model to explain how colony function improves with increased intracolony genetic diversity. The model predicts that workers have genetically based internal thresholds for responding to task stimuli and patrilineal variation in these internal thresholds generates division of labor. In support of the response threshold model, patriline-based differences in behavior have been widely reported for honey bees such as guarding (Giray et al. 2000), nest thermoregulation (Jones et al. 2004), feeding larvae (Chapman et al. 2007), grooming (Frumhoff and Baker 1988), and corpse removal (Robinson and Page 1988).

Division of labor, whereby workers specialize on subsets of colony tasks (Oster and Wilson 1978), emerges in part from age-related task partitioning; young adult workers handle in-hive tasks such as comb building and brood care (i.e., “nursing”), and older workers perform peripheral tasks such as guarding and foraging (Winston 1987). Moreover, the network of repeated interactions between workers of various genetic and phenotypic dispositions modulates task partitioning (Robinson 1992). Worker interactions provide a mechanism for all individuals to assess the needs of the colony, primarily determined by the colony’s food supply (Ribbands 1952). Trophallaxis, the transfer of food from one worker to another, serves as a primary mechanism for the assessment of colony state and allocation of nutrition resources (Crailsheim 1998). As the primary digesters and distributors of protein in a honey bee colony, nurse bees play a central role in

trophallaxis. Nurse bees consume and digest stored pollen and distribute it as a proteinaceous jelly to all colony members, including the queen, larvae, drones, and foragers (Crailsheim 1991).

The transition to foraging behavior is accompanied by reduced pollen consumption (Crailsheim et al. 1992) as well as reduced levels of total protein in the hemolymph (Fluri et al. 1982). To support their high activity levels, foragers consume some pollen directly; however, they also receive protein via trophallaxis with nurse bees (Crailsheim 1991). Nutritional and physiological shifts in workers suggest that changes in nutritional status may initiate and regulate foraging behavior (Toth and Robinson 2005). Mechanisms that modulate the foraging population are likely integral to colony fitness.

Of note, colonies comprised of multiple patrines are often associated with robust foraging populations [reviewed by Mattila and Seeley (2014)]. Among honey bees, patriline-based differences have been shown to influence a number of foraging-related activities including type of forage (Oldroyd et al. 1992a), foraging distance (Oldroyd et al. 1993), foraging time of day (Kraus et al. 2011), scouting (Dreller 1998; Mattila and Seeley 2011), and dance communication (Mattila and Seeley 2010). Variation in behavioral states within a multiple patriline colony thus enhances the overall foraging effort. Indeed, when compared to honey bee colonies with reduced intracolony genetic diversity, honey bee colonies with greater intracolony genetic diversity are more efficient at pollen foraging, resulting in greater amounts of pollen collected and increased brood production (Mattila and Seeley 2007; Eckholm et al. 2011).

Large amounts of protein are provided to larvae by nurse bees (Crailsheim 1990). Nurse bees increase the feeding frequency and feeding duration of young larvae when stored pollen is available, resulting in higher larval protein content (Schmickl and Crailsheim 2002). Pollen foraging success therefore directly affects colony growth. However, we speculated that the ability to convert inbound food resources to colony growth may be—like foraging effort—augmented by intracolony genetic diversity. To that end, we established colonies headed by queens instrumentally inseminated with either multiple ($n=20$) or

single ($n=1$) drones to construct two treatments of intracolony genetic diversity. We then investigated the effects of “high” versus “low” intracolony genetic diversity on pollen consumption and as well as physiological factors associated with protein digestion and uptake by assessing the nutritional status of nurses, pollen foragers, and larvae collected from each treatment. Specifically, we compared nurse bee midgut protease concentration, larval total soluble protein, and hemolymph total soluble protein concentration in nurses and pollen foragers between our two treatments. We found significant differences in nearly all of these physiological parameters, and suggest that improved worker nutritional status may be yet another example of the positive influence of patriline diversity on colony phenotype.

2. METHODS

We established colonies of either high or low intracolony genetic diversity using Carniolan-derived honey bee queens instrumentally inseminated by a commercial breeder (Glenn Apiaries, Fallbrook, CA, USA). One group of queens was each inseminated with 1.0 μL of semen collected from a unique Carniolan-derived drone (i.e., one unique drone per queen). The other group of queens was each inseminated with a 1.0- μL mixture of semen collected from 20 unique Carniolan-derived drones (i.e., 20 unique drones per queen). This approach has been demonstrated to produce single-drone-inseminated (SDI) queens whose worker progeny are genetically similar, and multiple-drone-inseminated (MDI) queens whose worker progeny are genetically diverse (Haberl and Moritz 1994; Tarpy and Seeley 2006; Mattila and Seeley 2010). To minimize colony variability due to individual queen genetics, queens were supersisters—daughters of a common queen mother and sired by the same drone father ($G=0.75$). Drones were selected at random from a pool of over 1,000 drones collected from 20 different colonies maintained by the breeder. Prior to shipping, inseminated queens were monitored at the breeder apiary to ensure oviposition had begun.

Instrumentally inseminated queens arrived at the Carl Hayden Bee Research Center (CHBRC) in Tucson, AZ, USA on 1 May 2012. Queens were introduced under push-in cages into queenless colonies of equal strength in nine-frame, single-story hives. Hive

locations were randomized in the apiary. Over the subsequent weeks, workers in each colony were supplanted by the offspring of the inseminated queen. This approach allowed us to establish colonies of low intracolony genetic diversity headed by the SDI queens, and colonies of high intracolony genetic diversity headed by the MDI queens. Colonies were examined weekly to assess overall colony health. Any colony that killed or superseded its instrumentally inseminated queen was removed from the study.

We performed two experiments using the aforementioned colonies during the summer of 2012 at the CHBRC. In the first experiment, we assessed the effect of the patriline diversity on pollen consumption using same-aged, caged workers sourced from either MDI or SDI colonies. We measured pollen consumption after 7 days, and also assessed their digestive activity and protein uptake by measuring midgut tissue protease concentration and hemolymph total soluble protein concentration, respectively. In the second experiment, we considered the effect of patriline diversity on nutritional status at the colony level in the apiary. Specifically, we measured midgut tissue protease concentration for nurse bees, hemolymph total soluble protein concentrations for both nurse bees and pollen foragers, and total soluble protein for late-instar larvae. Methodological details for the experiments are outlined below.

Bioassay cages We placed newly emerged workers from either MDI colonies ($n=11$) or SDI colonies ($n=10$) into bioassay cages ($11.5 \times 7.5 \times 16.5 \text{ cm}^3$). We then measured pollen consumption as well as midgut protease concentration and hemolymph protein concentration for each treatment (described in the following sections). We obtained newly emerged workers by removing frames of capped brood from MDI and SDI colonies and placing them in emergence cages (separated by colony) in a climate-controlled environmental room (34°C , 70 % RH). After 24 h, 100 newly emerged workers from each emergence cage were collected and transferred into the bioassay cages. While there are limitations to understanding colony-level phenomena using cages, this approach offered a very controlled method for measuring pollen consumption without the confounding effects of other colony parameters such as colony size, worker age, presence of the queen and brood, and access to other food resources. To establish a baseline of hemolymph protein concentration and midgut protease concentration for each treatment, an

additional 24 newly emerged workers from each emergence cage were ice-anesthetized for hemolymph collection and midgut dissection.

Bioassay cages containing the newly emerged workers were provisioned ad libitum with water and 50 % sucrose syrup, along with a preweighed amount of pulverized corbicular pollen. The cages were placed in an incubator (34 °C, 65 % RH) for 7 days. The weight of the unconsumed pollen in each cage was determined, and then subtracted from the starting pollen weight to estimate the amount of pollen consumed. Twenty-four workers from each cage were then ice-anesthetized for hemolymph collection and midgut dissection.

Worker sampling in the apiary To assess nutritional status between the two primary worker castes, 24 nurse bees and 24 pollen foragers were collected from each MDI colony ($n=9$) and each SDI colony ($n=8$) in the apiary and used for hemolymph extraction and total soluble protein analysis. Additionally, nurse bee midguts were dissected to assess midgut protease concentration (described below). Returning foragers observed with pollen loads in their corbiculae were collected at the hive entrance using forceps and immediately placed in an ice-cold 50-mL conical centrifuge tube. Frames with developing larvae and workers presumed to be nursing (Winston 1987) were temporarily removed from each hive, and workers seen with their heads in cells containing larvae were collected with forceps and placed in an ice-cold 50-mL conical centrifuge tube similar to the pollen foragers. Within an hour after ice-anesthetization, hemolymph was extracted and nurse bee midguts dissected.

Hemolymph collection and total soluble protein analysis We compared hemolymph protein concentrations between treatments as a measure of protein uptake in a manner similar to other studies (e.g., Bitondi and Simoes 1996; Cremonese et al. 1998; Cappelari et al. 2009; DeGrandi-Hoffman et al. 2013). Hemolymph was extracted from ice-anesthetized workers (newly emerged bees, 7-day-old caged bees, nurse bees, and pollen foragers) using 100- μ L microcapillary pipettes (Kimble Glass, Inc.) which had previously been held over a Bunsen burner flame and then pulled apart to form a sharp tip. The sharpened microcapillary pipette was inserted into the lateral portion of the thorax. Approximately 2 μ L of hemolymph from each worker was first drawn into the microcapillary pipette and dispensed

onto clean wax paper. Then, 1 μ L of hemolymph was collected from the wax paper using a pipette and transferred into a 2-mL microcentrifuge tube along with 9 μ L phosphate-buffered saline (PBS) containing 1 % EDTA-free Halt Protease Inhibitor Cocktail (Thermo Scientific). This process was repeated until 24 worker hemolymph samples had been collected and pooled from each colony or cage. Pooled hemolymph samples were stored at -70 °C until analyzed.

Hemolymph total soluble protein concentrations were estimated using a bicinchoninic acid (BCA) protein assay (Thermo Scientific). Absorbance was measured at 562 nm using a Synergy HT spectrophotometer (BioTek Instruments, Inc.). Protein quantity was then estimated using a protein concentration standards curve generated from serial dilutions of bovine serum albumin (BSA).

Total midgut protease concentration To assess differences in colony-level protein digestion between treatments, we measured protease concentration in pooled midgut tissue of young workers. Midguts were dissected from newly emerged bees, 7-day-old caged bees, and nurse bees, for which we had previously collected hemolymph. Twenty-four excised midguts from each colony or cage were placed into a 2-mL reinforced microvial (BioSpec Products, Inc.) containing 1,500 μ L 50 mM borate buffer (pH 8.5; Thermo Scientific) along with two 2.3-mm diameter chrome-steel beads (BioSpec Products). Midguts were then homogenized for 30 s in a mini beadbeater (BioSpec Products). Samples were centrifuged at 10,000 $\times g$ for 5 min, and then 5 μ L of supernatant from each sample was added to a clean 2-mL centrifuge tube containing 995 μ L borate buffer and stored at -20 °C until analyzed. Total midgut protease concentration for each sample was determined using the QuantiCleave Protease Assay Kit (Thermo Scientific), following the manufacturer's instructions. The kit utilizes succinylated casein, which, in the presence of protease, is cleaved at peptide bonds. TPCK-treated trypsin is included with the kit as a protease standard against which sample protease concentration were compared.

Larvae collection and total soluble protein analysis As a measure of colony protein investment in brood, we also collected 24 larvae from each colony in the apiary and measured total soluble protein concentration. Using soft forceps, we placed larvae (estimated

to be at or near the final instar) in an ice-cold 15-mL conical centrifuge tube. After ice-anesthetization, larvae from each colony were weighed together, then transferred into 2-mL reinforced microvials along with two 2.3-mm diameter chrome steel beads and homogenized for 30 s in the mini beadbeater. After homogenization, larvae were transferred back into a 15-mL conical centrifuge tube, diluted with 10 mL PBS containing protease inhibitor as previously described, and vortexed for 30 s. A 10- μ L aliquot of the slurry was diluted with 990 μ L PBS+protease inhibitor, which was then used for total soluble protein analysis using the BCA protein assay.

Data analysis Protein consumption, nurse midgut protease concentration, larval total soluble protein, and caste hemolymph protein concentration were compared between treatments using two-sample *t* tests. In cases of unequal variance between treatments, we applied Welch's correction and adjusted the degrees of freedom accordingly. To account for the effects of worker age in our bioassay cage study, midgut protease concentration and hemolymph protein concentration were analyzed using two-way analysis of variance (ANOVA). A two-way ANOVA was also used to show the interaction between caste and treatment on hemolymph protein concentration. Simple linear regression was used to illustrate the linear association between hemolymph protein concentration and pollen consumption (Zar 1999).

3. RESULTS

Pollen consumption—caged workers After 7 days in bioassay cages, the average amount of pollen consumed by same-age workers sourced from MDI colonies was approximately 52 % greater than the amount consumed by caged, same-age workers sourced from SDI colonies. This difference in pollen consumption was statistically significant (two-sample *t* test; $t_{19}=2.28$; $p=0.04$; Figure 1).

Total midgut protease concentration—newly emerged and caged workers A two-way ANOVA showed a significant effect of treatment on midgut protease concentration for newly emerged and

caged workers ($F_{1,38}=7.99$; $p=0.008$). MDI workers had lower midgut protease concentration than SDI workers. The effect of age was not significant ($p=0.53$), and there was no significant interaction between treatment and age ($p=0.88$) (Table I).

Hemolymph total soluble protein—newly emerged and caged workers A two-way ANOVA showed no significant effect of treatment on hemolymph protein concentration for newly emerged and caged workers ($F_{1,38}=0.38$; $p=0.54$). The effect of age was not significant ($p=0.054$), and there was no significant interaction between treatment and age ($p=0.49$) (Table I). We found a linear relationship between hemolymph protein concentration and protein consumption ($F_{1,17}=10.38$; $p=0.005$). The coefficient for treatment was not significant, and there was no significant interaction between consumption and treatment. However, when hemolymph protein concentration was regressed against pollen consumption separately by treatment, the coefficient for pollen consumption was significant for the SDI treatment ($F_{1,8}=16.16$; $p=0.004$), but not for the MDI treatment ($F_{1,9}=1.53$; $p=0.25$; Figure 2). We also noted that hemolymph protein concentration from newly emerged workers showed no linear association pollen consumption for either MDI ($F_{1,9}=0.13$; $p=0.72$) or SDI ($F_{1,8}=0.67$; $p=0.44$) treatments, suggesting that initial hemolymph protein concentration alone may not be good predictor of pollen consumption.

Hemolymph total soluble protein—nurses and pollen foragers The average hemolymph protein concentration for nurse bees was slightly lower for MDI colonies compared to SDI colonies, although this difference was not significant ($t_{15}=0.987$; $p=0.34$). Forager hemolymph protein concentration, however, was significantly higher among MDI colonies than among SDI colonies ($t_{6,288}=2.94$; $p=0.03$). A two-way ANOVA on worker hemolymph protein concentration revealed a significant interaction between caste and treatment ($F_{1,28}=5.27$; $p=0.03$). Caste differences in hemolymph protein concentration were thus dependent upon colony genetic diversity. Post hoc comparisons using Tukey's HSD test

Figure 1 Pollen consumption after 7 days by 100 caged workers sourced from MDI colonies (*left*) and SDI colonies (*right*). On average, the amount of pollen consumed by caged MDI workers ($\bar{X} = 2.51 \pm 0.26$ g) was approximately 52 % greater than the amount of pollen consumed by caged SDI workers ($\bar{X} = 1.65 \pm 0.27$ g; two-sample t test; $t_{19}=2.28$; $p=0.035$). Group means are indicated with an X .

indicated that the mean hemolymph protein concentration for SDI nurses was significantly higher than SDI foragers. However, mean hemolymph protein concentrations between MDI nurses and MDI foragers were not significantly different (Table II, Figure 3).

Total midgut protease concentration—nurses The average midgut tissue protease concentration for

nurses from MDI colonies was significantly lower than the average midgut protease concentration for nurses from SDI colonies ($t_{11.554}=3.25$; $p=0.007$; Figure 4).

Total soluble protein—late-instar larvae The average total soluble protein per larva from MDI colonies was significantly higher than for SDI colonies ($t_{12}=3.02$; $p=0.01$; Figure 5).

Table I. Estimated means ($\pm$ SEM) for each measure at days 0 and 7 between multiple-drone-inseminated (MDI) and single-drone-inseminated (SDI) workers in the bioassay cage study.

Measure	Treatment	Age		p value
		Day 0 $\bar{X}(\pm$ SEM)	Day 7 $\bar{X}(\pm$ SEM)	
Midgut protease concentration (μ g/ml)	MDI	0.97 ($\pm$ 0.36)	1.15 ($\pm$ 0.36)	0.008*
	SDI	1.95 ($\pm$ 0.37)	2.24 ($\pm$ 0.37)	
Hemolymph total soluble protein (μ g/ml)	MDI	194.7 ($\pm$ 16.9)	240.6 ($\pm$ 16.9)	0.54
	SDI	217.5 ($\pm$ 17.7)	239.0 ($\pm$ 17.7)

Figure 2 Regression analyses of hemolymph total soluble protein on pollen consumption for caged MDI workers (*left*) and caged SDI workers (*right*). The relationship for caged MDI workers was relatively weak ($R^2=0.15$), while the relationship for caged SDI workers was stronger ($R^2=0.67$).

4. DISCUSSION

In our study, MDI colonies produced nurse bees that consumed more protein, pollen foragers with greater hemolymph protein concentration, larvae with greater total protein, and smaller differences in hemolymph protein concentration between nurses and pollen foragers when compared to SDI colonies. Our findings suggest that MDI colonies are better nourished than SDI colonies because they consume and distribute more protein in response to the social context within the hive. As such, improved nutritional status among workers may be yet another example of colony-level benefit gained from multiple mating by the queen.

The flow of nutrients into a honey bee colony begins with pollen foragers and continues on through to larvae being fed by nurse bees. Schmickl and Crailsheim (2002) found that nursing frequency and duration were correlated with the amount of available pollen, resulting in higher larval protein content in well-nourished colonies. MDI colonies collect more pollen than SDI colonies (Mattila and Seeley 2007; Eckholm et al. 2011). Thus, more nutrients flow into MDI colonies, affording increases in both brood rearing and brood protein content. Our bioassay cage experiment, however, demonstrated that when given equal access to pollen, same-aged workers from MDI colonies consumed significantly more pollen than same-aged workers from SDI colonies. This

Table II. Estimated means ($\pm$ SEM) for hemolymph total soluble protein concentration ($\mu\text{g/ml}$) for nurses and pollen foragers from multiple-drone-inseminated (MDI) and single-drone-inseminated (SDI) colonies.

Treatment	Nurse	Forager	p values	
	$\bar{X}(\pm\text{SEM})$	$\bar{X}(\pm\text{SEM})$	Tukey HSD	Interaction
MDI	314.5 (± 15.7)	257.0 (± 15.7)	0.07	0.03*
SDI	342.8 (± 16.6)	207.9 (± 19.2)	<0.001*	
p values (t test)	0.34	0.03*

Figure 3 Interaction plot showing caste differences in hemolymph protein concentration from MDI and SDI colonies. In both treatments, nurse bee hemolymph protein concentration was higher than pollen foragers. However, the interaction between caste and treatment was significant (two-way ANOVA; $F_{1,28}=5.27$; $p=0.03$) such that the difference in hemolymph protein concentration between castes was only significant for SDI colonies (Table II). Group means are indicated with an X.

result suggests that access to colony protein stores alone may not explain colony growth. Rather, the genetic diversity among workers within a colony appears to influence the rate at which available protein is consumed and distributed among nestmates, including larvae.

We measured protease concentration from dissected midguts to compare relative protein digestion between our two treatments. While mechanisms for digestive enzyme synthesis and secretion are complex, various studies in other insect systems have shown a postprandial decrease in protease concentration. Dadd (1956) showed that for starved *Dytiscus* beetles, protease initially accumulates in the midgut epithelium, and then remains low after feeding. Consumption of soluble protein stimulates trypsin release into the midgut lumen (Blakemore et al. 1995). When the midgut is empty, secretion stops and the enzyme again accumulates in the epithelial tissue

(Dadd 1956). In both *Anopheles* and *Aedes* mosquitoes, expression levels of early trypsin genes are downregulated after blood feeding (Müller et al. 1995). Thus, in our study, we interpret lower midgut tissue protease concentration as evidence of greater protein consumption, and higher midgut tissue protease concentration as evidence of reduced protein consumption. In nurse bees sampled from colonies in the apiary, we found midgut tissue protease concentration to be significantly lower among MDI colonies compared to SDI colonies. This finding suggests greater and, potentially, more frequent protein consumption by nurses from MDI colonies. This was consistent with our bioassay cage study, where caged MDI workers consumed more pollen and had lower midgut protease concentration than did caged SDI workers. Importantly, newly emerged workers sourced from MDI colonies also had lower protease concentration than newly emerged

Figure 4 Midgut protease concentration of nurse bees from MDI colonies ($\bar{X}=7.66\pm1.59$ $\mu\text{g/mL}$), and SDI colonies ($\bar{X}=17.79\pm2.68$ $\mu\text{g/mL}$). MDI nurses had significantly lower midgut protease concentration than SDI nurses (Welch's two-sample t test; $t_{11,554}=3.25$; $p=0.007$). Group means are indicated with an X .

workers from SDI colonies. Our results suggest that even newly emerged workers in the presence of multiple patriline may more actively begin protein consumption than newly emerged workers comprised of a single patriline.

Worker hemolymph protein concentration is affected by ingested food quantity (Bitondi and Simoes 1996; Cremonese et al. 1998), age (Fluri et al. 1982; Crailsheim 1986), and genetic differences (Zakaria 2007). In our bioassay cage experiment, consumption differed significantly between treatments. We were therefore somewhat surprised to find that average hemolymph protein concentrations between our bioassay cage treatments were virtually identical. There was a strong, positive linear relationship between pollen consumption and hemolymph protein concentration among our SDI bioassay cages. While workers in our MDI bioassay cages consumed more pollen, the relationship between pollen consumption and hemolymph protein concentration was rather weak. This finding was somewhat unexpected,

but may simply have been an artifact of our experiment. Namely, we had one consumption measure and one pooled hemolymph protein measure per cage; any genetic linkage between pollen consumption and hemolymph protein concentration may have been obscured by our pooling of hemolymph samples from multiple patrilines within our MDI treatment.

Despite significant differences in nestmate protein allocation between our two treatments in the apiary, we noted the conspicuous similarity in nurse hemolymph protein concentration between them, as with our bioassay cage study. Given the central role of nurse bees in protein processing and significant differences in protein consumption between treatments, we certainly expected notable differences in their hemolymph protein concentration. It may be the case that worker hemolymph protein concentration simply adheres to temporal homeostatic set points, as many physiological traits shift in an age-dependent manner (Toth et al. 2005). However, hemolymph protein was

Figure 5 Average total soluble protein per larva from MDI colonies ($\bar{X}=1,632.4\pm67.7$ µg/mL), and SDI colonies ($\bar{X}=1,300.9\pm89.3$ µg/mL). Late instar larvae from MDI colonies had significantly more soluble protein than late instar larvae from SDI colonies (two-sample t test; $t_{12}=3.02$; $p=0.01$). Group means are indicated with an X .

significantly higher for MDI foragers than SDI foragers, suggesting more than just worker ontogeny regulates the trait. The “leaky bucket” analogy could characterize an alternative explanation for similar nurse hemolymph protein concentrations between treatments, whereby the volume of liquid contained within a hole-riddled bucket is a function of both inflow and outflow: MDI nurses consume more protein than SDI nurses, and both larvae and foragers in MDI colonies have higher protein titers than their SDI counterparts. Nurse hemolymph protein titers may look similar between treatments merely because MDI nurses distribute more protein than SDI nurses. In our bioassay cage study, however, workers could not offload protein to either larvae or foragers. Perhaps in a queenless, broodless, yet genetically diverse environment like our MDI cages additional protein resources are more readily diverted to other tissues such as fat body, hypopharyngeal glands, or ovaries. Regardless, some social aspect of the MDI environment—perhaps during larval

development or via interactions among workers—increases protein consumption. It may be that well-fed larvae develop into healthier, hungrier adults or that increased interactions among genetically diverse workers boost their protein metabolism. Sampling of other tissues (e.g., fat body, hypopharyngeal glands, and ovaries) and observation of trophallactic interactions could help untangle these possibilities.

Hemolymph protein concentration was lower among foragers compared with nurses within both MDI and SDI treatments. Reduced forager hemolymph protein is consistent with other studies (e.g., Fluri et al. 1982; Crailsheim 1986), and suggests that these two major behavioral castes are distinguishable as a function of hemolymph protein concentration. Between treatments, however, hemolymph protein concentration was significantly higher among foragers from MDI colonies compared to foragers from SDI colonies. Further, the difference in hemolymph protein between nurses and foragers was significantly

smaller among MDI colonies than SDI colonies. Foragers receive considerable amounts of protein via trophallaxis with nurse bees (Crailsheim 1991), and since our results suggest MDI nurses are processing greater amounts of protein than SDI nurses, they may also be distributing more protein to the forager population.

Division of labor is a fundamental characteristic of honey bee colony organization, which is regulated by worker-worker interactions (Huang and Robinson, 1992, 1996) as well as worker nutritional state (Toth and Robinson 2005) and nutrient uptake (Schulz et al. 1998). Trophallaxis has long been proposed as a primary mechanism for worker perception of colony needs, which modifies worker physiology and ultimately drives task performance. For example, the social inhibition model for honey bee division of labor proposes that foragers interacting with nurses invoke an inhibitory effect, suppressing behavioral development (Huang and Robinson 1999). Since the transition to foraging is accompanied by reduced lipid stores (Toth and Robinson 2005) and reduced hemolymph protein concentration (Crailsheim 1986), the transfer of nutrition resources from nurses to foragers may serve to regulate the foraging force, which in turn provides adequate resources for nurse bees to rear brood. Since the foraging effort directly affects colony growth and development, mechanisms to efficiently modulate the foraging population are integral to colony fitness. In our study, pollen foragers from MDI colonies had significantly higher hemolymph protein titers than pollen foragers from SDI colonies. Since MDI hives collect more pollen (Mattila and Seeley 2007; Eckholm et al. 2011), and greater food availability can lead to earlier foraging activity (Seeley 1985), higher hemolymph protein among MDI foragers may be indicative of earlier onset of foraging behavior. However, since nurses from MDI colonies are both consuming and distributing more protein than nurses from SDI colonies, the increased protein demand by a genetically diverse nurse population may be expediting the pollen foraging effort as well.

Many traits that improve with increased intracolony genetic diversity are also energetically expensive, and require access to energy-rich

molecules for proper function. For example, maintaining immunocompetence is physiologically costly (Schmid-Hempel 2005). While greater intracolony genetic diversity reduces the variance in disease resistance (Schmid-Hempel 1998), an immune response itself is often dependent upon nutritional status (Schneider 2009; Ponton et al. 2011). Indeed, even baseline immunocompetence in honey bees is associated with nutrition (Alaux et al. 2010). Like all animals, honey bees are resource-limited, and selection should favor those phenotypes which can most efficiently access nutrition resources to maximize both individual and colony fitness. As such, greater protein consumption and improved colony nutritional status may represent a cornerstone of the adaptive significance of honey bee polyandry.

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La diversité génétique à l'intérieur d'une colonie d'abeilles (*Apis mellifera*) influence le statut nutritionnel des ouvrières Polyandrie extrême/nutrition/abeille/approvisionnement/protéine

Die genetische Diversität innerhalb von Völkern der Honigbiene (*Apis mellifera*) beeinflusst den Ernährungszustand der Arbeiterinnen genetische Diversität innerhalb des Volks/extreme Polyandrie/Ernährung der Honigbiene

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