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Trophic aspects of caste determination in *Halictus ligatus*, a primitively eusocial sweat bee

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Abstract. Caste determination in primitively eusocial sweat bees is thought to be due to an interacting suite of factors, including size of the larval provision mass, time of year, and social context of the nest into which a young female emerges. Newly emerged gynes are significantly fatter than newly emerged workers, suggesting the existence of larval caste determination cues. Since photoperiod, temperature, and interactions with nestmates were unlikely to affect larval caste determination, we compared the sizes and contents of larval provision masses destined to produce either workers or gynes. Gyne-destined larvae consumed pollen masses that were larger and contained slightly more sugar than those of worker-destined larvae. We suggest that sugar content is one cue which prompts the development of fat reserves in gyne-destined females but not in worker-destined females. The amount of fat possessed by a newly emerged female influences her chances of successfully entering diapause shortly after emergence. Therefore, small, lean females may be more susceptible to behavioural control by queens and more likely to become workers, while large, fat females would be more likely to become gynes.

Key words: Caste determination – Primitively eusocial – Sweat bee – Sugars

Introduction

In social insects, larval nutrition is one of the factors that influences reproductive caste (de Wilde and Beetsma 1982; Wheeler 1986; Hunt 1991). In the advanced eusocial Hymenoptera, larval nutritional cues are linked to endocrine control of caste differentiation (Wirtz 1973; de Wilde 1976), and these insects are defined by the occurrence of morphologically and behaviourally distinct castes. Caste affiliation is permanent, and queens are larger than workers, live longer, are mated and virtually monopolize oviposition. Workers are smaller, have shorter lifespans, do not mate, and spend much of their time in activities related to nest and brood maintenance.

The mechanisms of caste determination in primitively eusocial insects are not obvious. In primitively eusocial sweat bees, caste determination appears to be associated with a suite of interacting factors including larval nutrition, day length, temperature, mating, and the social context of the nest (Michener 1974, 1990). The queen and worker castes of primitively eusocial sweat bees are not morphologically and behaviourally discrete. In general, queens are females that found nests, are relatively large, are almost always mated, monopolize oviposition, produce workers and are behaviourally dominant. Workers are bees produced by queens, do not found nests, are relatively small, are often unmated, lay few eggs and are behaviourally submissive to queens. In temperate populations, an important difference between gynes (potential foundresses) and workers is that the former hibernate through the winter before founding nests the following spring, while workers do not live past the end of the summer in which they emerge. Caste-switching, at least in the behavioural sense, does occur. For instance, upon the death of a queen, one of her daughter workers may become a replacement queen; thereafter, her behaviour is indistinguishable from that of the original queen.

Sweat bees are mass provisioners, and a larva consumes its entire pollen mass prior to pupation. In those species in which queenlike behaviour is associated with larger size, the quantity of food provided to female larvae must influence adult reproductive behaviour (Michener 1974). In species where workers and gynes emerge at different times of the year, photoperiod and temperature might also influence caste. In *Lasioglossum zephyrum*, and probably other species with high levels of queen mortality during the colony cycle, the social context within the nest is a major determinant of caste, and physical disturbance of her nestmates by the queen inhibits queen-like behaviour and induces worker-like behaviour (reviewed by Michener 1990). In one species,

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L. marginatum (Plateaux-Quénu 1960), mating induces overwintering behaviour and subsequent nest founding. A similar phenomenon has been suggested for *Halictus rubicundus* (Yanega 1989).

In this study, we investigated factors affecting caste determination in the primitively eusocial sweat bee, *Halictus ligatus*. Although total food consumed, day length, temperature, and social context are certainly correlated with caste induction to some extent, none of these factors can provide female larvae or newly emerged adults with strictly reliable caste-induction cues. In temperate populations, queens are conspicuously larger than the workers in their own nests, but there is extensive overlap between the size distributions of queens and workers in the population as a whole (Knerer 1980; Litte 1977; Packer 1986a). This bimodal distribution in size of foundress queens and workers reflects the bimodal size distribution of the pollen masses that produce them (Chandler 1955; Michener and Bennett 1977; Packer 1986a; Boomsma and Eickwort 1993), but for individuals of intermediate size, food quantity alone cannot be the sole determinant of caste. Day length and temperature also are insufficient as caste determining cues. In temperate regions, the large reproductive brood females and the smaller workers are often produced at different times of year, but in the tropics and subtropics both workers and foundresses of distinctly different sizes may be produced simultaneously (Michener and Bennett 1977; Packer and Knerer 1986a). The social context of the nest also may provide unreliable cues. For instance, the presence or absence of queen and workers is dependent on patterns of adult survival and the phenology of colony development, which may both change from year to year (Richards 1994).

We studied newly emerged adults of a population of *Halictus ligatus* in southern Ontario, and found that gynes possessed considerable fat stores, whereas workers did not. If amount of stored fat influences a female's ability to enter diapause very soon after emergence, then fat stores could affect a newly emerged female's subsequent reproductive behaviour, since diapause is a prerequisite to founding a nest. Gadagkar et al. (1991) have suggested that in the primitively eusocial vespid wasp *Ropalidia marginata*, larval sugar consumption influences fat deposition during development. We examined provision masses of *H. ligatus* to determine whether there might be differences in sugar content that might directly or indirectly lead to differential development of fat stores in juvenile females. We suggest that sugar contents of larval provisions do influence the deposition of fat, and this in turn biases the reproductive activities of young females, affecting their tendencies to behave as either workers or gynes.

Methods

Halictus ligatus is a widespread, nearctic sweat bee common in southern Ontario. In 1990 we studied an aggregation of 250–300 nests on Highway 50, 4 km north of Victoria, Ontario. This is the aggregation studied earlier by Packer (1986a, b, 1988; Packer and Knerer 1986b). The following section outlines pertinent aspects of the biology of this population; further details can be found in the aforementioned papers.

Basic social cycle at Victoria. In late May and early June, foundresses emerge from their hibernaculae, establish nests, and begin foraging to provision their worker broods. In 1990, this activity lasted from early June until early July. Adult workers began to emerge in mid-July. Workers foraged to provision the reproductive brood from about 18 July until late August. Provisioning of brood entails the excavation of a brood cell and the collection of sufficient pollen and nectar to form into a pollen ball. This is followed by oviposition, and closure of the brood cell. Larvae consume their entire pollen balls before defecation and pupation. Adult workers often mate (Packer 1986a) and therefore are capable of laying both haploid and diploid eggs. Nest excavations during the early part of the summer (prior to mid-July) yielded provision masses, larvae, and pupae of the developing worker brood, including about 16% males (Richards 1994). Excavations during the latter part of the summer yielded provision masses, larvae, and pupae of the reproductive brood, both males and gynes. Occasionally a callow adult of the first brood was found after mid-July. These first brood individuals were identified by the large gap in age between them and the younger individuals of the second brood.

Size and fat contents of newly emerged adults. When nests were excavated, pupae and larvae that had consumed their entire pollen masses were transferred into wax-lined petri dishes to be raised to adulthood in the laboratory. Adults raised in the laboratory or collected in the field were frozen in individual microfuge tubes at −80° C within 36 h of emergence. Those collected in the field were newly emerged, lacked hardened wings, and had not yet left their brood cells. Weight and head width measurements were made on thawed bees wiped clean of condensation. Head widths were measured with an ocular micrometer accurate to 0.1 mm. Female caste was assessed in terms of head width and wing length: workers are considerably smaller than their queens and in 1990 emerged several weeks before the gynes in their own nests (Richards 1994). Therefore caste assessments were unambiguous.

To dessicate the bees, each one was placed in a small aluminum envelope open on one side to prevent dust from settling on it and dried at 60° C for 5 days (this period is sufficient to achieve constant weight). To extract total body fat, dessicated bees were soaked in 2–3 ml petroleum ether for 5 days, then air-dried at room temperature for several hours and again placed in a 60° C oven for at least 12 h. Body fat was measured as weight loss after ether extraction. In the laboratory, the bees were handled only with surgical gloves or forceps to prevent damage and to ensure that dry weights were as accurate as possible. Wet and dry weights were measured to 0.1 mg.

Size and contents of provision masses. Pollen masses producing males, workers or gynes were identified by size and shape. Workers and male-producing pollen masses are small and almost spherical, whereas those producing gynes are large and have a peculiar saddle shape (Chandler 1955; Michener and Bennett 1977; Packer 1986a; Boomsma and Eickwort 1993). Gynes are produced only in the second brood. We could not distinguish between male and female-producing pollen masses in the worker brood (about 16% of this brood is male), but the distinction was obvious in the reproductive brood. Upon excavation, intact pollen masses with eggs (which are about 2 mm in length) or small larvae (<3 mm) were collected in individual 1.5-ml microfuge tubes and stored on ice for transport to the laboratory.

Pollen balls are composed of a mixture of pollen and nectar. Nectar is composed primarily of sugars (especially sucrose, glucose and fructose) and water, with trace amounts of amino acids, proteins, lipids, and other organic and inorganic substances (Baker and Baker 1973, 1975). Pollen is rich in protein. Observations of foraging bees indicated that they forage primarily on a daisy, *Chrysanthemum leucanthemum*, and yarrow, *Achillea millefolium*. We

Table 1. Morphological characteristics of newly emerged workers, gynes, and males collected in 1990. Measurements are given as the mean ± SD. Groups significantly different in size (SNK test) are indicated by *S* (small), *M* (medium) or *L* (large). Note that head widths of males and females are not statistically comparable because those of males are proportionately smaller relative to body size

Sex or caste	*n*	Head width[1] (mm)	Wet weight[2] (mg)	Dry weight[3] (mg)	Fat content[4] (mg)	Relative fat content[5] (%)
Workers	14	2.53±0.16 *S*	19.68±2.93 *S*	5.64±1.14 *S*	0.64±0.51 *S*	17.7 *L*
Gynes	23	2.75±0.17 *L*	27.30±6.72 *L*	7.66±2.06 *L*	1.39±0.54 *L*	17.8 *L*
Males	9	2.24±0.11	20.30±2.36 *S*	6.59±1.08 *S,L*	1.18±0.40 *M*	17.7 *S*

[1] One-way ANOVA: $F=14.56$, $df=1,35$, $P<0.01$

[2] One-way ANOVA: $F=11.75$, $df=2,43$, $P<0.01$; SNK: $MSE=26.8$, $df=43$

[3] One-way ANOVA: $F=6.56$, $df=2,43$, $P<0.01$; SNK: $MSE=2.78$, $df=43$

[4] One-way ANOVA: $F=5.05$, $df=2,43$, $P<0.05$; SNK: $MSE=2.00$, $df=43$

[5] One-way ANOVA: $F=9.33$, $df=2,43$, $P<0.01$; SNK: $MSE=0.003$, $df=43$

do not have estimates of the nectar sugar concentrations of these flowers in the field. Pollen masses were weighed on a Mettler AE100 balance (accurate to 0.1 mg). After wet weight was measured, the dry weight of each pollen ball was obtained following desiccation for 48 h at 80° C in a drying oven. A subsample of pollen masses was redried to ascertain that 48 h was sufficient to achieve constant dry weight. Note that desiccation causes the water in the nectar to evaporate, leaving the sugar and other nectar constituents as part of the dry contents of the pollen mass.

To measure sugar content of larval provisions, each desiccated pollen ball was put in a 1.5 ml eppendorff tube with 200 µl of distilled water. It should be noted that this amount of water was 5–10 times the amount originally removed from the pollen ball by desiccation. Once the pollen ball was sufficiently softened, it was crushed with forceps in order to completely suspend the solid matter. The suspension was spun in a microcentrifuge for 2 min to separate the pollen from the liquid contents. A 25-µl aliquot was used to measure the sugar concentration of this solution with a Bausch and Lomb refractometer for solutions between 0 and 32% sugar. The total amount of sugar within each pollen ball could then be calculated. Not all pollen masses desiccated were available for analysis of sugar content.

Statistical analyses. Most statistical analyses were conducted using SAS, version 6.0. Although significant differences in adult head width of bees from different nests have been detected with large samples (Richards 1994), internest differences in adult and pollen ball sizes were not detectable with the smaller samples used in this study.

For adult bees, we investigated the effects of dry weight and caste or sex on both absolute and relative fat content. Size comparisons among workers, gynes, and males were done using one-way analysis of variance (ANOVA) in conjunction with the Student-Newman-Keuls (SNK) test for multiple comparisons among means. Using analysis of covariance (ANCOVA), absolute fat content (dependent variable) was analysed with respect to the nonfat portion of dry weight and caste or sex (male, gyne or worker).

For pollen masses, we used ANCOVA to investigate the relationship between sugar content (dependent variable) vs. dry weight of pollen masses and caste or sex (worker, gyne, or male). To investigate whether sugar content could be used to *predict* the caste of a pollen mass, we used two linear maximum likelihood models:

sex = sugar content + dry weight

sex = sugar content/dry weight

This type of analysis is a hybrid of linear regression and frequency tests of homogeneity. The independent variables are continuous but the response variable is categorical. The model is analysed as the likelihood that a pollen mass will produce a male, worker, or gyne given its sugar content and dry weight. The effects of each independent variable are unaffected by the order in which they are entered into the model (unlike regression), and are statistically evaluated with a log-likelihood χ^2 (*G*) test.

Results

Adults

Head width is a common measure of body size in sweat bees. Gynes collected in 1990 were significantly larger than workers (Table 1), but there was considerable overlap in the two head width distributions. Gynes were also the heaviest and contained the most fat compared to workers and males (Table 1). The absolute amount of fat stored by newly emerged gynes, workers, and males was compared to non-fat dry weight (Fig. 1). Absolute fat content was greater in larger bees, and gynes and males were fatter than workers (Table 2a), so in general, gynes and males were proportionately fatter than workers (Table 1). These results are not simply due to the large size of gynes. A comparison of individuals of similar size (i.e. <7.0 mg nonfat dry weight) confirms that workers contain less fat than gynes or males (Table 2b).

Table 2. Analysis of covariance (ANCOVA) showing the effect of nonfat weight and sex or caste on variation in stored fat content of workers, gynes, and males. A: All individuals. B: Individuals weighing less than 7.0 mg

Source	*df*	MS	*F*	*P*
A.				
Model	3	3.21	21.99	$P<0.01$
Non fat weight	1	8.14	55.76	$P<0.01$
Sex or caste	2	0.75	5.11	$P=0.01$
Error	42	0.15		
B.				
Model	3	1.53	10.83	$P<0.01$
Nonfat weight	1	2.98	21.12	$P<0.01$
Sex	2	0.80	5.69	$P<0.01$
Error	34	0.14		

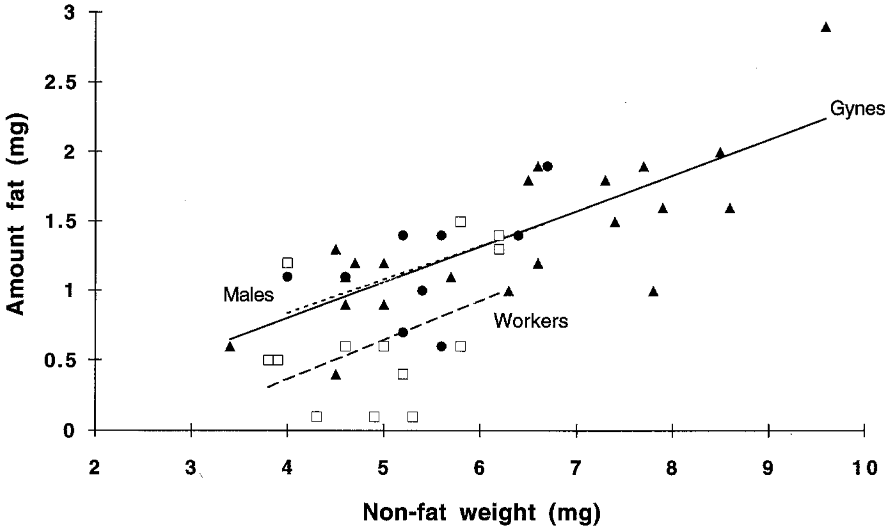

Fig. 1. Relationship between absolute fat stored and non-fat dry weight of adult *Halictus ligatus* males, gynes and workers in 1990; $r^2=0.611$. Gyne-producing pollen balls are represented by *closed triangles*, workers by *open squares*, and males by *closed circles*

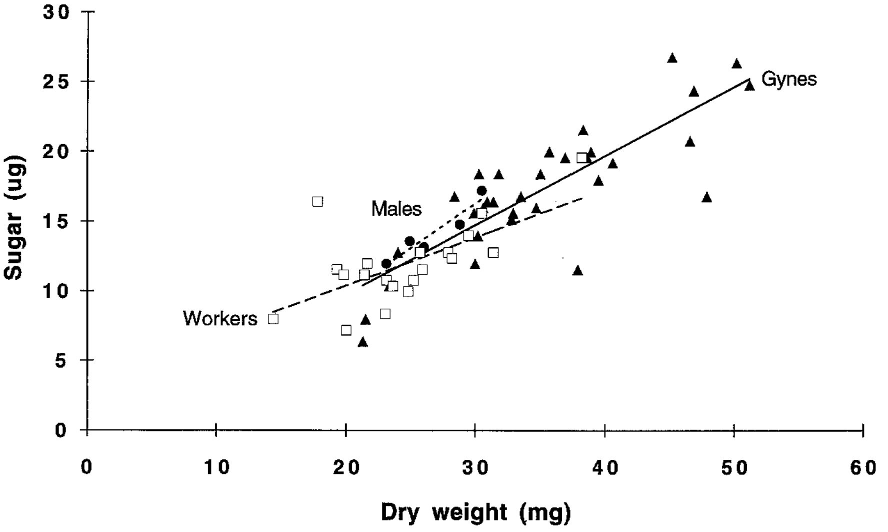

Fig. 2. Relationship between sugar content and dry weight of *H. ligatus* pollen balls producing males, gynes, and workers in 1990; $r^2=0.366$. Gyne-producing pollen balls are represented by *closed triangles*, workers by *open squares*, and males by *closed circles*

Table 3. Characteristics of *Halictus ligatus* pollen masses excavated at Victoria, Ontario in 1990. Measurements are given as the mean ± SD (*n*). Groups significantly different in size (SNK test) are indicated by *S* (small) or *L* (large)

Caste or sex	Wet weight[1] (mg)	Dry weight[2] (mg)	Sugar content[3] (μg)
Worker brood	42.44± 9.18 (52) *S*	23.87±5.88 (52) *S*	11.75±2.78 (19) *S*
Gynes	61.20±10.99 (33) *L*	33.92±8.22 (33) *L*	17.75±4.48 (30) *L*
Males	47.58± 7.27 (5) *S*	26.66±2.98 (5) *S*	14.16±1.97 (5) *S*

[1] One-way ANOVA: $F=37.03$, $df=2,87$, $P<0.01$; SNK: $MSE=0.096$, $df=87$

[2] One-way ANOVA: $F=22.48$, $df=2,87$, $P<0.01$; SNK: $MSE=45.56$, $df=87$

[3] One-way ANOVA: $F=14.76$, $df=2,51$, $P<0.01$; SNK: $MSE=14.43$, $df=51$

Pollen masses

There was considerable overlap in the size ranges of worker and reproductive brood pollen masses (Table 3). On average, gyne pollen masses were almost 50% heavier and contained more sugar than those of workers or males. Sugar content of pollen masses generally scaled with size (Fig. 2, Table 4). Since gyne pollen masses were the largest, they also contained the most sugar.

If larval sugar consumption directly or indirectly influences the amount of fat deposition in developing brood, then this would generally explain the observation that gynes are fatter than workers. Indeed, when all worker and gyne-producing pollen masses are considered, overall size (i.e. dry weight) is a better predictor of caste than sugar content (effect of dry weight: $G=4.02$, $df=1$, $P=0.0450$; effect of sugar: $G=11.37$, $df=1$, ns). However, the size ranges of adult females overlap as do the size ranges of their larval provision masses. In this intermediate size range, some factor other than overall provision mass size must influence the fat content of the developing larvae.

Table 4. ANCOVA showing the effect of dry weight and sex or caste on variation in absolute sugar content of larval provision masses. A: All provision masses. B: Gyne and worker provision masses of intermediate size (smaller than the largest worker provision mass and larger than the smallest gyne provision mass)

Source	*df*	MS	*F*	*P*
A.				
Model	3	338.2	114.89	$P<0.01$
Dry weight	1	1003.9	341.08	$P<0.01$
Sex	2	5.3	1.79	ns
Error	50	147.2		
B.				
Model	2	132.2	61.93	$P<0.01$
Dry weight	1	255.5	119.67	$P<0.01$
Sex or caste	1	8.9	4.19	$P=0.05$
Error	25	53.4		

We used ANCOVA to determine whether sugar content varied among gyne and worker-producing pollen masses in the intermediate size range (i.e. those bigger than the smallest gyne pollen mass and smaller than the largest worker pollen mass). In this size range, sugar content was significantly higher in gyne than in worker pollen masses (Table 4b), implying that proportional sugar content was also higher. For female-producing pollen masses of intermediate size, dry weight is not a good predictor of caste ($G=0.52$, $df=1$, ns), while absolute sugar content is close ($G=3.37$, $df=1$, $P=0.07$). Relative sugar content (absolute sugar content/dry weight) was a good predictor of caste ($G=3.43$, $df=1$, $P=0.0506$).

Discussion

The possession of fat stores at emergence is clearly correlated with the behaviour of *Halictus ligatus* females after emergence: gynes are fat and diapause, while workers are lean and remain in the natal nest. Possession of larger fat stores apparently enables a young female to survive the 8–10 months spent in overwintering diapause. On the other hand, a lack of fat stores must impose a severe limit on the ability of a newly emerged female to begin hibernating shortly after emergence. A lean female could spend several days foraging and depositing fat following emergence, but this option is limited by two important aspects of the reproductive biology of this species. First, as in other sweat bees (Kukuk and May 1991), queens exert behavioural control over workers through aggressive behaviour (Packer and Richards, unpub.), and the small size of workers relative to queens puts them at a distinct disadvantage. Second, overwintering hibernaculae are dug as deep extensions of the nest below the brood-rearing areas (Packer and Knerer 1986b), so a female cannot dig one undetected by other bees. If a small, first brood female emerged without sufficient fat stores, her chances of feeding sufficiently and of excavating a hibernacula, free of interference from the queen, would be very poor. In fact, by producing small, lean daughters, queens deprive them of the opportunity to become gynes, coercing them into remaining in the nest as foragers. Since these first brood females never establish their own nests in the year of emergence, their reproductive options become limited to acting as workers.

What factors could influence the differential deposition of fat stores in developing female larvae? Juvenile sweat bees consume their pollen masses, grow, and develop in separate brood cells. In *Halictus* nests, the cells are closed, so the larvae develop in the dark and have little or no contact with other members of the nest or with the outside environment. Factors such as photoperiod or contact with other bees can have no influence before emergence. Soil temperature during development also proves to be an unreliable caste-determining cue, since temperatures and brood developmental rates may vary considerably from year to year (Richards 1994). The absolute amount of food consumed by female larvae is obviously important, since large amounts of food generally produce gynes and small amounts of food generally produce workers, but for females of intermediate size (i.e. large workers and small gynes), the absolute amount of food also is not a sufficient clue. Our data show that there must be some other factor that biases female development, and we suggest that this cue is found in the amount of sugar consumed with the larval pollen mass. Increased consumption of sugars by larvae also gives rise to adults with increased fat stores in the primitively eusocial wasp *Ropalidia marginata* (Gadagkar et al. 1991).

It is well known that in different populations of *H. ligatus*, gyne-destined larvae feed on large, distinctively saddle-shaped provision masses, whereas workers and males develop on smaller, spherical pollen masses (Chandler 1955; Packer 1986a; Boomsma and Eickwort 1993). This indicates that foragers are capable of discriminating between provision masses meant to produce either caste or sex, and do have the opportunity to deliberately manipulate the sugary contents, namely the nectar. In this study, we detected approximately a two-fold difference in the sugar concentrations of worker and gyne-producing pollen masses, using a volume of liquid many times greater than the total volume of nectar added to the pollen mass and also greater than the total hemolymph volume of a developing larva. It seems very likely that both foragers and larvae would be able to taste such a difference. Most nectar is added to the provision mass after all the pollen has been collected (Plateaux-Quénu 1983), and since bees can concentrate nectar solutions, sweat bee foragers should be able to control the amount of sugar in a pollen mass quite precisely. *Halictus ligatus* is widespread throughout North America, encountering many different flowers whose nectar sugar contents differ. The sugar content of a larval provision mass may be the only reliable caste-determining cue across the species' geographically and climatically diverse range.

A nutritional caste-determining cue could explain how workers and gynes could be produced at the same time and how they could overlap in size, since pollen masses of similar size but different sweetness could produce fatter or leaner females. Another aspect of the biology of this particular population of *H. ligatus* might also be explained by nutritional caste determination. Spring foundresses occur in two different size classes (Packer 1986b). Most spring foundresses are large females, and solitary foundresses are virtually always larger than the workers they eventually produce. However, some spring foundresses are tiny, and in some multiple-foundress associations, subordinate foundresses are smaller than the workers in their own nests. Some very small foundresses might have arisen from larvae that consumed very small pollen masses such as are sometimes produced in the worker brood and which contain relatively large amounts of sugar, so that they developed fat stores as proportionately large as those of gynes. If such females emerged towards the end of the worker brood period, just before the emergence of the first gynes, they might be more likely to escape the behavioural domination of the queen. Such individuals could enter hibernal diapause, becoming foundresses the following spring rather than remaining in the maternal nest as workers. In this way they would resemble *H. rubicundus* worker brood females that mate and overwinter, becoming foundresses the following year (Yanega 1989).

A third interesting aspect of preimaginal caste-biasing in this bee, is that it is essentially reversible after emergence, given the right social circumstances. We propose that queens can effectively prevent small, lean workers from feeding sufficiently to store hibernatory fat, and this encourages them to remain in the nest and assist their mother. On the other hand, a worker emerging into a nest without a queen or other workers would have the opportunity to feed and construct an overwintering hibernacula. Indeed, this does occasionally happen. In 1990, worker brood mortality was very high (Richards 1994), and in at least two nests, a single worker eventually emerged into an orphan nest. Free of interference, these workers did not begin foraging, instead constructing overwintering hibernaculae, exactly as if they had been gynes.

Developmental switches leading to caste differentiation have been proposed for honeybees, bumblebees, ants, and termites (de Wilde and Beetsma 1982; Wheeler 1986). Juvenile hormone and ecdysone are the two morphogenetic hormones critical in the regulation of development in insects, and Hartfelder (1990) has formulated two general hypotheses concerning the relationship between larval nutrition and hormone modulation leading to caste differentiation in social insects. First, caste-specific composition of larval food may affect hormone modulation by causing a specific haemolymph composition. Second, the achievement of a critical larval size could result in a specific program of hormone modulation leading to caste determination. Sugar consumption is known to have a phagostimulatory effect on bee larvae (Asencot and Lensky 1976). By consuming more food with more sugar, larvae may experience an excitatory effect of the nervous system. This may translate into modulation of juvenile hormone titre, high levels leading to gyne development and low levels leading to worker development (Wheeler 1986; de Wilde and Beetsma 1982).

Our small sample of males indicates that while their pollen masses may contain as little sugar as the average worker pollen mass, newly emerged adult males seem to contain fat stores as large (relative to their size) as those of young gynes. This is puzzling, but our sample sizes for males are very small. However, the pollen masses definitely identified as male were all produced in the second brood, at about the same time as the gyne pollen masses, and this supports the view that foragers deliberately add more sugar (nectar) to gyne pollen masses, since they are evidently capable of discriminating among the different types.

The most important aspect of this work is the discovery that gynes emerge with proportionately more stored fat than workers, signifying the existence of a pre-imaginal cue besides total food consumed, that affects female development in these bees. It is possible that the cue is something other than the sugar content of larval food. One problem is that the amount of sugar detected in larval provisions was small, although the amounts in gyne and worker-producing pollen masses were statistically different. Another is that the composition of flower nectar is highly variable, changing with time of day, season, or location (Dafni 1992). The different sugar contents of gyne and worker provision masses might be associated with other compositional differences that we were not able to examine, for instance, changes in amino acid composition. However, regardless of the nature of the cue affecting fat deposition, it does bias the reproductive options of female sweat bees towards worker or foundress roles. It therefore plays a fundamental role in caste determination in this species.

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