

## **Honey supplementation and its developmental consequences: evidence for food limitation in a paper wasp, *Polistes metricus***

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**ABSTRACT.** 1. *Polistes metricus* Say (Hymenoptera: Vespidae, Polistinae) field colonies were supplemented with dilute honey during the pre-emergence and early post-emergence phases of colony development.

2. Supplementation did not increase number of nest cells constructed or rates of loss to predation or foundress disappearance compared with controls.

3. Colonies receiving honey supplementation produced first offspring earlier in the season than control colonies. The difference is due to a shorter time span between founding and first emergence.

4. Foundresses of supplemented and control colonies did not differ in wing length or per cent body fat.

5. Offspring of both supplemented and control colonies had shorter wing lengths than did foundresses of supplemented colonies. Offspring of control colonies had shorter wing lengths than did foundresses of control colonies but not foundresses of supplemented colonies.

6. The per cent body fat of offspring from control colonies was lower than that of all foundresses and of offspring from supplemented colonies.

7. The per cent body fat of offspring from supplemented colonies was higher than that of all foundresses.

**Key words.** *Polistes*, development, nourishment, caste, larva, lipid, reproductive potential, social behaviour.

### **Introduction**

Honey storage by paper wasps (Vespidae, Polistinae) occurs in at least two patterns. The better known pattern is in tropical, swarm-founding polistine wasps that store honey in

vacant nest cells, usually in dry seasons. The behaviour is particularly prominent in *Brachygastra* (e.g. White, 1841) and *Polybia* (e.g. Rau, 1933; Hunt *et al.*, 1987). It is probable that this honey constitutes a food reserve for the entire colony, which remains active all year. Strassmann (1979) documented a temperate zone variation on this pattern in the pleiometrotic (multiple foundress) annual paper wasp *Polistes annularis* in Texas. In this instance, honey is stored in vacant nest cells at the end of the reproductive season, and gynes, which over-

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winter away from the nest, then emerge from hibernacula on warm winter days and return to their natal nest to feed on the stored honey. In a second, less well known pattern, honey droplets are sometimes observed in pre-emergence nests of species in the primitively social genera *Mischocyttarus* (Vesey-Fitzgerald, 1938; Jeanne, 1972) and *Polistes* (Janet, 1903; Rau, 1928, 1939; Heldmann, 1936). In this case the honey droplets are in nest cells also occupied by an egg or small larva. Heldmann (1936) speculated that *Polistes gallicus* is prudent in this honey storing habit, because nests accumulated a large honey supply prior to adverse weather. Rau (1928) proposed that newly eclosed larvae receive their first feedings from the droplets, but an adult would be a necessary intermediary since larvae typically cannot reach the droplets nor has self feeding ever been observed in polistine larvae. Jeanne (1972) suggested that the honey served primarily as a food reserve for adult *Mischocyttarus drewseni*, though he observed adults feeding nectar to larvae.

To date, no study has documented utilization patterns of honey in colonies containing both larvae and adults, and no assessment has been made of the impact of honey storage and use on individual and colony development. The present study was designed to address the developmental consequences of honey use by a paper wasp. Honey was placed experimentally into nests of *Polistes metricus*, and variables of colony development and the size and fat content of adult colony members were then contrasted with controls that did not receive supplemental honey.

## Materials and Methods

The study was conducted at Washington University's Tyson Research Station near Eureka, St Louis County, Missouri, in spring 1985. The research station is largely mature second-growth oak-hickory forest (described quantitatively by Hampe, 1984) with clearings maintained as early successional oldfields by biennial mowing. Six species of *Polistes* have been recorded there (unpublished data). Of those, *P. metricus* is the most abundant and nests both on buildings and vegetation. *P. metricus* is largely haplometrotic (single foundress) in eastern Missouri, although

two (sometimes) or three (rarely) foundresses per nest can be observed (unpublished data).

Nest boxes had been placed at the study site in autumn 1984 to be used by foundresses the following spring. The boxes were approximately 13 cm in each dimension, with a plywood top, fibreboard sides, and open bottom. Each was placed c. 1.5 m above the ground on a wooden stake. Boxes were separated by distances of c. 10 m and were erected in oldfield areas known by prior observation to have substantial populations of *P. metricus*. Boxes were employed in order to: (1) facilitate observations, (2) standardize near-nest environmental variables, and (3) facilitate management of potential nest predators. After nest founding, a 25 cm barrier of Tanglefoot® (a sticky, grease-like product) was maintained at the base of each stake to prevent ants and deer mice (*Peromyscus* spp.) from gaining access to nests. During the experiment, all nests boxes were examined twice weekly to remove spiders and webs.

On 16 April, fifty nest boxes, each containing a single foundress and nest stalk, were numbered and assigned alternately to supplemented and control treatments, yielding an initial sample size of twenty-five colonies for each treatment. Over the next 3 days, all fifty foundresses were marked with paint spots for individual recognition. Supplemented and control colonies were spatially intermingled, so effects of competition for food resources both among study colonies and between them and any colonies on vegetation could realistically be assumed to be uniform.

Supplemented colonies were provided twice weekly with slightly dilute, unprocessed *Apis* honey (c. 60% sugar in sucrose equivalents as determined using a hand refractometer). A 5  $\mu$ l droplet was placed, using a Hamilton 25  $\mu$ l glass syringe, into half of the total number of nest cells. Using nest maps, droplets were placed into the same cells at each application, with every other new cell being added to the group of supplemented cells. A two-cell nest thus received 5  $\mu$ l of supplemental honey; a twelve-cell nest received 30  $\mu$ l; a twenty-cell nest received 50  $\mu$ l; etc. Supplementation began at the two-cell stage and continued twice weekly until all colonies were collected. Total supplementation over the 61-day duration of the experiment averaged  $539 \pm 37 \mu$ l (SE) per colony ( $n=20$ ). Adult wasps were removed from nests during supplementa-

tion; anaesthetization was rarely employed. When necessary, groups of more than seven adults were lightly anaesthetized with ether for removal. All adults were released near the nest box immediately after supplementation.

Colonies were collected in the early mornings of 28 June to 1 July except for five nests that had been recent victims of bird predation. Those colonies were collected on 6 July, when adults not seen on 1 July (the probable date of predation) had returned. Adults were collected by etherization and were placed immediately into prelabelled vials of Kahle's solution. All nests were collected and final maps of them made.

Total wing length of each wasp, which is a correlate of other indices of body size (Haggard & Gamboa, 1980), was measured from tip to tegula using a dial caliper accurate to 0.01 cm. Total non-cuticular lipids were determined for each wasp using a variation of the Sullivan & Strassmann (1984) adaptation of the Folch *et al.* (1957) method. Each wasp was placed into a preweighed boat and dried under a 60 W lamp for 48 h to remove excess preservative. Liquid nitrogen was then added to each boat, and the wasps were freeze-dried in a Labconco Freeze Dry-5 lyophilizer at  $-50^{\circ}\text{C}$  and 0.5 torr for 24 h. After constant weight was thus achieved, the boats were promptly weighed using a Mettler H20T analytical balance to yield the dry weight. Next, each wasp was placed into a ground-glass homogenizer and ground by hand for 5 min in 2.0 ml of 2:1 chloroform:methanol solvent. The tissue was then rinsed, using 10 ml additional solvent, into a dry, preweighed centrifuge tube. The homogenate was vortexed at medium speed for 30 s and then centrifuged at 2000 g for 10 min. After centrifuging, the supernatant (containing the fat) was carefully pipetted off and discarded. Two 3.0 ml washes were then performed in the same manner as the extraction. Total contact time between tissues and solvent was approximately 2 h, and a final solvent volume of 18 ml was reached. Tubes containing residues were dried in an oven at  $50^{\circ}\text{C}$  for 72 h and then reweighed to yield the residue weight. The fat content was calculated by subtracting the residue weight from the dry weight.

## Results

No colonies were lost to predation by ants or deer mice. Seven of fifty colonies were victims of

apparent bird predation, in which the lateral margin of the nest was punctured and pupae and large larvae were removed from their cells leaving eggs and small larvae undamaged. Such predation affected five supplemented and two control colonies (Fisher exact probability test [Siegel, 1956],  $P>0.1$ ). Two supplemented and four control colonies failed because of foundress death or abandonment (Fisher exact probability test,  $P>0.4$ ).

Nests that remained active until collection did not differ in number of nest cells (supplemented =  $37.5 \pm 2.3$  [SE],  $n=20$ ; control =  $42 \pm 3.6$ ,  $n=19$ ;  $t=1.025$ ,  $P>0.2$ ). Completed nest cells are *c.* 2 cm high. To assess possible difference in cell construction, cells less than 1 cm high were valued 0.5 and cells greater than 1 cm high were valued 1.0, and difference between groups was again tested. The adjusted cell numbers did not differ (supplemented =  $32 \pm 2.1$ ; control =  $34 \pm 3.2$ ;  $t=0.61$ ;  $P>0.4$ ).

Census data for the time span of first offspring emergence reveal that the number of colonies with emerged offspring is significantly greater for the supplemented treatment on four dates in June (Fig. 1). The number of days from nest founding until first observation of emerged offspring was significantly less for supplemented colonies ( $53.9 \pm 1.30$ ,  $n=20$ ) than controls ( $58.8 \pm 1.01$ ,  $n=22$ ) ( $t=2.914$ ,  $P<0.01$ ).

Wing measurements (Table 1) revealed no significant size difference within category (foundress, offspring) between treatment (supplemented, control). Supplemented foundresses

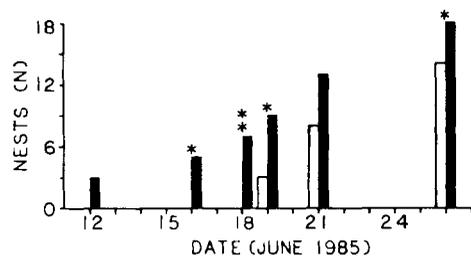


FIG. 1. Cumulative number ( $N$ ) of nests with emerged offspring for control (open bars) and supplemented (solid bars) treatments on six census dates in June 1985. Nests were collected beginning 28 June, with no additional colonies having emerged offspring. Asterisks indicate significance level ( $*P<0.05$ ;  $**P<0.01$ ) for difference in number of supplemented versus control colonies. 16, 18 and 19 June: Fisher exact probability test (Siegel, 1956); 21 and 26 June:  $\chi^2$ .

TABLE 1. Total wing length in centimetres and non-cuticular body fat as a percentage of total body dry weight for *Polistes metricus*. All values are given as mean  $\pm$  one standard error. Samples sizes are given in parentheses.

	Foundress		Offspring	
	Supplemented	Control	Supplemented	Control
Wing length (cm)	1.57 $\pm$ 0.03 (n=10)	1.51 $\pm$ 0.02 (n=12)	1.47 $\pm$ 0.001 (n=71)	1.48 $\pm$ 0.01 (n=56)
Body fat (%)	20.7 $\pm$ 1.5 (n=10)	18.5 $\pm$ 1.3 (n=12)	25.4 $\pm$ 0.5 (n=69)	14.5 $\pm$ 0.7 (n=50)

were significantly larger than both supplemented offspring ( $t=3.45$ ;  $P<0.001$ ) and control offspring ( $t=2.83$ ;  $P<0.01$ ); control foundresses averaged larger than control offspring ( $t=1.22$ ;  $P<0.05$ ) but not supplemented offspring ( $t=0.94$ ;  $P>0.05$ ). Mean non-cuticular fat as a per cent of total dry body weight (Table 1) did not differ between treatments for foundresses, but offspring of the two treatments differed significantly from each other ( $t=12.63$ ;  $P<0.0001$ ) and from foundresses. Offspring of control colonies had lower fat percentages than both supplemented foundresses ( $t=3.76$ ;  $P<0.001$ ) and control foundresses ( $t=2.62$ ;  $P<0.02$ ), while offspring of supplemented colonies had higher fat percentages than both supplemented foundresses ( $t=3.01$ ;  $P<0.01$ ) and control foundresses ( $t=4.82$ ;  $P<0.001$ ).

## Discussion

Use of nest boxes and control of potential predators assured as uniform conditions as could be reasonably provided for unrestrained colonies. In these circumstances, supplemented and control colonies did not differ in rates of bird predation or unexplained failure. Supplemented and control nests did not differ in size at the collection dates. Offspring were smaller than foundresses, which is in accord with known patterns for *P. metricus* (Haggard & Gamboa, 1980), though the difference between control foundresses and supplemented offspring was not significant.

Supplementation with *Apis* honey yielded two important differences. Supplemented colonies had significantly earlier dates of first emergence (with concomitantly shorter time spans between founding and emergence), and offspring of supplemented colonies had significantly higher non-

cuticular fat than offspring of control colonies. Indeed, offspring of supplemented colonies had significantly higher fat than did foundresses.

Early emergence is a likely correlate of higher offspring productivity from paper wasp colonies in seasonal environments where environmental factors may limit productivity at the season's end. High percentage of non-cuticular fat is a correlate (and sometimes experimental determinant [Eickwort, 1969]) of reproductive potential in paper wasps. Low body fat percentages in early offspring of the control colonies is typical for the species (Haggard & Gamboa, 1980). In supplemented colonies, the very high body fat percentages in early offspring, which are presumed always to be workers in *P. metricus*, is strikingly different from the norm. That the body fat of those offspring exceeds that of foundresses is an unprecedented result.

The honey of honeybees (*Apis*) is never consumed by the honeybee larvae. Wasp larvae, however, will readily accept honey that is experimentally offered (e.g. Hunt *et al.*, 1987). The enhanced development of offspring in supplemented colonies therefore could have been due to direct effects of the dilute *Apis* honey being fed by adults to the larvae. Or, if the adult wasps were the principal (or only) consumers of the supplemental nourishment, they may subsequently have reduced solicitation for larval saliva (Hunt, 1988) or withheld less arthropod provision haemolymph for their own nourishment (Hunt, 1984) so that the supplementation had indirect effects on larval development.

This experiment has not addressed the amounts and seasonality of the honey that is naturally stored by *P. metricus*. Over 10 years of observation of the species in eastern Missouri has revealed honey storage to be a common feature in pre-emergence nests in April and May. The amount and duration of storage

appear to vary from year to year, which suggests that environmental variables affect the behaviour. However, a few pre-emergence nests seen in July (possibly founded by that season's offspring) also contained honey, whereas post-emergence nests have not been seen to contain honey in July. Therefore colony development stage may also affect the behaviour. J. P. Spradbery (personal communication) has recently found honey stores in post-emergence nests of *Polistes h. humilis* in Australia, which underscores both the variability of the behaviour and how poorly it is understood.

Experimental supplementation continued beyond the usual period of naturally observed honey storage in *P. metricus*, but the quantity of supplementation (c. 0.5 ml total per nest per 61 days) was modest. This pattern and amount of supplementation yielded data to show that indicators of both colony success (i.e. early first emergence) and individual reproductive potential (i.e. high percentage body fat) may be food limited in natural conditions.

Food limitation of larvae has long been hypothesized to play a role in caste determination in primitively social paper wasps such as *Polistes* (Roubaud, 1916; Hunt, 1989). In recent studies, trophic factors have been reported to contribute to pre-imaginal determination of caste in *Polistes gallicus* (Grechka, 1986), and larval food limitation is also likely to be contributory to the pre-imaginal determination of caste in *Ropalidia marginata* revealed by Gadagkar *et al.* (1988).

The present study is the first experimental demonstration of food limitation in paper wasps and the first study to indicate potential colony effects as well as individual effects of such food limitation. Whether naturally occurring food limitation has an environmental basis (limited resources) or behavioural basis (limited foraging time investment by colony adults) cannot be answered by these data. Neither can these data address the behavioural repertoires of early offspring with high fat content or their subsequent reproductive activities. These subjects merit future investigation.

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