Food Quantity Affects Traits of Offspring in the Paper Wasp *Polistes metricus* (Hymenoptera: Vespidae)

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ABSTRACT The effects of food quantity on the morphology and development of the paper wasp *Polistes metricus* Say are studied, and experimental results are compared with predictions of the parental manipulation hypothesis. Food deprivation led to smaller female offspring. By hand feeding larvae we used a technique that counteracts the queen's hypothesized ability to restrict food provisioning. Hand feeding larvae did not result in larger offspring, but their abdomen was wider and heavier and the hand-fed wasps survived longer in a cold test. We infer that hand-fed colonies produced more gynes and fewer workers than did control colonies. Results of a restricted nourishment treatment do not support the differential feeding hypothesis, because in fasting colonies the emergence of all larvae was delayed by a month, and we did not detect discriminatory feeding of particular larvae for faster emergence. Although fasting colonies produced fewer offspring, the sex ratio did not show significant differences from the other groups. These data suggest that *Polistes metricus* colonies are partly able to respond to different nutritional conditions by allocating excess food to increase the number of gynes at the expense of workers.

KEY WORDS *Polistes metricus*, caste, nutrition, parental manipulation, phenotypic plasticity, social wasp

"ONE CHALLENGE OF **developmental evolutionary biology is to demand more precision in pinpointing the actual effects on phenotype ontogeny. . . . This requires attention to the mechanisms of regulation.**" (West-Eberhard 1996, p. 293).

Eusocial Hymenoptera are characterized by reproductive division of labor in which direct reproduction is mainly carried out by a queen or queens, while colony maintenance is mainly carried out by functionally sterile female workers. Superimposed on reproductive division of labor in most eusocial species is some form of specialization among workers, such that not all individuals are equally likely to perform a given task (for reviews of this field see Robinson 1992, Gordon 1996, Karsai and Wenzel 1998, Bourke and Ratnieks 1999). In this article we address the first type division of labor (the differences between workers and reproductives), where among the main questions are how and why future queens and workers differ, and how we can detect those differences?

In most highly eusocial insects, there are marked and consistent morphological differences between the reproductive and sterile castes. Differential larval nutrition has long been proposed as the fundamental process that gives rise to differential development of brood leading to morphologically distinct castes. Paul Marchal (1896, 1897) first postulated that the physical divergence of the queen and worker castes of Vespinae is based on nutritional discrimination during larval growth. Wheeler (1986) proposed that nutritional switches channel females of highly eusocial species into discrete developmental pathways (castes). Wheeler (1986) proposes that key to the evolution of an efficient reproductive division of labor has been the regulation of growth and development in the larval stage through factors intrinsic to the society. These factors fall into two classes. The first involves the capacity of larvae to initiate development changes in response to nutritional factors. The second involves the capability of adults to regulate larval nutrition itself. Although the physiological basis of this channeling is not yet fully understood, a simple link is suggested by Wheeler (1986) in which the nutritional history of larvae is translated into endocrine signals that coordinate subsequent patterns of differentiation. If nutrition is sufficient, juvenile hormone (JH) level will rise and it initiates and coordinates the expression of gyne-specific development (see also West-Eberhard 1996, for a more evolutionary context).

In most primitively eusocial holometabolous species, however, there are few, if any, consistent morphological differences between the reproductive and worker castes. This may suggest that the caste of an

individual is not fixed at eclosion, thus leaving room for flexibility in which social factors, such as presence or absence of brood, can lead to physiological changes in newly emerged adult females that then lead to caste differentiation. Solís and Strassmann (1990) argue that unpredictable social and ecological environments favor caste flexibility over preimaginal determination in the paper wasp *Polistes exclamans* Viereck. In Wheeler (1986, figure 3) *Polistes* is shown to be different from other social insects in two manners. First, JH plays a role in caste determination in the early adult stage, and second, in other taxa gynes have high and workers low levels of JH, but in *Polistes* both have low levels with only the colony queen having a high level. It is difficult to understand what these differences may mean, because there are apparently no available studies of JH function in solitary wasps, and JH not only seems to regulate reproductive division of labor but also division of tasks among workers (Robinson 1992) and social dominance behavior (Röseler et al. 1985) as well. However, there is accumulating evidence that there is some preimaginal biasing of caste in at least some primitively eusocial species (Grechka 1986; Grechka and Kipyatkov 1883; Rossi and Hunt 1988; Gadagkar et al. 1990, 1991; Hunt 1991; but see Field and Foster 1999). O'Donnell (1998) suggests that the same developmental mechanism may play a role in caste determination throughout Vespidae, in species of all grades of social complexity.

Wheeler (1986) presented a developmental view of the evolution of insect societies as a bridge between evolutionary theories (Hamilton 1964a, 1964b; Lin and Michener 1972; West-Eberhard 1975) and theories focused on insect societies as superorganisms (Oster and Wilson 1978). We agree with Wheeler (1986) that evolutionary and mechanistic views are not necessarily contradictory. Understanding mechanisms by which insect societies are integrated and regulated can lead us to a more reliable formulation of evolutionary hypotheses. West-Eberhard (1996) proposed an explanation of the origin of reproductive castes by "reciprocal deletion," where the ancestral reproductive cycle is decoupled into two parts, with one part expressed in workers and the other in queens (see also West-Eberhard 1987, but see O'Donnell 1996). Each caste lacks the "deleted" set of traits expressed in the other, and the two complementary forms co-operate in the colony, compensating each other's deficiencies. Although nutrition has long been regarded as a crucial element of the mechanism by which this phenotypic plasticity manifests itself (reviewed in Hunt 1994, O'Donnell 1998), there are few studies where the effect of food quantity on *Polistes* paper wasps (a crucial model group for evolutionary studies) has been reported. O'Donnell states (1998, p. 336): " The opinion expressed by Pardi (1948) for *Polistes gallicus* that caste determination 'is independent of nutrition received during larval life' has been very influential to vespid biologists, but it clearly needs to be reexamined. . . . In particular, studies are needed that employ measurements of relative sizes and shapes of structures in all three major body sections (head, meso-

soma and metasoma) and compare them among queens and workers" (O'Donnell 1998, p. 331). The aim of the current study is to respond to these needs by providing simple experimental results on the effect of food quantity on the morphology and development of the paper wasp *Polistes metricus* Say and to compare these results to predictions of the parental manipulation hypothesis (Alexander 1974).

Materials and Methods

Laboratory Conditions and Organisms. On 14 May 1998, single-foundress colonies of *Polistes metricus* that had been freely founded in open nest boxes (15 by 15 by 15 cm) in the field at Shaw Arboretum in Franklin County, MO, were brought into individual cages in the laboratory. All nests contained only eggs, small-, and medium-sized larvae and had a single queen at this time. The cages (30 by 30 by 30 cm) were made from clear plastic and plastic screen with a 10 by 10-cm opening in the top. Wasps had built their nests on the inner side of the wooden top of the field box. This top was removed and was placed as a lid on the hole of the cage. In this way, removing the nest from the substrate where it was initiated originally was unnecessary. The laboratory was a room without windows where ceiling fluorescent lights plus four photo-floods in reflectors were on between 0600 and 2100 hours CDT. Temperature fluctuated between 25 and 30°C. The cages were moved daily in a rotation pattern ($\approx$2m) to ensure equal exposure to room variation for all cages over the course of the experiment. Each cage had ad libitum water supply by a pet bird water-feeder, a lump of sucrose candy, and a large sheet of blotter paper for building material. A separate dish served to hold the caterpillar food (*Trichoplusia ni* Hübner) that was refreshed regularly (see details below).

From the 37 colonies three groups were separated randomly that differed only in the quantity of caterpillars the larvae were provisioned. The 10 control colonies received three large caterpillars every day. Because this quantity was almost never consumed, we considered this group to be provisioned ad libitum. The 14 fasting colonies received a single medium-sized caterpillar twice a week. We considered this group to be underfed colonies. In 13 hand-fed colonies we provided ad libitum caterpillar food as we did in control colonies, but in addition every fourth and fifth instar was fed individually each day by the experimenters. Caterpillars were freshly macerated, and this pulp was put by forceps to the mouth of the larva that then generally accepted the food. This was repeated until the larva refused to eat more. Hand feeding was done in the late afternoon when the activity of the queen had ceased. This approach allowed us to provide the maximum food for the larvae even if the queen decreased its own feeding rate responding somehow indirectly to the manipulation. Thus, we considered hand feeding as supplemental food above the feeding by the queen under ad libitum feeding conditions.
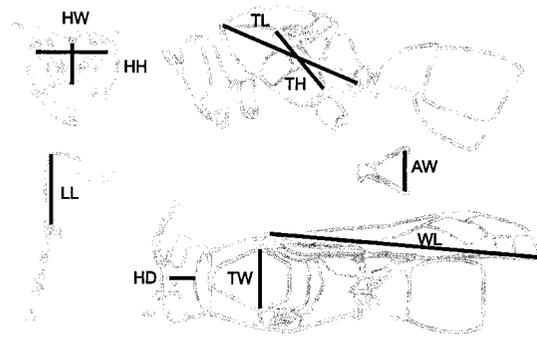
Fig. 1. Point to point measurements on the wasp. Head width (HW), head height (HH), head depth (HD), mesosomal length (TL), mesosomal height (TH), mesosomal width (TW), second metasomal segment width (AW), metathoracic femur length (LL), and wing length (WL).

Data Collection and Evaluation. All nests were monitored at least once daily. If a larva spun a cocoon, an identification number was written by marker on the pupal cap to make it possible to follow the fate of every pupated individual. When the new wasp emerged it was identified by the missing numbered pupal cap, and the wasp was removed. The majority of wasps were frozen at $-20°C$, but every second, fifth, and ninth emerged wasp was used for a cold tolerance test (cold tolerance is a way to estimate whether a given individual will develop into gyne or worker). This cold tolerance test was based on similar principles to those used by Solís and Strassmann (1990), but instead of waiting for 16 d after emergence, we put the wasp into a 4–6°C refrigerator the same day that it emerged. The cold tolerance test measured the number of days the wasp survived at 4–6°C. These wasps were kept individually in the dark, each in a small plastic petri dish, the top of which contained a wet blotter paper to keep humidity high. The wasps were checked daily, and dead wasps were removed and the date of death was registered.

The frozen wasps (that were not used for the cold test) were used for morphometric measurements. Using a microscope equipped with a video camera, the magnified picture of the wasp was video captured when the wasp was aligned using reference points, and the picture was frozen. Point to point measurements were made on the image using DIAS (Digital Image Analysis Software, Corporation Squared, Tamarac, FL). Data were collected on: head width, head height, head depth, mesosomal length, mesosomal height, mesosomal width, second metasomal segment width, metathoracic femur length, and wing length (Fig. 1). After the measurements, the metasoma (gaster) of the wasp was clipped where it is connected to the mesosoma. The gasters were then dried to constant weight by holding at 50°C for several weeks and weighed using an analytical balance with mg precision. Only female wasps were measured. Males were only taken into account in this study for sex ratio results.

Because data were often not normally distributed, and we had to reject the homogeneity of variance in a few cases, nonparametric statistics were used. If not mentioned otherwise, two-tailed tests were used. Principal component analysis (PCA) was used to examine the relationships of morphological parameters and to reduce the nine measured variables to interpretable factors. The data structure fulfilled the conditions of PCA (Kaiser-Meyer-Olkin measure of sampling adequacy = 0.9562; Bartlett test of sphericity = 1,603.46, $P < 0.001$). We attempted to carry out discriminant analysis, but the conditions of that analysis were not fulfilled (Box's $M = 301.16$, $P < 0.05$).

Restrictions and Conditions. Because all offspring were removed immediately after emergence, this study diverges from the natural situation in which postemergent colonies generally have one or more workers, males, and/or gynes on the nest (e.g., Haggard and Gamboa 1980, Metcalf 1980, Dropkin and Gamboa 1981, on the same species). Because our study concentrated on the effect of food, we believe that the simplification of the situation was a benefit contra the more complex (and largely unknown) natural case where adults dynamically vary in number and kind on the nest. We believe the two approaches complement each other.

We did not take into account any group or colony effects. Daughters of the same queen are closely related, thus if there are any genetic or other colony level effects we expect that offspring wasps from the same colony may be grouped along some variables (Sullivan and Strassmann 1984). However, our data have a time scale; several months elapsed between the first and last emergences. For that reason we cannot calculate average values between the colonies. Instead, for morphometric studies we concentrated only on the first 10 offspring, which was the range that was available for all three treatment groups. We examined the difference between early (first to third offspring) and later (fourth to 10th offspring) broods, dividing offspring into these cohorts on the basis of the results of Mead et al. (1994). Because our question was how the quantity of food affects the characteristics of individuals, we believe that considering every offspring wasp individually is an acceptable first step.

The full set of specimens (foundresses and offspring) used for morphometric measurements has been deposited in the Museum of Natural History of the University of Missouri-St. Louis.

Results

The morphological parameters showed high correlation with each other ($0.68 < r < 0.94$, $N = 120$). PCA reduced the variables into a single factor that explained 85% of the total variance in the data. This factor explained the majority of variance of every variable (factor loadings > 0.82), which is why we interpreted this factor as general 'size.' In the second PCA factor the head height variable possessed the largest factor loading, but this factor had very low eigenvalue (0.38) and explained only 4.2% of the total variance. This result suggests that none of the mea-

Table 1. Differences (mean ± SD) in morphometric parameters (mm) in *P. metricus*

Parameter	Control (N = 54)	Fasting (N = 35)	Hand-fed (N = 31)
AW	3.55 ± 0.17	3.27** ± 0.23	3.67** ± 0.15
HD	0.91 ± 0.06	0.81** ± 0.05	0.90 ± 0.06
HH	1.64 ± 0.08	1.49** ± 0.10	1.66 ± 0.09
HW	3.58 ± 0.12	3.36** ± 0.15	3.57 ± 0.13
LL	3.76 ± 0.15	3.30** ± 0.22	3.75 ± 0.19
TH	4.38 ± 0.23	3.91** ± 0.28	4.45 ± 0.17
TL	7.47 ± 0.36	6.62** ± 0.46	7.54 ± 0.34
TW	3.15 ± 0.16	2.77** ± 0.21	3.21 ± 0.11
WL	15.87 ± 0.50	14.24** ± 0.90	15.90 ± 0.58

Mann-Whitney test was used to compare the experimental groups to the control (**, $P < 0.01$). Acronyms for parameters in Fig. 1.

Fig. 2. Pupa development time and cold tolerance. Columns show the average values of control (stippled), fasting (empty), and hand-fed (black) offspring. Sample size: N above the graph, standard deviation: pillars on the column; **, $P < 0.05$ (Mann-Whitney test between the control and the given group).

sured variables (or their combinations) show definite discrepancy from others.

The size of offspring of control colonies (Mann-Whitney $U = 1,025.5$ and 1,056.0 for head width and mesosomal length respectively, $P > 0.1$, $N = 112$), and the size of offspring of hand-fed colonies (Mann-Whitney $U = 580.5$ and 551.0 for head width and mesosomal length respectively, $P > 0.1$, $N = 79$) did not significantly differ from the queens (27 queens emerged in the field were measured), but the offspring of fasting colonies were significantly smaller (Mann-Whitney $U = 301.5$ and 163.5 for head width and mesosomal length respectively, $P < 0.001$, $N = 81$).

Offspring of fasting colonies were significantly smaller than those of control colonies (Table 1). Hand-fed and control colonies showed no significant size difference, except that hand-fed wasps had significantly wider gasters. The gasters of hand-fed offspring were significantly heavier (29.6 ± 4.04 mg, $N = 31$; Mann-Whitney $U = 270.0$, $P < 0.001$, one-tailed test) and those of the fasting offspring were significantly lighter (17.09 ± 4.42 mg, $N = 35$; Mann-Whitney $U = 191.0$, $P < 0.001$, one-tailed test) than the gasters of the control offspring (24.1 ± 3.86 mg, $N = 54$).

First pupation occurred on 28 May and the first three larvae spun cocoons within a few days thereafter in both control (5.4 ± 2.1 d, $N = 24$) and hand-fed colonies (4.8 ± 2.8 d, $N = 17$) (Mann-Whitney $U = 171.5$, $P > 0.2$). However, in fasting colonies the first pupations were delayed significantly (Mann-Whitney $U = 0$, $P < 0.001$) by more than a month (39.8 ± 15.9 d, $N = 33$) and with considerable variance among the fasting colonies in their date of first pupation. A consequence of this increased larval development time was that the fasting offspring emerged much later than in the other two groups. Time spent in the pupal stage was shorter in fasting colonies and longer in hand-fed colonies compared with the controls (Fig. 2). When freshly emerged wasps were used for the cold tolerance test, we detected no difference in survival between control and fasting wasps, but hand-fed wasps survived significantly longer in cold conditions (Fig. 2).

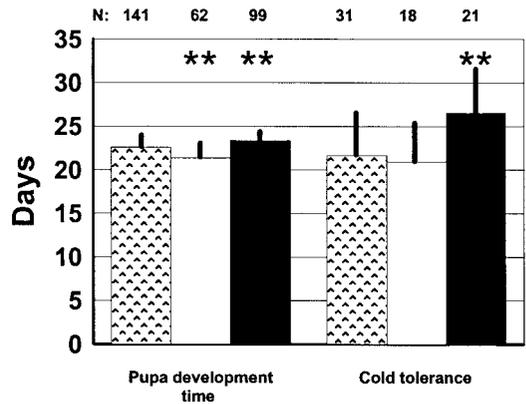To test whether early offspring were different from others, we divided the wasps into two cohorts: cohort 1 = first through third offspring, cohort 2 = fourth through 10th offspring. Fasting colonies showed no differences between the two cohorts in morphology, pupal development time, or cold tolerance (Table 2). However, both control and hand-fed colonies showed significant between-cohort difference both in pupal development time and in cold tolerance (Table 2). The average values of gaster weight increased between cohorts in control and hand-fed but not in fasting colonies (Table 2), while the width of the gaster did not change significantly between cohorts in any treatment (Table 2). The significant difference between hand-fed and control colonies in gaster weight remained significant after the wasps were divided into cohorts (Table 2). Furthermore, cohort 1 wasps belonging to hand-fed colonies had a larger mesosoma width (4.49 ± 0.11 mm, $N = 11$) than did cohort 1 control wasps (4.32 ± 0.28 mm, $N = 16$; Mann-Whitney $U = 39.5$, $P < 0.05$, one-tailed). In cohort 2, hand-fed wasps tolerated cold longer (28.36 ± 6.30 d, $N = 11$) than did cohort 2 control wasps (23.17 ± 5.93 d, $N = 18$; Mann-Whitney $U = 47.5$, $P < 0.05$, one-tailed), while this was not the case for cohort 1 wasps (Mann-Whitney $U = 15.5$, $P > 0.2$).

The sex ratio of total offspring was female biased: sex ratio = 0.743 (355 females and 123 males). Females included both workers and gynes (to differentiate them on the basis of male emergence as in Metcalf 1980, would have resulted in circular reasoning in this study). All colonies produced females, thus all queens were inseminated, but several colonies did not produce males or produced only very few. Less food resulted in differences both in the number of females (Mann-Whitney $U = 1.5$, $N = 21$, $P < 0.001$) and males (Mann-Whitney $U = 17.5$, $N = 21$, $P < 0.01$) but not in sex ratio (Mann-Whitney $U = 44.5$, $N = 21$, $P > 0.2$) in fasting colonies, while control and hand-fed colonies did not differ in offspring number or in sex ratio (Fig 3). In control and hand-fed colonies the first male

Table 2. Differences between cohorts of offspring

Parameter	Cohort 1	Cohort 2	MW 1-2[a]
AW in fasting colonies	3.28 ± 0.16, N = 17	3.30 ± 0.25, N = 16	**119.5 NS**
AW in control colonies	3.49 ± 0.17, N = 16	3.57 ± 0.16, N = 31	**177.0 NS**
AW in hand-fed colonies	3.62 ± 0.10, N = 11	3.67 ± 0.16, N = 17	**64.0 NS**
Abwei in fasting colonies	17.5 ± 3.6, N = 17	17.6 ± 4.8, N = 16	**118.0 NS**
Abwei in control colonies	22.1 ± 3.0, N = 16	24.7 ± 3.9, N = 31	**150.0 ****
Abwei in hand-fed colonies	27.4 ± 3.9, N = 11	30.4 ± 3.8, N = 17	**56.0 ***
MW control and hand-fed[b]	**27.5****	**72.0****	
PupaT in fasting colonies	21.4 ± 1.7, N = 33	21.4 ± 2.3, N = 40	**313.0 NS**
PupaT in control colonies	21.9 ± 1.2, N = 24	23.3 ± 1.0, N = 57	**265.5 ****
PupaT in hand-fed colonies	22.8 ± 1.6, N = 17	24.4 ± 0.9, N = 36	**135.0 ****
MW control and hand-fed[b]	**137.5***	**438.0****	

Cohort 1: first through third wasps emerged, cohort 2: fourth through tenth wasps emerged. From the examined 12 parameters, only weight of the gaster (Abwei) and pupa development time (PupaT) showed significant differences between cohorts (MW 1-2 column). Nonsignificant parameters, except AW (second metasomal segment width), are not presented here. If there is significant difference between the cohorts, then the hand-fed and the control groups are compared by cohorts as well.

[a] Cells shows mean $\pm$ SD and sample size, or value of Mann-Whitney U (bold characters).

[b] MW control and hand-fed under the given variable.

**, $P < 0.05$; *, $P < 0.1$, NS, $P > 0.1$.

emerged 70 and 80 d after the first pupation respectively, while in fasting colonies this happened after 105 d. This is a somewhat shorter delay than that we observed in the case of the emergence of first fasting workers (39.8 ± 15.9 d, N = 33). In both control and hand-fed colonies males emerged only after the 10th offspring, while in fasting colonies male offspring emerged after the fourth offspring.

Discussion

In all species of social insects, caste differences among colony members have proved to be phenotypic rather than genetic (Wilson 1985). Among the three major evolutionary theories of worker altruism (see West-Eberhard 1981; Gadagkar 1985), the hypothesis of parental manipulation of the brood (Alexander 1974) provides a simple link between a nutrition mechanism and an evolutionary theory. If the adults underfeed some larvae, then they create a class of individuals with low direct reproductive value. These

Fig. 3. Boxplots of male and female production of the colonies and the sex ratio (on secondary y-axis). Control colonies (stippled, N = 10), fasting colonies (empty, N = 11) and hand-fed colonies (black, N = 8). Box show range between 25 and 75th percentiles, thick line shows median, whiskers: minimum and maximum value that is not outlier, dots: outliers (values > 1.5 box-lengths from 75th percentile).

individuals are forced into a 'handicapped loser' reproductive category, where indirect reproduction is the most profitable alternative. Kin selection in this case may explain "the conformity of manipulated offspring to the helper role" (West-Eberhard 1981, p.12). However, Sullivan and Strassmann (1984) found no evidence that any females of *Polistes annularis* L. are forced into being subordinates due to inadequate feeding as larvae. They documented that the variance in size among foundresses on spring nests was not significantly greater than that of all females from their natal nests. Metcalf and Whitt (1977) concluded that their results do not support the parental manipulation hypothesis for *Polistes metricus* (the same species we report in this article).

We believe that our experiment is the first to have directly tested the possibility of parental manipulation in *Polistes* using a technique that counteracts the queen's hypothesized ability to restrict food provisioning. From the parental manipulation, hypothesis one can derive a simple prediction: the queen should underfeed some larvae (mainly the first ones), even if food is abundant. In our experiment, hand-fed colonies were provided with food in addition to that which the queen brought to the nest. Control colonies had ad libitum food, i.e., the queen was able to give the quantity of food to the larvae that she wanted. Therefore, detected differences between those groups can be contrasted as the effect of parental manipulation. We found no significant size difference between the control and hand-fed offspring, except that the width of the second metasomal segment and the weight of the gaster were larger in hand-fed wasps. Fasting colonies had significantly smaller offspring than those with ad libitum food. These results suggest that the queen does not underfeed larvae in *Polistes metricus* to gain small sized workers, but instead provides them with enough food to develop into a queen-sized individual. The hand feeding of larvae seems to have resulted in a little extra nutrition that was turned into fat (see later).
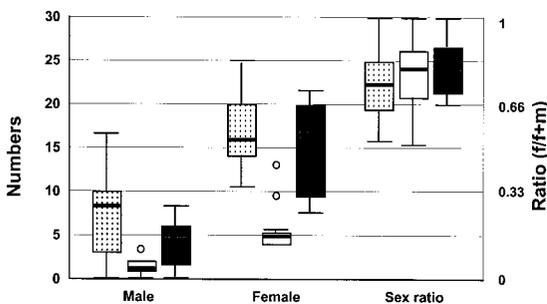
Our finding does not necessary contradict the commonly found pattern that in *Polistes* the first workers are smaller than females that emerge later in the season (West-Eberhard 1969, Haggard and Gamboa 1980, Turillazzi 1980, Miyano 1983). This pattern may due to variation of food availability in the field (O'Donnell 1996). For example, more or larger caterpillars may be available later in the season (we are not aware of a specific study of this pattern), or later in the colony life (after worker emergence) the larger number of foragers available may result in the collection of more food per larva. However, our experiment suggests that the pattern of naturally occurring size difference is not the result of parental manipulation. As in most insects, differences in adult size can result from variation in environmental factors such as temperature (Wigglesworth 1965, Plowright and Jay 1977, Nijhout 1981) as well as in nutrition during larval development. Some primitively eusocial species have harnessed environmental variation of resources to produce substantial variation in offspring size (Knerer and Atwood 1966). However, we cannot exclude the possibility that this natural phenotypic variation can have evolutionary consequences for fertility and in turn for social evolution. Our experiment simply suggests that this variation is not the result of direct parental manipulation in *P. metricus*, but rather it is the consequence of external parameters.

Polistes wasps seem to show isometric growth and have a unimodal body size distribution, and size is not considered as an absolute indicator of reproductive potential (Eickwort 1969, Turillazzi and Pardi 1977, Dropkin and Gamboa 1981, Röseler et al. 1984). In eusocial wasps, allometric caste differences, if they exist, are most pronounced in the structures of the gaster (Blackith 1958). Even if there are no visible external caste differences there may exist differentiation on a physiological level. *P. exclamans* future queens are indistinguishable from workers, but they show a strikingly greater development of the parietal fat bodies located beneath the abdominal tergites (Eickwort 1969). This has been used as an indirect indicator of caste status of temperate-zone independent founding wasps (Litte 1977, Haggard and Gamboa 1980, Strassmann et al. 1984). In the current study, hand-fed offspring had wider and heavier gasters. This increase of mass in our case is also due to larger fat bodies (I.K. and J.H.H., unpublished data).

Strassmann et al. (1984) showed that *Polistes* gynes are more tolerant of cold than are workers. Although the offspring wasps in the current study were those logically destined to be workers (i.e., the first 10 emerged females), the hand-fed wasps were able to survive in the cold longer than control wasps. They lived even longer than wasps predicted to be gynes of *P. exclamans* under similar condition in the Solís and Strassmann (1990) experiment. However, in our case this cold tolerance difference does not originate from a nutrition difference or social interaction after the individual emerged (see Solís and Strassmann 1990), but from the nutrition differences the larvae had received. It seems that the wasps carried through the

surplus food they received from larval stage as an energy storage, and we propose that this allowed them to survive longer. The two results are not necessary contradictory. Surely fat quantity and behavior may change as the physiology of the newly eclosed adult may also change in response to social and environmental influences after emergence. This may ensure the very important individual level flexibility that characterizes societies composed of small numbers of individuals (Karsai and Wenzel 1998).

It is evident that it is to the queen's benefit to have workers as soon as possible, because these workers may provide help and, more importantly, they forage, thus eliminating the most dangerous tasks for the queen. This logically suggests the possibility of differential feeding (mainly if food is scarce), where some larvae are preferentially fed to bring them to maturity quickly. West-Eberhard (1969) suggested that larvae near the nest center are better fed in *P. fuscatus*, but Strassmann and Ferreira Orgren (1983) did not find differences in feeding of larvae in different zones of the nests in *P. exclamans.* However, Mead et al. (1994) found that first hatched larvae (age and not position is important) are preferentially fed in *P. dominulus* Christ, and this was irrespective of the food conditions. Miyano (1981, 1983, 1990) also showed that the first offspring grow to adults quickly. Our results do not support the differential feeding hypothesis, because the first emergences in fasting colonies were delayed by more than a 1-mo interval. Considering the quantity of food we used, differential feeding and/or larval cannibalism could have made it possible for queens of fasting colonies to rear one or two larvae into adults at about the same time as in control colonies, but this flexibility seems to be missing in *P. metricus.* The finding that the sex ratio did not show significant differences among the groups and that males emerged later in time but earlier in the brood sequence in fasting than in control and hand-fed colonies also suggests the lack of differential feeding. It seems that the fates of fasting larvae were random: they were reared or died (presumably cannibalized) irrespective of their sex, and all surviving larvae developed slowly. However, they spent less time as pupae than the other two groups. This last finding agrees with West-Eberhard's (1969) finding in *P. fuscatus* (F.) that the length of the pupal period of a given individual is an inverse function of the length of its larval period. It is possible that the large flexibility in larva development time contributes to the lack of physical castes in *Polistes.* It seems that the larvae resist developing into very small individuals by extending their larva stage in case of food deprivation.

It is somewhat surprising that hand feeding of larvae did not result in a greater number of offspring than emerged in control colonies. Miyano (1998) found that egg laying capacity was not affected by the amount of available flesh food (insect tissue) in *P. chinensis antennalis* Pérez. In our study, for both control and hand-fed colony types the caterpillar food was ad libitum, thus the queen supposedly laid eggs at her maximum capacity. Because of the ample food the

frequency of oophagy was also minimal (Mead et al. 1994). The extra food that larvae received by hand feeding seems to have been used to increase the quality of the female offspring (the number of males did not show differences between control and hand-fed colonies, and quality differences were not tested). When we separated the offspring into cohorts, we found that the weight of the gaster of the first three hand-fed wasps was already heavier than of the control wasps. Although control wasps showed a significant increase in gaster weight between the first and second cohorts, they remained significantly lighter than hand-fed wasps. In view of these differences, together with the significant difference in cold tolerance, we infer that hand-fed colonies produced more gynes and fewer workers than did control colonies.

We hope that these findings will foster more studies on the nutritional mechanisms involved in caste regulation. We find especially important the need to track the behavioral fates of food-manipulated females. We further hope that nutritional data within an appropriate theoretical framework, such as state-dependent life history models (McNamara and Houston 1996), will soon contribute answers to both proximate and ultimate questions on social evolution.

Acknowledgments

References Cited

Alexander, R. D. 1974. The evolution of social behavior. Annu. Rev. Ecol. Syst. 5: 325–383.

Blackith, R. E. 1958. An analysis of polymorphism in social wasps. Insectes Soc. 5: 263–272.

Bourke, A.F.G., and F.L.W. Ratnieks. 1999. Conflict and caste determination in social insects. Behav. Ecol. Sociobiol. 46: 287–297.

Dropkin, J. A., and G. J. Gamboa. 1981. Physical comparisons of foundresses of the paper wasp *Polistes metricus* (Hymenoptera: Vespidae). Can. Entomol. 113: 457–461.

Eickwort, K. R. 1969. Differential variation of males and females in *Polistes exclamans.* Evolution 23: 391–405.

Field, J., and W. Foster. 1999. Helping behaviour in facultatively eusocial hover wasps: an experimental test of the subfertility hypothesis. Anim. Behav. 57: 633–636.

Gadagkar, R. 1985. Evolution of insect sociality—a review of some attempts to test modern theories. Proc. Indian Acad. Sci. (Anim. Sci.) 94: 309–324.

Gadagkar, R., S. Bhagavan, K. Chandrashekara, and C. Vinutha. 1991. The role of larval nutrition in pre-imaginal biasing of caste in the primitively eusocial wasp *Ropalidia marginata* (Hymenoptera: Vespidae). Ecol. Entomol. 16: 435–440.

Gadagkar, R., S. Bhagavan, R. Malpe, and C. Vinutha. 1990. On reconfirming the evidence for preimaginal caste bias in a primitively eusocial wasp. Proc. Indian Acad. Sci. (Anim. Sci.) 99: 141–150.

Gordon, D. M. 1996. The organization of work in social insect colonies. Nature (Lond.) 380: 121–124.

Grechka, E. O. 1986. The regulation of seasonal development and caste determination in Polistes wasp, pp. 43–73. *In* V. B. Zinchuk [ed.], Essays in memory of N. A. Kholodkoskii. Nauka, Leningrad (C. Starr and L. D. Kassianoff, translators).

Grechka, E. O., and V. Y. Kipyatkov. 1983. Seasonal developmental cycle and caste differentiation in the social wasp *Polistes gallicus* L. (Hymenoptera, Vespidae). I. Phenology and life cycle regulation. Entomol. Rev. 62: 19–31.

Haggard, C. M., and G. J. Gamboa. 1980. Seasonal variation in body size and reproductive condition of a paper wasp, *Polistes metricus* (Hymenoptera: Vespidae). Can. Entomol. 112: 239–248.

Hamilton, W. D. 1964a. The genetical evolution of social behaviour. I. J. Theor. Biol. 7: 1–16.

Hamilton, W. D. 1964b. The genetical evolution of social behaviour. II. J. Theor. Biol. 7: 17–52.

Hunt, J. H. 1991. Nourishment and the evolution of the social vespidae, pp. 426–450. *In* K. G. Ross and R. W. Matthews [eds.], The social biology of wasps. 12. Cornell University Press, Ithaca, NY.

Hunt, J. H. 1994. Nourishment and social evolution in wasps *sensu lato*, pp. 211–244. *In* J. H. Hunt and C. A. Nalepa [eds.], Nourishment and evolution in insect societies. 7. Westview Press, Boulder, CO, and Oxford and IBH, New Delhi.

Karsai, I., and J. W. Wenzel. 1998. Productivity, individual-level and colony-level flexibility, and organization of work as consequences of colony size. Proc. Natl. Acad. Sci. U.S.A. 95: 8665–8669.

Knerer, G., and C. E. Atwood. 1966. Polymorphism in some Nearctic halictine bees. Science (Wash. DC) 152: 1262–1263.

Lin, N. L., and C. D. Michener. 1972. Evolution of sociality in insects. Q. Rev. Biol. 47: 131–159.

Litte, M. 1977. Behavioral ecology of the social wasp, *Miscocyttarus mexicanus.* Behav. Ecol. Sociobiol. 2: 229–246.

Marchal, P. 1896. La reproduction et l'évolution des guepes sociales. Arch. Zool. Exp. Gen. 4: 1–100.

Marchal, P. 1897. La castration nutriciale chez les Hyménopteres sociaux. C. R. Soc. Biol. (Paris) 556–557.

McNamara, J. M., and A. I. Houston. 1996. State-dependent life histories. Nature (Lond.) 380: 215–221.

Mead, F., C. Habersetzer, D. Gabouriaut, and J. Gervet. 1994. Dynamics of colony development in the paper wasp *Polistes dominulus* Christ (Hymenoptera, Vespidae): the influence of prey availability. J. Ethol. 12: 43–51.

Metcalf, R. A. 1980. Sex ratios, parent-offspring conflict, and local competition for mates in the social wasps *Polistes metricus* and *Polistes variatus.* Am. Nat. 116: 642–654.

Metcalf, R. A., and G. S. Whitt. 1977. Relative inclusive fitness in the social wasp *Polistes metricus.* Behav. Ecol. Sociobiol. 2: 353–360.

Miyano, S. 1981. Brood development in *Polistes chinensis antennalis* Pérez. I. Seasonal variation in duration of immature stages and an experiment on thermal response of egg development. Bull. Gifu Prefect. Mus. 2: 75–83.

Miyano, S. 1983. Number of offspring and seasonal changes of their body weight in a paper wasp, *Polistes chinensis antennalis* Pérez (Hymenoptera, Vespidae), with reference to male production by workers. Res. Popul. Ecol. 25: 198–209.

Miyano, S. 1990. Number, larval duration and body weights of queen-reared workers of a Japanese paper wasp, *Polistes chinensis antennalis* (Hymenoptera, Vespidae). Nat. Hist. Res. 1: 93–97.

Miyano, S. 1998. Amount of flesh food influences the number, larval duration, and body size of first brood workers, in a Japanese paper wasp, *Polistes chinensis antennalis* (Hymenoptera: Vespidae). Entomol. Sci. 1: 545–549.

Nijhout, H. F. 1981. Physiological control of molting insects. Am. Zool. 21: 631–640.

O'Donnell, S. 1996. Reproductive potential and division of labor in wasps: are queen and worker behavior alternative strategies? Ethol. Ecol Evol 8: 305–308.

O'Donnell, S. 1998. Reproductive caste determination in eusocial wasps (*Hymenoptera: Vespidae*). Annu. Rev. Entomol. 43: 323–346.

Oster, G. F., and E. O. Wilson. 1978. Caste and ecology in the social insects. Princeton University Press, Princeton, NJ.

Pardi, L. 1948. Dominance order in *Polistes* wasps. Physiol. Zool. 21: 1–13.

Plowright, R. C., and S. C. Jay. 1977. On the size determination of bumblebee castes. Can. J. Zool. 55: 1133–1138.

Robinson, G. E. 1992. Regulation of division of labor in insect societies. Annu. Rev. Entomol. 37: 637–665.

Röseler, P.-F., and A. Röseler, I., Strambi. 1985. Role of ovaries ecdysteroids in dominance hierarchy establishment among foundresses of the primitively social wasp, *Polistes gallicus.* Behav. Ecol. Sociobiol. 18: 9–13.

Röseler, P.-F., I. Röseler, A. Strambi, and R. Augier. 1984. Influence of insect hormones on the establishment of dominance hierarchies among foundresses of the paper wasp, *Polistes gallicus.* Behav. Ecol. Sociobiol. 15: 133–142.

Rossi, A. M., and J. H. Hunt. 1988. Honey supplementation and its developmental consequences: evidence for food limitation in a paper wasp, *Polistes metricus.* Ecol. Entomol. 13: 437–442.

Solís, C. R., and J. E. Strassmann. 1990. Presence of brood affects caste differentiation in the social wasp, *Polistes exclamans* Viereck (Hymenoptera: Vespidae). Funct. Ecol. 4: 531–541.

Strassmann, J. E., R. E. Lee, R. R. Lojas, and J. G. Baust. 1984. Caste and sex differences in cold-hardiness in the social wasps *Polistes annularis* and *Polistes exclamans* (Hymenoptera: Vespidae). Insect. Soc. 31: 291–301.

Strassmann, J. E., and M.C.F. Orgren. 1983. Nest architecture and brood development times in the paper wasp, *Polistes exclamans* (Hymenoptera: Vespidae). Psyche 90: 237–248.

Sullivan, J. D., and J. E. Strassmann. 1984. Physical variability among nest foundresses in the polygynous social wasp, *Polistes annularis.* Behav. Ecol. Sociobiol. 15: 249–256.

Turillaza, S. 1980. Seasonal variation in the size and anatomy of *Polistes gallicus* (L.) (Hymenoptera: Vespidae). Monït. Zool. Ital. (N.S.) 14: 63–75.

Turillazzi, S., and L. Pardi. 1977. Body size and hierarchy in polygynic nests of *Polistes gallicus* (L.) (*Hymenoptera Vespidae*). Monit. Zool. Ital. 11: 101–112.

West-Eberhard, M. J. 1969. The social biology of Polistine wasps. Miscellaneous Publications Museum of Zoology, University of Michigan 140: 1–101.

West-Eberhard, M. J. 1975. The evolution of social behavior by kin selection. Q. Rev. Biol. 50: 1–33.

West-Eberhard, M. J. 1981. Intragroup selection and the evolution of insect societies, pp. 3–17. *In* R. D. Alexander and W. Tinkle [eds.], Natural selection and social behavior. Chiron, Concord, MA.

West-Eberhard, M. J. 1987. Flexible strategy and social evolution, pp. 35–51. *In* Y. Ito, J. L. Brown, and J. Kikkawa [eds.] Animal societies: theories and facts. Scientific Societies Press, Tokyo.

West-Eberhard, M. J. 1996. Wasp societies as microcosms for the study of development and evolution, pp. 290–317. *In* S. Turillazzi and M. J. West-Eberhard [eds.], Natural history and evolution of paper-wasps. 17. Oxford University Press, Oxford.

Wheeler, D. E. 1986. Developmental and physiological determinants of caste in social *Hymenoptera:* evolutionary implications. Am. Nat. 128: 13–34.

Wigglesworth, V. B. 1965. The principles of insect physiology, 6th ed. Methuen, London.

Wilson, E. O. 1985. The sociogenesis of insect colonies. Science 228: 1489–1498.