

Research article

## Lipid stores, ovary development, and brain gene expression in *Polistes metricus* females

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**Abstract.** In order to gain insights into the mechanistic basis of caste and behavioral differences in *Polistes* paper wasps, we examined abdominal lipid stores and ovary development in *Polistes metricus* females in four groups: foundresses, queens, workers, and gynes. Queens had the largest ovaries, followed by foundresses, workers, and gynes. Gynes had 6x higher lipid stores than the other groups, and lipid stores were lower in foragers (foundresses, workers) than non-foragers (queens, gynes). Lipid levels and ovary development were negatively correlated across the four groups, but removing gynes from the analysis revealed a significant positive correlation for foundresses, workers, and queens, suggesting different energy allocation strategies for gynes vs. other groups. Expression levels of 9 genes (including three in the insulin pathway), examined in a previous study, correlated with either lipid stores or ovary development. These correlative results suggest important relationships between nutrition, reproduction, and division of labor in primitively social insects. We also show that it is possible to assign *P. metricus* females to one of the four female groups on the basis of wing wear (an indicator of foraging experience), lipid stores, and ovary development, which can facilitate caste-specific collections for future studies.

**Keywords:** *Polistes metricus*, ovary development, caste, nutrition, foraging behavior, gene expression.

### Introduction

*Polistes* paper wasps have been a model system for studying social evolution for well over 50 years (Pardi, 1948; West-Eberhard, 1996). They are “primitively eusocial” due to small colony size, overt conflict over reproduction, and little or no morphological difference between queens and workers (Wilson, 1971). Nonetheless, categories of females with very different behavioral profiles can be clearly recognized on temperate *Polistes* colonies during different phases of colony development (Reeve, 1991). Individuals or small groups (foundresses) initiate nests, lay eggs, and rear a first set of offspring. The first offspring emerge as workers, and a foundress becomes the primary egg-laying queen of the colony. Females emerging late in the season, called gynes, are the wasps that mate, overwinter, and become the foundresses of the next colony cycle.

Although morphological caste differences are absent, some physiological differences between *Polistes* females are known. Dominance behavior among foundresses and between the queen and workers has been extensively studied, and it is well-established that behaviorally dominant individuals have larger ovaries than subordinates (Pardi, 1948; West-Eberhard, 1969; Dropkin and Gamboa, 1981; Roseler et al., 1984). Dominant females have higher levels of juvenile hormone (JH) than subordinates, and JH treatment increases dominance behavior (Roseler et al., 1984), ovary development (Bohm, 1972), and guarding behavior (Giray et al., 2005). Gynes possess large, well-developed fat bodies with higher levels of storage proteins than workers

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(Sullivan and Strassmann, 1984; Hunt et al., 2003). Despite the wealth of natural history information about *Polistes* (Evans and West-Eberhard, 1970; West-Eberhard 1996), there is much still to be learned about the mechanistic basis of caste and behavioral differences in *Polistes* on both physiological and molecular levels (Sumner, 2006; Hunt, 2007; Toth et al., 2007).

Nutritional and reproductive conditions and interactions between the two can profoundly affect the behavior of individuals and, thus, colony division of labor (Markiewicz and O'Donnell, 2001). In social insects in general, reproductive potential is associated with adequate nutrient reserves (Wheeler, 1986). Individuals that forage usually have low nutritional state and reduced reproductive capacity. *Polistes* foraging behavior may be regulated by dominance interactions (Gamboa et al., 1990; Pardi, 1948). Subordinate individuals tend to perform disproportionately more foraging behavior, and when foragers return to the nest they may be aggressively solicited by other females, usually the queen or dominant workers, to share food with them (West-Eberhard, 1969). Thus, dominant individuals have a trophic advantage because they receive food from subordinates and spend less time in energetically-demanding foraging behavior. It is still not known whether differences in nutritional state among *Polistes* females regulate division of labor for foraging, as in several advanced eusocial insect species (reviewed in Toth and Robinson, 2005). Understanding relationships among nutrition, reproductive state, and behavior may provide important insights into how *Polistes* division of labor is organized.

We examined abdominal lipids and ovary development in four groups of female *Polistes metricus* representing both reproductive and worker castes. Gynes, foundresses, and queens represent sequential behavioral states that an individual of the reproductive caste passes through in her lifetime, and workers are the nonreproductive caste. We used sensitive methods to measure lipids and ovaries and looked for correlations between the two physiological measures between and within groups. We also examined relationships between these physiological traits and published data from the same set of wasps on expression (mRNA levels) of 32 genes previously associated with division of labor among worker honey bees. An earlier study showed that *P. metricus* foundresses, queens, workers, and gynes differ significantly in expression of several of these genes in brains and associated tissues in the head (Toth et al., 2007). We sought to examine relationships among gene expression, lipid levels, and ovary development in order to better understand the mechanisms that underlie caste and behavioral differences in *Polistes*.

We also supplemented the physiological and gene expression data with information on tattered wing margins, which we determined to be an indicator of foraging experience, and with microsatellite data to confirm that workers were the daughters of the queens. From all these measures we were able to determine a

suite of criteria that could be used to assign individual wasps' group status without extensive behavioral observations.

## Materials and methods

### Wasps

The same individual wasps in this study were examined for brain gene expression in a previously published study (Toth et al., 2007). *Polistes metricus* were collected May–August 2005 from two sites in east central Illinois—Allerton Park in Piatt County and the Vermilion River Observatory (VRO) in Vermilion County. The sites are approximately 100 km apart, and we thus likely sampled different populations. In February and March, 2005, we placed 50 open-bottom wooden nest boxes (15 cm on each side) at each site and allowed foundresses to establish colonies in them (Rabb, 1960). Occupancy was approximately 40% Allerton and 50% at VRO. After colony establishment, 1 cm wire mesh was affixed to the bottoms of boxes to prevent predation by birds and mammals. We collected  $N = 10\text{--}13$  wasps per group per site, totaling 86 individuals from 45 nests. Individuals representing different groups were collected from the same colonies when possible, in order to minimize possible effects of genetic heterogeneity on our variables of interest. We could not control this completely, because foundress removal precludes further sampling.

Foundresses were marked on the thorax with Testor's enamel. From the beginning of worker emergence (the last week in June in our populations) through gyne emergence (mid August), newly emerged wasps were individually marked every 2–3 days so that approximate age was known for each. Only wasps that were paint-marked as foundresses were subsequently collected as queens. Only females that emerged after the emergence of the first males were collected as gynes (Noonan, 1978; Metcalf, 1980; Suzuki, 1993). Because the social and genetic context (e.g., a worker's relatedness to reproductive individuals) may affect a worker's physiological state, we used microsatellite analysis (see *Microsatellite analyses* below) to verify that workers were the daughters of the queen on their nest, eliminating the possibility that they were produced by undetected usurpers (Gamboa, 1978; Klahn, 1988).

Wasps were collected between 0600 and 0830 hours, prior to the initiation of foraging. All colony members were anaesthetized using carbon dioxide gas for one minute or less, and collected wasps were placed directly onto dry ice, then stored at  $-80^{\circ}\text{C}$  until analysis.

### Female groups

The *Polistes* colony life cycle encompasses differences among females in reproductive activity, behavioral role, and foraging status (Table 1). 1) **Foundresses**: During the spring founding phase, foundresses construct nests, lay eggs, forage, and provision larvae to produce a first batch of offspring. Although there is a low rate of cooperative founding in our populations (1–8%, A.L.T., pers. obs.), we focused only on solitary foundresses, which are active egg-layers and foragers. 2) **Workers**: The first female offspring emerge in mid-summer and become workers, taking over nest construction and foraging. Most workers forage to some extent, but dominant individuals generally forage less (Pardi, 1948). We collected only workers with evidence of wing wear in order to focus on the most actively foraging workers (see *Assessment of foraging experience using wing wear* below). 3) **Queens**: After the emergence of workers, the foundress takes on the role of a queen specializing on reproduction (Pardi, 1948; West-Eberhard, 1969; Dew and Michener, 1981). 4) **Gynes**: In late summer, colonies produce a second set of female offspring, gynes, that will mate and overwinter and then attempt to found colonies the following year. While on the nest in late summer and autumn, these wasps generally do not forage (West-Eberhard, 1969).

**Table 1.** The four *Polistes* female groups examined in this study can be classified by foraging state (foraging or non-foraging) and reproductive state (reproductive or non-reproductive). Note that foundresses, queens, and gynes are all members of the reproductive “caste” in different phases of the colony cycle, whereas workers are members of the worker “caste”.

	Foraging	Non-foraging
Reproductive	Foundress	Queen
Non-reproductive	Foraging worker	Gyne

#### Lipid analysis

We quantified lipids by a colorimetric assay with a sulphophosphovanillin reagent, a method that has provided highly consistent and sensitive results for honey bees (Toth and Robinson, 2005; Toth et al., 2005), with two modifications. First, tissue was homogenized in Kontes tissue grinders as previously but using an electric screwdriver rather than the standard pestle due to the extreme toughness of the wasps' cuticles. Second, only 30  $\mu$ l of lipid extract were used in each assay run (as opposed to 100 $\mu$ l for honey bees) because of much higher lipid levels in wasps.

#### Measurement of ovary development

Ovaries were dissected under a dissecting microscope from freshly collected abdomens, placed in 100% ethanol for storage, and subsequently examined under a compound microscope with camera attachment. Ovaries were photographed, and all visible ovarioles and mature oocytes were measured using Image Pro Plus Software. The largest five measurements were averaged to provide a single measure of “mean maximum ovariole/oocyte length” for each individual.

#### Gene expression data

Gene expression for 32 genes was examined in dissected brains from the same wasps for which lipid and ovary measurements were taken. The RNA levels of each gene were measured using real time quantitative RT-PCR as described in Toth et al. (2007). In that study, we found evidence that 4 genes were likely to be expressed in contaminating tissues surrounding the brain. For the current study, we examined all possible correlations between either lipid or ovary measures and expression of each of the 32 genes, including those expressed in non-brain tissue.

#### Assessment of foraging experience using wing wear

Wing wear has been used to indicate foraging experience in other species (Breed et al., 1990; Gobbi et al., 2006). To investigate whether wing wear – small tears or irregular edges on wing margins – was associated with foraging activity in *Polistes metricus*, we observed 5 worker phase colonies in nest boxes at VRO on 18 July (14:50–16:10), 19 July (14:00–16:25), 20 July (14:13–15:01) and 21 July (10:00–12:05 and 13:38–15:27) 2005. All females were individually paint marked. Two or three colonies were observed simultaneously, with all 5 colonies observed for a total of 13 hours (each set alternately every 30 minutes by two observers). We recorded the number of foraging trips and presence and (if possible) type of foraging load for each wasp (prey or pulp). The day after the end of these observations, all individuals were anaesthetized with CO<sub>2</sub> and scored for the presence/absence of wing wear using a 5x magnifier.

For all collected foundresses, queens, workers and gynes described above, wing margins were examined under a dissecting microscope and scored for wing wear presence/absence. The presence or absence of wing wear was most informative for the worker group, as there is known

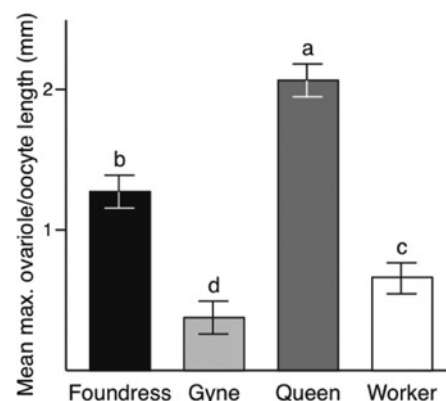
to be a great deal of variability in foraging activity of workers on *P. metricus* nests (Dew and Michener, 1981). When collecting workers, we selected the worker with the most visibly worn wings from each colony.

#### Microsatellite analyses

In 19 of the 23 worker phase colonies (no tissue samples were available for workers from the remaining four colonies), we genotyped queens and workers at 6 microsatellite loci: Pbe128, Pbe203, Pbe205, Pbe269, Pbe440, and Pbe424 (Strassmann et al., 1997) following previously published protocols (Henshaw et al. 2004). We estimated relatedness between the workers and the queens on each colony using the computer program Relatedness 5.0 (Goodnight and Queller, 1999a; Goodnight and Queller, 1999b), and assessed the most likely pedigree relationship between workers and queens using the program Kinship 1.3 (Goodnight and Queller, 1999a).

#### Statistical analyses

We used R statistical software for all analyses. We compared means for wing wear/foraging rate with unpaired t-tests assuming unequal variance. Proportions of wasps with and without wing wear observed foraging were compared using Fisher's exact test (fisher.test function). Mixed model ANOVA for ovary, lipid, and individual gene comparisons were performed using the lme function (library NLME). For lipid and ovary ANOVA, our linear model considered group (foundress, gyne, queen, or worker) as a fixed factor and colony nested within site as a random factor. Post hoc comparisons were made using the pairwise.t.test function with a Bonferroni correction. Pearson correlations between lipid and ovary measures, between lipid measures and gene expression, and between ovary measures and gene expression were performed using the cor.test function.

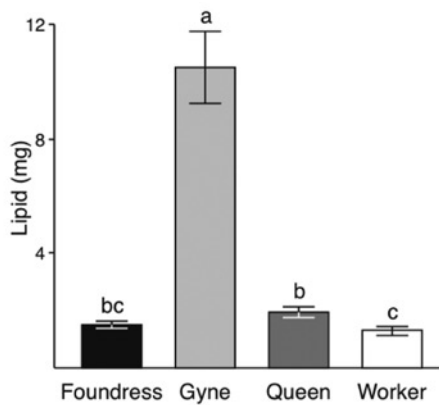


**Figure 1.** Ovary measurements of females from the four behavioral groups (foundresses (n=22), gynes (n=14), queens (n=21), and workers (n=20)). Different letters above bars represent significant differences,  $p < 0.05$ .

## Results

### Ovary development

We found marked differences in ovary development between our groups of interest ( $F_{3,26} = 109.10$ ,  $p < 0.0001$ , Fig. 1). In Bonferroni-adjusted pairwise comparisons, all four groups differed significantly from each other, with queens > foundresses > workers > gynes (Fig. 1). In five



**Figure 2.** Abdominal lipid measurements of females from the four behavioral groups (foundresses (n=22), gynes (n=20), queens (n=20), and workers (n=22)). Different letters above bars represent significant differences,  $p < 0.05$ .

gynes, ovaries were extremely small and no ovarioles could be found for measurement, so these were excluded from the analysis, but they can be considered to show very low levels of ovary development.

#### Abdominal lipid stores

Groups differed significantly in abdominal lipids ( $F_{3,26} = 104.3017$ ,  $p < 0.0001$ , Fig. 2). Gynes had significantly higher lipid stores (at least 6x) than other behavioral groups. Lipid stores in queens were much lower than in gynes but significantly (approximately 2x) higher than in workers, which had the lowest lipid stores. Foundresses were intermediate between queens and workers. Lipid stores were not normalized to body size, but this is unlikely to greatly affect the results, because *Polistes metricus* females vary in size by approximately 10% (Haggard and Gamboa, 1980), whereas group differences in lipid stores were on the order of 100–600%.

#### Relationship between ovary development and lipid stores

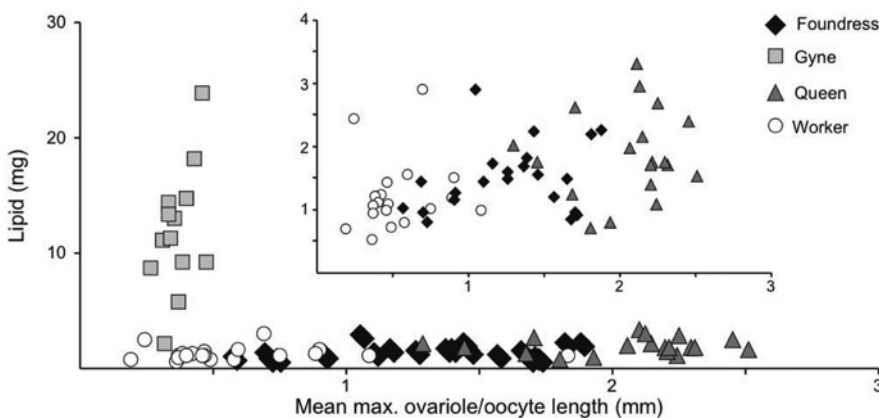
There was a significant negative relationship between ovary development and lipid stores (Pearson correlation analysis:  $R = -0.33$ ,  $P = 0.007$ ), although the relationship was clearly nonlinear (Fig. 3). However, when gynes, with their enormous lipid stores, are considered separately, a different picture emerges. For gynes alone, there is a marginally non-significant positive correlation between ovary development and lipid stores ( $R = 0.49$ ,  $P = 0.07$ ). For foundresses, queens, and workers combined there is a significant positive correlation ( $R = 0.40$ ,  $P = 0.001$ , Fig. 3, inset).

#### Correlations between gene expression and physiological measures

Of the 32 genes examined in Toth et al. (2007), 9 showed significant correlations with physiological measures (Table 2); all 9 of these were previously shown to be significantly differentially regulated across the groups. *Pmtctp*, *PmInR1*, and *PmInR2* were positively correlated with lipid levels, and *Pmsh3 $\beta$* , *PmCG11971-like*, and *Pmg5sd* were negatively correlated with lipid levels. *PmVg* was positively correlated with ovary development, and *PmILP2* and *Pmmcp* negatively correlated with ovary development. Of these, *PmVg* and *Pmg5sd* showed evidence of being expressed in non-brain tissues in the head (Toth et al., 2007). A list of correlation coefficients, significance values, and descriptions of genes with physiological correlates (and their putative functions) are given in Table 2.

#### Wing wear as an indicator of foraging experience

All foundresses and queens had noticeable wing wear, and all gynes did not. Our worker behavioral observations showed a clear differentiation between the foraging activity of workers with and without wing wear. Of 25



**Figure 3.** Relationship between lipid stores and ovary development for all four behavioral groups (n=76), Pearson correlation coefficient ( $R = -0.33$ ,  $P = 0.007$ ). Inset: Relationship between lipid stores and ovary development for foundresses, queens, and workers (n= 62,  $R = 0.40$ ,  $P = 0.001$ ).

**Table 2.** Significant correlations between brain\* gene expression and ovary and lipid measures. \* Indicates genes that are likely to be expressed in non-brain tissues in the head (Toth et al., 2007). *Polistes metricus* gene names were assigned on the basis of orthology to *Apis mellifera* genes and putative functions were assigned on the basis of similarity to *Drosophila melanogaster* genes, as in Toth et al. 2007.

Physiology-gene correlation	Gene description	Pearson R	P-value
Lipid- <i>Pmtctp</i>	Translationally controlled tumor protein	0.58	<0.001
Lipid- <i>PmInR2</i>	Insulin-like receptor 2	0.46	<0.001
Lipid- <i>PmInR1</i>	Insulin-like receptor 1	0.34	0.002
Lipid- <i>Pmsh3β</i>	Copper/zinc superoxide dismutase	-0.25	0.021
Lipid- <i>PmCG11971</i>	Unnamed, nucleic acid and zinc binding	-0.23	0.031
Lipid- <i>Pmg5sd*</i>	Glutamate-5-semialdehyde dehydrogenase	-0.21	0.048
Ovary- <i>PmVg*</i>	Vitellogenin, yolk protein	0.31	0.006
Ovary- <i>PmILP2</i>	Insulin-like peptide 2	-0.26	0.029
Ovary- <i>Pmmcp</i>	Monocarboxylate porter	-0.25	0.032

workers from 5 colonies, we found 15 with and 10 without wing wear. The presence of wing wear was always associated with foraging activity. All 15 workers with wing wear were observed making trips off the nest, as compared to 4 of 10 workers without wing wear, a significant difference (Fisher's exact test  $p=0.001$ ). All 6 wasps that were never observed foraging had no wing wear. Wasps that were observed foraging rarely (on only one occasion) sometimes had wing wear (3/7=42.9% with wing wear). All 12 wasps that were observed foraging more than once had wing wear. This suggests that wasps with more foraging activity also have more wing wear. We pooled all observations of trips off the nest, including returns with prey, pulp, or no visible forage (presumably water or nectar), and found the average foraging rate (defined as trips off nest per hour) to be significantly higher in individuals with wing wear than without wing wear ( $t_{15,02} = -4.6472$ ,  $p = 0.0003$ , Fig. 4).

#### Microsatellite analyses

Wasp pairs from all 19 analyzed colonies had relatedness estimates that overlapped 0.50 (data not shown), the value expected for mothers and daughters. The average relatedness between workers and queens of the same colony was  $0.49 \pm 0.07$  (mean  $\pm$  95% confidence interval). Likelihood analysis found that all pairs were significantly more likely to be related than unrelated (data not shown). The analysis could not reject a mother-daughter relationship for any wasp pair.

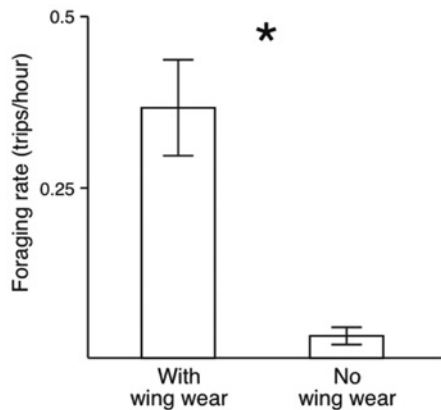
#### Discussion

Even though caste differences in primitively eusocial insects are less well-defined than in advanced social groups (Wilson, 1971), we found significant differences in physiological state of *Polistes metricus* females in both the amount of stored abdominal lipid and the extent of ovary development. At the same time, our finding that physio-

logical measures overlap to some extent between reproductive and worker castes (e.g., lipid stores in workers and queens, ovary measures in workers and foundresses) confirms that caste differences in *Polistes* are less distinct than in many advanced eusocial species. This physiological flexibility reflects and/or allows the totipotency of adult *Polistes* females, which have the capacity to act as either workers or reproductives depending on the social environment (Reeve et al., 1998; Starks, 2001; Strassmann et al., 2004; Hunt and Amdam, 2005).

We found the highest ovary development in queens, consistent with previous studies of *Polistes* (Pardi, 1948; West-Eberhard, 1969; Roseler et al., 1984). Ovary development in foundresses was also high, but significantly lower than that of queens. This suggests that after workers emerge in *P. metricus* colonies and the queen ceases to forage, she is able to increase ovary development and egg-laying over her previous role as a foraging foundress.

Our measure of mean maximum ovariole/oocyte length proved to be a sensitive measure of ovary development that allowed us to detect slightly, but significantly, higher ovary development in workers than gynes. Differences in ovary size between gynes and workers have not been previously reported, but prior evidence nonetheless suggested extremely different reproductive physiology in these two groups. If isolated, gynes do not initiate new nests, and they fail to show ovary development when placed in warm, long day conditions that induce ovary development in workers (Bohm, 1972). On the other hand, workers' ovaries grow with JH application, workers will initiate new nests in isolation (Bohm, 1972), and queen removal causes ovarian activation in workers (Strassmann et al., 2004). Our data show that gynes exhibit lower ovary development and less inter-individual variation in this measure than workers, which supports the hypothesis that they are physiologically constrained from ovary development, i.e., in a state of reproductive diapause (Hunt and Amdam, 2005; Hunt, 2007; Hunt et al., 2007). The low ovary development of gynes could also reflect that they were collected at a



**Figure 4.** Average foraging rate was significantly higher for worker wasps with wing wear ( $n=15$ ) compared to wasps without wing wear ( $n=10$ ),  $t=-4.65$ , \* designates  $p=0.0003$ .

young age relative to the other groups; some ovarian activation has been observed in gynes found in aggregations preparing to overwinter in *Polistes dominulus* (Dapporto et al., 2006). In general, the slightly higher level of ovary development we found in workers compared to gynes, supports the hypothesis that workers are in a “reproduce now” physiological state (Hunt and Amdam, 2005; Hunt, 2007; Hunt et al., 2007) that is repressed by social interactions with other individuals, including the queen and dominant workers (Pardi, 1948) as well as the larvae (Marchal, 1897; Hunt, 2007).

Lipid stores were lowest in *P. metricus* foraging groups (workers, foundresses) and extremely high in gynes. Although queens had approximately 2x the lipid stores of workers, queens were quite low in comparison to gynes. Because we measured lipids on abdomens from which ovaries had been removed, our lipid measurements do not account for lipids contained in the ovaries. In queens, stored nutrients are likely to be directed to the ovaries, therefore it is probable that the overall lipid stores of queens if ovaries were included would be greater than what we report here. Even so, our finding of high lipid stores in gynes coincides with previous studies demonstrating high storage protein levels (Hunt et al., 2003) and the appearance of large fat bodies in gynes compared to other groups (Eickwort, 1969; Strassmann et al., 1984; Karsai and Hunt, 2002). Thus, our results support the idea that gynes invest in energy storage, as opposed to reproductive development, as an adaptation for overwintering (Strassmann et al., 1984).

Our lipid results also generally agree with the well-documented correlation between low nutritional state and foraging behavior in social insects (Porter and Jorgenson, 1981; Tschinkel, 1987; 1998; Blanchard et al., 2000; Markiewicz and O'Donnell, 2001; Toth and Robinson, 2005; Toth et al., 2005). Although in this case we did not demonstrate a causal relationship between lipid depletion and foraging behavior (see Toth et al. 2005), our correlative results provide support for the

hypothesis that nutritional differences may be a common mechanism underlying division of labor for foraging (Toth and Robinson, 2005, 2007; Smith et al., 2008). Because of the known trophic advantage of behaviorally dominant females in *Polistes* and other primitively eusocial insects, it is likely that a gradient in nutritional status associated with dominance status may help to organize division of labor in these societies.

The negative relationship between reproductive development and nutrient storage, which is only apparent when considered across all four female groups, suggests different strategies of nutrient allocation exist for different groups. Gynes invest heavily in nutrient storage, whereas queens and foundresses invest in ovary development. Workers are the most nutrient deprived and have low levels of both lipid stores and ovary development. Within foundresses, workers, and queens, there is a positive correlation between ovary development and lipid stores, which suggests that in behavioral groups that have immediate reproductive potential (as opposed to gynes in a state of reproductive diapause), ovary development may be limited by available nutrients.

Our results also show connections between nutritional and reproductive physiology and the expression of 9 genes, all of which were significantly differentially regulated between the four female groups (Toth et al., 2007). These results suggest there may be connections between physiological state and the expression of behaviorally-relevant genes in the brain. However, it is important to note that two of these genes, *PmVg* and *Pmg5sd* showed evidence of being expressed in non-brain tissues in the head (Toth et al., 2007; see also Corona et al., 2007). The positive correlation between ovary development and expression of *PmVg* suggests that, unlike in honey bees (Page and Amdam, 2007), vitellogenin may retain its ancestral reproductive role in *Polistes*. Two metabolic enzymes (*Pmg5sd*, *Pmmcp*) were among the differentially regulated genes, highlighting a possible connection between physiological processes and metabolism in the brain and surrounding tissues. Interestingly, we found a negative correlation between ovary development and expression of *PmILP2* (although the correlation only explained 26% of the variation), but positive correlations between lipid levels and *PmInR1* and *PmInR2*. This suggests that in *P. metricus* the insulin-like growth factor 1 (IIS) pathway may play a role in the allocation of energy to nutrient storage and reproduction, as is known for other insects (Wu and Brown, 2006). The IIS pathway has also been implicated in both queen-worker (Wheeler et al., 2005; Patel et al., 2007) and worker-worker (Ament et al., 2008) division of labor in honey bees. Genes in the IIS pathway thus are good candidates for follow-up studies on how nutrition and reproduction can affect brain function and social behavior in *Polistes* and other social insects.

Our microsatellite analyses verified that workers were all daughters of queens. This is an important observation, as nest-switching, nest usurpation, and multiple egg-

layers have been observed in other *Polistes* species and even in other populations of *P. metricus* (Gamboa, 1978; Klahn, 1988). This result gives further strength to our queen and worker group assignments and suggests that nest takeover events were limited in our populations of *P. metricus*.

Wing wear is a good indicator of foraging experience in *P. metricus* females. Our behavioral observations showed that workers with wing wear had an average 10x greater foraging rate than contemporaneous nestmates with no wing wear. Even in a relatively short survey of behavior, all workers with wing wear were observed foraging, while far fewer workers with no wing wear were observed foraging. This agrees with previous studies indicating that most *Polistes* workers engage in foraging behavior, but that the tendency to forage varies greatly across individuals (Dew and Michener, 1981). The fact that wing wear was present on all foundresses and queens but no gynes, which do not usually forage, further demonstrates that wing wear can serve as an accurate and easily measured indicator of foraging experience.

As a technical note, our results indicate it is possible to distinguish behavioral castes of morphologically identical *Polistes metricus* females on the basis of a few, relatively easily measured parameters. A flow chart (Fig. 5) that combines information on season of collection, presence/absence of wing wear, and upper and lower ranges for ovary and lipid measurements (that do not encompass regions of overlap between the groups) shows how it is possible to determine whether a given wasp is likely to be a foundress, queen, worker, or gyne. A similar method can likely be applied to other temperate *Polistes* species, although the exact values for ovary and lipid measurements may vary according to species. This method of group assignment may in some cases be preferable to extensive behavioral analyses in future studies.

## Acknowledgments

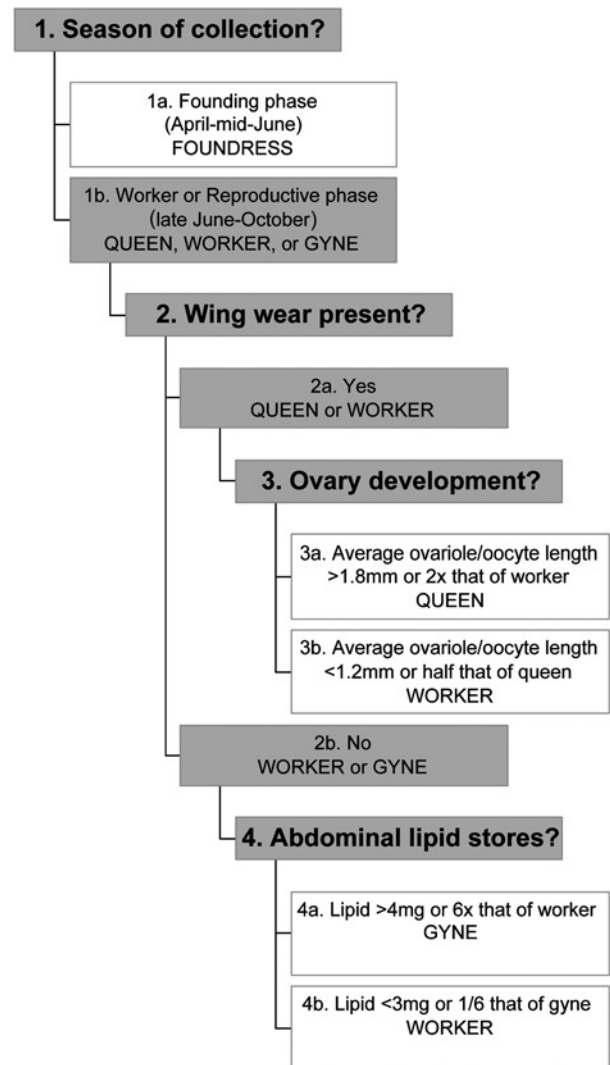
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## References

Ament S.A., Corona M., Pollock H.S. and Robinson G.E. 2008. Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. *Proc. Natl. Acad. Sci. USA* **105**: 4226–4231

Blanchard G.B., Orledge G.M., Reynolds S.E. and Franks N.R. 2000. Division of labour and seasonality in the ant *Leptothorax albipennis*: worker corpulence and its influence on behavior. *Anim. Behav.* **59**: 723–738

Bohm M.K. 1972. Effects of environment and juvenile hormone on ovaries of the wasp, *Polistes metricus*. *J. Insect Physiol.* **18**: 1875–1883



**Figure 5.** Flow chart for identifying *Polistes metricus* females as belonging to one of four groups (foundress, queen, worker, gyne) based on information on 1) season of collection, 2) wing wear, 3) ovary development, and 4) lipid stores.

Breed M.D., Robinson G.E. and Page R.E. 1990. Division of labor during honey bee colony defense. *Behav. Ecol. Sociobiol.* **27**: 395–401

Corona M., Velarde R.A., Remolina S., Moran-Lauter A., Wang Y., Hughes K.A. and Robinson G.E. 2007. Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. *Proc. Natl. Acad. Sci. USA* **104**: 7128–7133

Dapporto L., Palagi E., Cini A. and Turillazzi S. 2006. Pre-hibernating aggregations of *Polistes dominulus*: an occasion to study early dominance assessment in social insects. *Naturwissenschaften* **93**: 321–324

Dew H.E. and Michener C.D. 1981. Division of labor among workers of *Polistes metricus* (Hymenoptera, Vespidae) – laboratory foraging activities. *Insect. Soc.* **28**: 87–101

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

Eickwort K. 1969. Separation of the castes of *Polistes exclamans* and notes on its biology (Hym.: Vespidae). *Insect. Soc.* **16**: 67–72

Evans H.E. and West-Eberhard M.J. 1970. *The Wasps*. University of Michigan Press, Ann Arbor, Michigan. 265 pp

- Gamboa G.J. 1978. Intraspecific defense: advantage of social cooperation among paper wasp foundresses. *Science* **199**: 1463–1465
- Gamboa G.J., Wacker T.L., Scope J.A., Cornell T.J. and Shellman-Reeve J. 1990. The mechanism of queen regulation of foraging by workers in paper wasps (*Polistes fuscatus*, Hymenoptera, Vespidae). *Ethology* **85**: 335–343
- Giray T., Giovanetti M. and West-Eberhard M.J. 2005. Juvenile hormone, reproduction, and worker behavior in the neotropical social wasp *Polistes canadensis*. *Proc. Natl. Acad. Sci. USA* **102**: 3330–3335
- Gobbi N., Noll F.B. and Penna M.A.H. 2006. "Winter" aggregations, colony cycle, and seasonal phenotypic change in the paper wasp *Polistes versicolor* in subtropical Brazil. *Naturwissenschaften* **93**: 487–494
- Goodnight K.F. and Queller D.C. 1999a. Kinship 1.4 [Software].
- Goodnight K.F. and Queller D.C. 1999b. Relatedness 5.0 [Software].
- Haggard C.M. and Gamboa G.J. 1980. Seasonal variation in body size and reproductive condition of a paper wasp, *Polistes metricus* (Hymenoptera, Vespidae). *Can. Entomol.* **112**: 239–248
- Henshaw M.T., Robson S.K.A. and Crozier R.H. 2004. Queen number, queen cycling and queen loss: the evolution of complex multiple queen societies in the social wasp genus *Ropalidia*. *Behav. Ecol. Sociobiol.* **55**: 469–476
- Hunt J.H. 2007. *The Evolution of Social Wasps*. Oxford University Press, New York. 259 pp
- Hunt J.H. and Amdam G.V. 2005. Bivoltinism as an antecedent to eusociality in the paper wasp genus *Polistes*. *Science* **308**: 264–267
- Hunt J.H., Buck N.A. and Wheeler D.E. 2003. Storage proteins in vespid wasps: characterization, developmental pattern, and occurrence in adults. *J. Insect Physiol.* **49**: 14020–14025
- Hunt J.H., Kensing B.J., Kossuth J.A., Henshaw M.T., Norberg K., Wolschin F. and Amdam G.V. 2007. A diapause pathway underlies the gyne phenotype in *Polistes* wasps, revealing an evolutionary route to caste-containing insect societies. *Proc. Natl. Acad. Sci. U.S.A.* **104**: 14020–14025
- Karsai I. and Hunt J.H. 2002. Food quantity affects traits of offspring in the paper wasp *Polistes metricus* (Hymenoptera: Vespidae). *Envir. Entomol.* **31**: 99–106
- Klahn J. 1988. Intraspecific comb usurpation in the social wasp *Polistes fuscatus*. *Behav. Ecol. Sociobiol.* **23**: 1–8
- Marchal P. 1897. La castration nutritionnelle chez les Hyménoptères sociaux. *C.R. Soc. Biol. (Paris)*. **1897**: 556–557
- Markiewicz D.A. and O'Donnell S. 2001. Social dominance, task performance and nutrition: implications for reproduction in eusocial wasps. *J. Comp. Physiol. A* **187**: 327–333
- Metcalfe 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
- Noonan K.M. 1978. Sex ratio of parental investment in colonies of the social wasp *Polistes fuscatus*. *Science* **199**: 1354–1356
- Page R.E. and Amdam G.V. 2007. The making of a social insect: developmental architectures of social design. *Biosciences* **29**: 334–343
- Pardi L. 1948. Dominance order in *Polistes* wasps. *Physiol. Zool.* **21**: 1–13
- Patel A., Fondrk M.K., Kaftanoglu O., Emore C., Hunt G., Frederick K. and Amdam G.V. 2007. The making of a queen: TOR pathway is a key player in diphenic caste development. *PLoS ONE* **2**: e509
- Porter S.D. and Jorgensen C.D. 1981. Foragers of the harvester ant, *Pogonomyrmex owyheei*: a disposable caste? *Behav. Ecol. Sociobiol.* **9**: 247–256
- Rabb R.L. 1960. Biological studies of *Polistes* in North Carolina (Hymenoptera: Vespidae). *Ann. Entom. Soc. America* **53**: 111–121
- Reeve H.K. 1991. *Polistes*. In: *The Social Biology of Wasps* (Ross K.G. and Matthews R.G., Eds). Cornell University Press, Ithaca, New York. pp 99–148
- Reeve H.K., Peters J.M., Nonacs P. and Starks P.T. 1998. Dispersal of first "workers" in social wasps: Causes and implications of an alternative reproductive strategy. *Proc. Natl. Acad. Sci. USA* **95**: 13737–13742
- Roseler P.F., Roseler I., Strambi A. and Augier R. 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
- Smith C.R., Toth A.L., Suarez A.V. and Robinson G.E. 2008. Genetic and genomic analyses of the division of labour in insect societies. *Nature Rev. Genet.* **9**: 735–748
- Starks P.T. 2001. Alternative reproductive tactics in the paper wasp, *Polistes dominulus* with specific focus on the sit-and-wait tactic. *Ann. Zool. Fennici* **38**: 198–199
- Strassmann J.E., Barefield K., Solís C.R., Hughes C.R. and Queller D.C. 1997. Trinucleotide microsatellite loci for a social wasp, *Polistes*. *Mol. Ecol.* **6**: 97–100
- Strassmann J.E., Lee R.R., Rojas R.R. and Baust J.G. 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., Fortunato A., Cervo R., Turillazzi S., Damon J.M., and Queller D.C. 2004. The cost of queen loss in the social wasp *Polistes dominulus* (Hymenoptera: Vespidae). *J. Kans. Entomol. Soc.* **77**: 343–355
- Sullivan J.D. and Strassmann J.E. 1984. Physical variability among nest foundresses in the polygynous social wasp, *Polistes annularis*. *Behav. Ecol. Sociobiol.* **15**: 249–256
- Sumner S. 2006. Determining the molecular basis of sociality in insects: progress, prospects and potential in genomics. *Ann. Zool. Fennici* **43**: 423–442
- Suzuki T. 1993. Timing of insemination in terms of age of new reproductive females of a temperate-zone polistine wasp, *Polistes snelleni* (Hymenoptera, Vespidae). *Insect. Soc.* **40**: 342–343
- Toth A.L., Kantarovich S., Meisel A.F. and Robinson G.E. 2005. Nutritional status influences socially regulated foraging ontogeny in honey bees. *J. Exp. Biol.* **208**: 4641–4649
- Toth A.L. and Robinson G.E. 2005. Worker nutrition and division of labour in honeybees. *Anim. Behav.* **69**: 427–435
- Toth A.L. and Robinson G.E. 2007. Evo-devo and the evolution of social behavior. *Trends Genet.* **23**: 334–341
- Toth A.L., Varala K., Newman T.C., Miguez F.E., Hutchison S.K., Willoughby D.A., Simons J.F., Egholm M., Hunt J.H., Hudson M.E. and Robinson G.E. 2007. Wasp gene expression supports an evolutionary link between maternal behavior and eusociality. *Science* **318**: 441–444
- Tschinkel W.R. 1987. Seasonal life history and nest architecture of a winter-active ant, *Prenolepis imparis*. *Insect. Soc.* **34**: 143–164
- Tschinkel W.R. 1998. Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*: worker characteristics in relation to colony size and season. *Insect. Soc.* **45**: 385–410
- Turillazzi S. 1980. Seasonal variations in the size and anatomy of *Polistes gallicus* (L) (Hymenoptera, Vespidae). *Ital. J. Zool.* **14**: 63–75
- West-Eberhard M.J. 1969. The social biology of polistine wasps. *Misc. Publ. Museum Zool., Univ. Michigan* **140**: 1–101
- West-Eberhard M.J. 1996. Wasp societies as microcosms for the study of development and evolution. In: *Natural History and Evolution of Paper Wasps* (Turillazzi S. and West-Eberhard M.J., Eds). Oxford University Press, New York. pp 290–317
- Wheeler D.E. 1986. Developmental and physiological determinants of caste in social Hymenoptera: Evolutionary implications. *Am. Nat.* **128**: 13–43
- Wheeler D.E., Buck N. and Evans J.D. 2005. Expression of insulin pathway genes during the period of caste determination in the honey bee, *Apis mellifera*. *Ins. Mol. Biol.* **15**: 597–602
- Wilson E.O. 1971. *The Insect Societies*. Belknap Press of Harvard University Press, Cambridge, Mass. 548 pp
- Wu Q. and Brown M.R. 2006. Signaling and function of insulin-like peptides in insects. *Annu. Rev. Entomol.* **51**: 1–24