0014-4754/95/070749-04$1.50 + 0.20 © Birkhäuser Verlag Basel, 1995

Worker lipid stores decrease with outside-nest task performance in wasps: implications for the evolution of age polyethism

S. O'Donnell* and R. L. Jeanne

Department of Entomology, 1630 Linden Drive, University of Wisconsin, Madison (Wisconsin 53706, USA)
Received 22 July 1994; received after revision 5 November 1994; accepted 6 January 1995

Abstract. Recent models of energetically efficient division of labor in eusocial insects predict that risky tasks will be performed by workers with low nutrient content. We measured changes in workers' nutrient stores (chloroform:methanol extractable lipids) in relation to age-based division of labor in the eusocial wasp *Polybia occidentalis* to test this prediction. Distributions of age of first performance differed among task sets; tasks at increasing distance from the nest interior were performed later in life. However, individuals varied in the rate of passage through the task sequence. Weight of extractable lipids, corrected for differences in body weight, decreased with time elapsed since first performance of tasks outside the nest. Lipid content had a weaker negative relationship with adult age. Therefore, patterns of lipid decrease reflected individual differences in age polyethism. Age-based division of labor, with performance of risky tasks delayed until late in life by workers with depleted nutrient stores, may have evolved as an energy saving mechanism for insect colonies.
Key words. Divison of labor; *Polybia occidentalis*; Hymenoptera; Vespidae; age polyethism; lipid content; colony efficiency.

Age polyethism, a progression through a sequence of task specializations with age, is a common feature of division of labor among workers in diverse eusocial insect species[1,2]. Performance of riskier tasks (e.g., foraging) is typically delayed until late in worker life, although workers often differ in the rate of progression through the age-based task sequence[3–7]. Jeanne[8] developed a model linking colony-level energetics and behavioral differences among individual workers. This model predicts that, all else being equal, energetically expensive workers (e.g., those with high energy stores or nutritional content) will delay or avoid performing tasks associated with greater risk of mortality. The purpose of this study was to determine whether this prediction is upheld in *Polybia occidentalis*, a eusocial wasp with well-developed age polyethism[9]. We asked whether lipid content of *P. occidentalis* workers decreases with progression through the age-based task sequence, and whether lipid variation is better explained by individual differences in age polyethism than by adult age.

Polybia occidentalis workers progress through a series of task sets with age. Early task performance occurs inside the nest, then on the outer nest surface, and finally workers forage away from the nest. Foraging entails a high risk of mortality relative to in- and on-nest tasks[7]. Workers rarely revert to earlier task sets after advancing[9]. Within colonies, workers vary over a wide range in the age of first performance of task sets in the age sequence[7,9].

Lipid stores have been shown to vary among individuals and with adult age in eusocial insects[10,11]. Reproductive caste differentiation (e.g., differences between queens and workers) is based on variation in nutrition in many eusocial insect species (reviewed in refs 12, 13, see refs 14, 15 on wasps). Little is known about the relationship of nutrition to behavioral differentiation within worker castes[13], but food supplementation experiments by Rossi and Hunt[16] suggest that lipids are important as energy stores for wasp workers. We used weight of chloroform:methanol extractable lipids[17] as an index of nutrient content of individual workers.

Materials and methods

Wasp introductions, behavioral observations, and collections. Field work was conducted from 28 August to 18 October 1991 at Centro Ecologico la Pacífica in Guanacaste Province, Republic of Costa Rica. Two *P. occidentalis* colonies of moderate size (Colony A: 4 layers of comb, 953 unmarked adults; Colony B: 4 layers of comb, 381 unmarked adults at the end of the study) were chosen as observation colonies. Every 24 h all newly-emerged adults were removed from combs from other colonies maintained in the laboratory at ambient temperature. At 2-day intervals a cohort of 15 of these wasps was anesthetized with ether, marked with paint pens for individual recognition, and introduced into an observation colony to serve as subjects for the study. Newly-emerged ($\leq$24 h old) *P. occidentalis* adults are

* Corresponding author: Current address for correspondence: Department of Entomology, University of California, Davis (California 95616, USA), Fax +1 916 752 1537.

accepted when introduced into non-natal observation colonies[7].

On days alternating with worker introductions behavioral data were collected by an observer (S. O'Donnell) at the colony during continuous sessions of 1.5 h in the morning and 1 h in the afternoon. Behavioral acts of all visible wasps were recorded in scan samples of the nest entrance and the entire nest surface every 10 min (see ref. 18 for ethogram). All occurrences of marked forager arrivals at the nest were also recorded.

After three weeks of observations, the colonies were collected. Collections were made at night to ensure that all workers would be present. Nests were enclosed in a plastic bag with cotton soaked in ether; wasps did not contact liquid ether. Marked wasps were sorted from unmarked wasps and frozen at -10 °C until lipid extraction.

Lipid extractions. Frozen wasps were dried for 48 h at 60 °C under a vacuum of -70 kPa, which preliminary measurements showed was sufficient to reach constant dry weight. All weights were to the nearest 0.1 mg on a Mettler AE200 electronic balance. After dry weight was measured, the wasps were individually extracted at room temperature (approximately 22 °C) with aliquots of 5 ml chloroform-methanol (2:1 v/v) mixture[17] on a mechanical shaker. Wasps were extracted for 24 h each of four solvent aliquots, redried for 24 h, weighed, extracted once more, and final weight was measured (total solvent volume = 25 ml per wasp; total duration of contact with solvent = 120 h). In cases where the two post-extraction weights differed final weight was estimated by averaging. Lipid weight was calculated by subtracting final weight from dry weight.

Statistical analysis. We used survival analysis[19], which tests for differences in distributions of the time of occurrence of events, to examine differences in first age of performance among behavioral acts. To test the relationship between lipid content and variation in age polyethism we used linear multiple regression[20]. Multiple regression provides tests for associations between a dependent variable and independent variable(s) of interest after accounting for the effects of other independent variables (in this case, post-extraction weight).

Results

Changes in lipid content with task performance. Distributions of ages of first observed performance differed among inside-nest tasks, outside-nest tasks, foraging, and nest defense (fig. 1; Survival analysis Wilcoxon $X^2 = 187.01$, $df = 3$, $p < 0.001$). However, workers differed in their rates of behavioral development such that some individuals of the same age had spent different amounts of time performing particular tasks at the end of the study. Few of the 109 marked workers present in each colony upon collection had performed

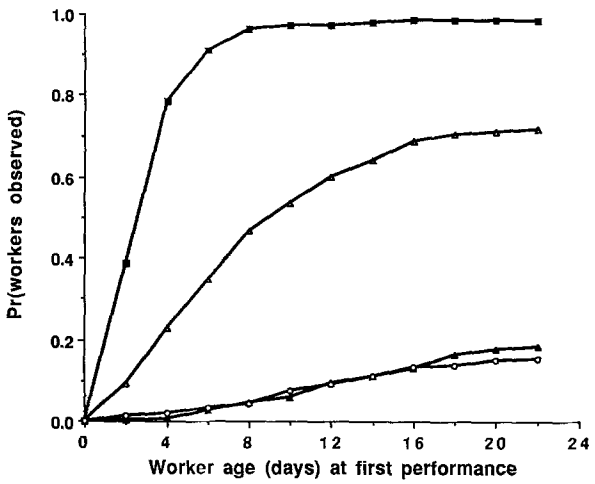

Figure 1. The cumulative proportion of workers observed plotted against ages of first performance of behavioral acts (abscissa) in four categories by *P. occidentalis* workers. Behavioral categories: In nest = acts performed inside nest, Outside nest = acts on nest surface, Foraging = arriving at nest with collected materials, Defense = aggressive behavior toward intruders. ■ = In nest; △ = outside nest; ▲ = foraging; ○ = defense.

foraging tasks by the end of the study (Colony A: 13 foragers; Colony B: 20 foragers). Time elapsed since first observed performance of acts on the nest surface (the task set typically performed prior to foraging) was used as an index of workers' extent of passage through the task sequence.

Lipid extractions were performed on 109 marked individuals from each colony. Total weight of extractable body lipids was positively correlated with post-extraction weight in both colonies (Colony A: $r = 0.29$, $p < 0.005$; Colony B: $r = 0.43$, $p < 0.001$). In the linear multiple regression analysis the interaction of (post-extraction weight) × (days outside) was not significant (Colony A: $t = 0.20$, $p > 0.75$; Colony B: $t = 1.01$, $p > 0.25$), and the interaction term was dropped from the regression model. After accounting for the effects of variation in post-extraction weight, lipid weight decreased significantly with increasing days outside in both colonies (Colony A: $t = -2.44$, $p < 0.05$; Colony B: $t = -3.95$, $p < 0.01$; fig. 2).

Changes in lipid content with worker age. In both colonies the effect of worker age on lipid weight, after accounting for variation due to post-extraction weight, was weaker than the effect of days outside; this relationship was not significant in one colony (Colony A: $t = 1.94$, $p > 0.05$; Colony B: $t = -3.80$, $p < 0.001$). Days outside was correlated with worker age at the end of the study in both colonies (Colony A: $r = 0.80$, $df = 107$, $p < 0.001$; Colony B: $r = 0.93$, $df = 107$, $p < 0.001$); therefore, worker age did not explain a significant amount of variance in lipid content after variation due to post-extraction weight and days outside were accounted for in the regression model (Colony A: $t = -0.01$, $p > 0.90$; Colony B: $t = 0.42$, $p > 0.75$).

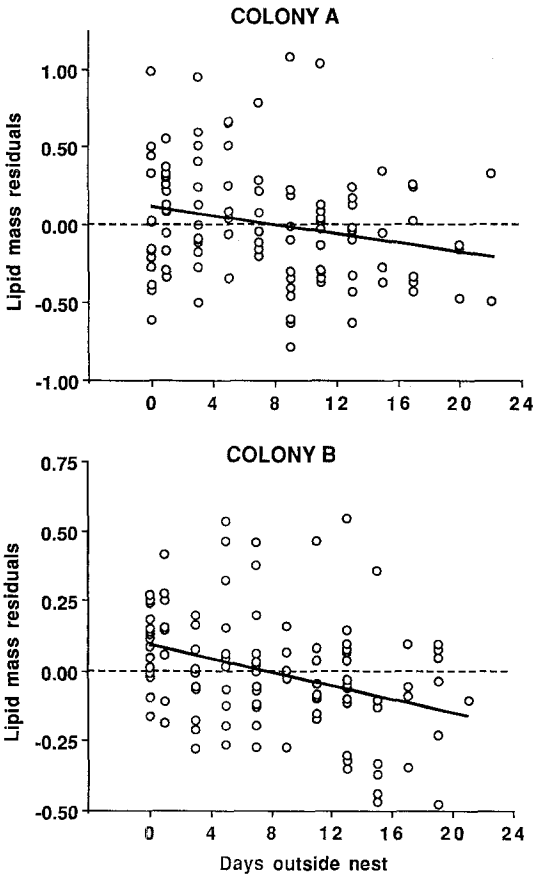

Figure 2. Lipid content of *P. occidentalis* workers plotted against the number of days elapsed since they were first observed performing acts outside the nest. Workers with zero days outside had performed only in-nest tasks. Lipid values shown are residuals (remaining unexplained variation in the dependent variable after regressing against an independent variable) of lipid weight after accounting for the effect of post-extraction body weight with linear multiple regression. The ordinate value of zero (horizontal dotted line) represents the mean of residuals; the solid line is the linear regression best fit of lipid residuals vs days outside.

Discussion

Lipid content, adjusted for body weight, declined with the number of days since first performance of acts outside the nest in *P. occidentalis* workers. Because this study employed individually marked workers of known age, we were able to separate the relationship of lipid content with variability in age polyethism from lipid content changes with worker age. The decrease in lipid content was better explained by the number of days elapsed since first outside-nest task performance than by adult age.

Models of adaptive energy investment by insect colonies predict that colony-level selection will maximize the gain in reproductive output per unit of energy expended to produce and maintain workers[2,8]. We propose that increased colony energetic efficiency may explain the nearly universal pattern of age polyethism in eusocial Hymenoptera in which risky tasks are performed last in the age sequence of tasks. Insect colonies would be more efficient if energetically-depleted workers performed the risky tasks because the energy contained in workers that die is generally lost to the colony. For example, Porter and Jorgensen[21] suggested that foraging *Pogonomyrmex owyheei* workers with reduced body weight were a 'disposable' caste. We predict that decreases in lipid content of workers will precede or coincide with the performance of tasks entailing high risk of mortality (see below) in a pattern analogous to deciduous trees taking in nutrients from leaves before they are abscised.

Few *P. occidentalis* workers had engaged in foraging by the end of this study; therefore, the decrease in lipids we measured preceded performance of high-risk and presumably energetically expensive tasks in most individuals. Similarly, MacKay[10] noted a gradual decrease in lipid content of harvester ant (*Pogonomyrmex* spp.) nest workers at increasing distance from the central brood area, suggesting that the decline in lipid stores preceded performance of risky foraging tasks in these ants as well. Martin[22] found that hornet (*Vespa affinis*) workers decreased in weight over 11–13 days after adult emergence, while similarly-aged queens in the same nests gained weight and developed copious fat bodies.

Although *P. occidentalis* lipid content declined before foraging, we could not determine whether outside-nest task performance caused or simply accompanied the reduction in workers' lipid stores. However, a drop in lipid content need not precede the onset of performance of risky tasks to support the hypothesis that age polyethism evolved through selection on colony energy budgets. The prediction is rather that lipid stores will not increase in those workers performing risky tasks. If lipid stores are of physiological value to workers[16], colony selection could act to adjust the net rate of lipid loss during task performance to coincide with mortality rates associated with that particular task. The amount of energy lost to the colony when a worker dies would then be balanced against that worker's expected longevity and future labor output[23].

A further comparative test of our colony efficiency hypothesis could be made based on the prediction that changes in lipid content will be less patterned with respect to age in eusocial insect species without strong age polyethism. Such species either have workers that switch frequently among tasks, as in *Polistes* spp. and *Vespula* spp. wasps[24,25], or workers that divide tasks among specialists independently of age. In the latter case workers specializing on risky tasks are predicted to have lower lipid content. Some ant species possess morphological worker castes that specialize on risky tasks such as foraging and defense[2], and these should be examined for differences in lipid content among castes. In some wasps, reproductive caste status may be determined after adult emergence[26]; therefore, changes in nutrient content with task performance do not necessar-

ily represent an adaption to selection for colony efficiency. An important alternative to consider is that moving from in-nest behavior (more queen-like behavior) to activities outside the nest (more worker-like behavior[27]) represents individuals' decreasing ability to succeed in reproductive competition in response to decreases in their nutrient stores. However, the changes in lipid content we observed occurred after the wasps had moved away from the brood combs to perform outside-nest tasks. Furthermore, reproductive caste differentiation in wasps may not involve differences in lipid stores[11], and the fact that worker ants which have little or no chance of personal reproduction exhibit similar decreases in lipid content with age polyethism[10,21] suggests that reproductive competition does not fully explain this pattern.

Acknowledgements. Thanks to Tom Phillips for use of his laboratory facilities. Stephen Martin kindly allowed us to examine his data on weight loss in hornet workers. Brian Yandell and the University of Wisconsin Animal Behavior Monday lunch group gave valuable advice on statistical analysis. Robert Page, Susan Bulova, and two anonymous reviewers made helpful comments on the manuscript. Research was supported by a tropical fellowship from the Organization for Tropical Studies/Pew Charitable Trust (to S. O'Donnell), USDA Hatch Grant 3544 (to R. L. Jeanne), and a postdoctoral fellowship from the US NSF-funded Animal Behavior Research Training Grant at the University of California at Davis and US NSF-DEB postdoctoral fellowship 9303244 (to S. O'Donnell).

1 Wilson, E. O., The Insect Societies. Harvard University Press, Cambridge, Massachusetts 1971.
2 Oster, G. F., and Wilson, E. O., Caste and Ecology in the Social Insects. Princeton University Press, Princeton, New Jersey 1978.
3 Dew, H. E., and Michener, C. D., Insectes Soc. *28* (1981) 87.
4 Winston, M. L., The Biology of Honey Bees. Harvard University Press, Cambridge, Massachusetts 1987.
5 Jaisson, P., Fresneau, D., and Lachaud, J.-P., in: Interindividual Behavioral Variability in Social Insects, p. 323. Ed. R. L. Jeanne. Westview Press, Boulder, Colorado 1988.
6 Calderone, N. W., and Page, R. E., Behav. Ecol. Sociobiol. *22* (1988) 17.
7 O'Donnell, S., and Jeanne, R. L., Anim. Behav. *44* (1992) 1021.
8 Jeanne, R. L., Monitore zool. ital. (N.S.) *20* (1986) 119.
9 Jeanne, R. L., Downing, H. A., and Post, D. C., in: Interindividual Behavioral Variability in Social Insects, p. 323. Ed. R. L. Jeanne. Westview Press, Boulder, Colorado 1988.
10 MacKay, W. P., J. Kansas Ent. Soc. *56* (1983) 538.
11 Solís, C. R., and Strassmann, J. E., Funct. Ecol. *4* (1990) 531.
12 Wheeler, D. E., Am. Nat. *128* (1986) 13.
13 Hunt, J. H., in: The Social Biology of Wasps, p. 426. Eds K. G. Ross and R. W. Matthews. Comstock Publishing Associates, Ithaca, New York 1991.
14 Sullivan, J. D., and Strassmann, J. E., Behav. Ecol. Sociobiol. *15* (1984) 249.
15 Gadagkar, R., Bhagavan, S., Chandrashekara, K., and Vinutha, C., Ecol. Ent. *16* (1991) 435.
16 Rossi, A. M., and Hunt, J. H., Ecol. Ent. *13* (1988) 437.
17 Christie, W. W., Lipid Analysis. Pergamon Press, New York, New York 1982.
18 O'Donnell, S., and Jeanne, R. L., Physiol. Ent. *18* (1993) 189.
19 SAS Users Guide: Statistics, version 5 edition. SAS Institute, Cary, North Carolina 1985.
20 Sokal, R. R., and Rohlf, F. J., Biometry, W. H. Freeman and Company, New York, New York 1981.
21 Porter, S. D., and Jorgensen, C. D., Behav. Ecol. Sociobiol. *9* (1981) 247.
22 Martin, S. J., Insectes Soc. *40* (1993) 363.
23 O'Donnell, S., and Jeanne, R. L., Behav. Ecol. (1995) in press.
24 Akre, R. D., Garnett, W. B., MacDonald, J. F., Greene, A., and Landolt, P., J. Kansas Ent. Soc. *49* (1976) 63.
25 O'Donnell, S., Ins. Soc. *42* (1995) 17–29.
26 West Eberhard, M. J., Science *200* (1978) 441.
27 West Eberhard, M. J., in: Natural Selection and Social Behavior: Recent Research and New Theory, p. 3. Eds R. D. Alexander and D. W. Tinkle. Chiron Press, New York, New York 1981.

SCIENTIFIC CORRESPONDENCE

EXPERIENTIA welcomes letters concerning articles which have appeared in our journal. Letters will be sent to the authors concerned to allow them the opportunity to reply. The correspondence will be published as rapidly as possible.