

# Methoprene accelerates age polyethism in workers of a social wasp (*Polybia occidentalis*)

SEAN O'DONNELL and ROBERT L. JEANNE Department of Entomology, University of Wisconsin, Madison, Wisconsin

**Abstract.** Topical applications of the Juvenile Hormone (JH) analogue methoprene to 1-day-old adult workers of the highly eusocial wasp *Polybia occidentalis* (Olivier) (Hymenoptera: Vespidae) accelerate the rate of age polyethism. Longevity of laboratory-reared wasps is negatively correlated with dose of topically applied methoprene. Doses of 25 µg methoprene or greater are lethal. Untreated wasps show marked age polyethism in the field. Age of first performance of nest in seven behavioural categories (in-nest, transition to outside, non-task on nest envelope, nest maintenance, foraged material handling, defence, and foraging) is negatively correlated with methoprene dose. Topical applications of methoprene accelerate age polyethism of highly eusocial bee and wasp workers, but do not have this effect on primitively eusocial bees and wasps, suggesting that JH control of age polyethism evolved independently in advanced species of Apidae and Vespidae.

**Key words.** Age polyethism, behavioural development, division of labour, Juvenile Hormone, Juvenile Hormone analogue.

## Introduction

Division of labour based on worker age, or age polyethism, is found in all families of eusocial Hymenoptera (Wilson, 1971). Species of bees (Apidae) and wasps (Vespidae) exhibit a range of social complexities, and in these families age polyethism is best developed in highly eusocial species which are characterized by permanently social or swarm-founded colonies (Roubik, 1989).

Juvenile Hormone (JH) has been implicated in the regulation of age polyethism in *Apis mellifera* (Robinson, 1987; Robinson *et al.*, 1989) with higher titres or higher doses of applied JH and JH analogues corresponding to accelerated behavioural development. JH and JH analogues did not have this effect when applied to primitively eusocial bees (*Bombus terrestris*; Röseler & Röseler, 1978. *B. impatiens* and *B. bimaculatus*; Cameron & Robinson, 1990) or wasps (*Polistes metricus*; Bohm, 1972. *P. annularis*; Barth *et al.*, 1975. *P. gallicus*; Röseler *et al.*, 1985). In primitively eusocial bees and wasps, JH and JH analogue manipulations augment ovary development and dominance status of treated individuals.

Cameron & Robinson (1990) hypothesized that JH plays a role in regulating age polyethism in highly eusocial

Hymenoptera. Vespid wasps are an important group for testing the generality of this hypothesis because this family, like the Apidae, includes primitively and highly eusocial species. Primitively eusocial Hymenoptera such as *Polistes* and *Rombus* species have relatively small colony sizes and their workers often do not exhibit strong age polyethism (Jeanne, 1991). In contrast, the swarm-founding wasp *Polybia occidentalis* (Olivier) (Hymenoptera: Vespidae) has larger average colony sizes and workers that exhibit marked age polyethism (Jeanne *et al.*, 1988). *P. occidentalis* is a highly eusocial insect which shares important characteristics of social biology with *Apis* species but not with more closely related *Polistes* species.

The purpose of this study was to test the influence of JH on age polyethism in a highly eusocial wasp, *P. occidentalis*, by topical application of the JH analogue methoprene to workers. We predicted that if JH plays a role in regulating age polyethism in this species, methoprene would cause a change in the rate of behavioural development of treated workers compared with controls.

## Materials and Methods

### *Effect of methoprene on longevity*

This experiment was conducted in Gamboa, Republic of Panama, August–October 1990, to determine the

Correspondence: Sean O'Donnell, Department of Entomology, University of Wisconsin, Madison, WI 53706, U.S.A.

non-lethal dose range of methoprene for later experiments. Workers emerging from laboratory-incubated combs were treated with varying doses (1, 5, 10, 25, 50, 100 and 1000 µg) of methoprene in acetone, acetone alone, or no treatment (controls). For this experiment 5 µl of methoprene solution or acetone was applied to the gaster of each treated worker with a microsyringe. Immediately after treatment, workers were introduced in cohorts of twenty to thirty individuals into 12 cm diameter × 9 cm deep glass dishes with gauze covers and filter paper lining. Water and honey were provided *ad libitum* and the lining was changed every 5 days. Each dish was examined daily; mortality was recorded and dead wasps were removed. Longevity data from the mortality experiment were analysed using the lifetable method of survival analysis (SAS Institute, 1985).

#### *Effect of methoprene on age polyethism*

**Worker treatments and introductions.** This experiment was performed in Guanacaste Province, Costa Rica, in July and August 1991. Four moderately-sized (352–539 adults upon collection) field observation colonies were selected in a 0.5 ha area. Workers eclosing from combs of other *P. occidentalis* nests incubated at ambient temperature in the laboratory were introduced into the observation colonies (Jeanne *et al.*, 1988; O'Donnell & Jeanne, 1992a). The incubated combs were cleared of adults daily so that all subject workers were 24 h old or less. Cohorts of twenty newly-eclosed workers were marked with paint for individual identification and each age cohort was divided evenly among two treatment groups (below). Stock solutions were prepared by dissolving the JH analogue methoprene (s-Methoprene ZR-2008; Sandoz Crop Protection, Inc.) in 2 ml acetone; solutions were stored in a freezer (–10°C) between uses to prevent concentration due to evaporation and were replaced after 7 days. Methoprene has shown JH activity in all insect species on which it has been tested (reviewed in Robinson, 1987). Treatments consisted of application of 2 µl of methoprene solution to the worker's gaster with a microsyringe. Treatment groups were based on dose applied: 1, 2 or 10 µg methoprene, acetone, or no application. Treatments were rotated until thirty wasps from each of the five treatment groups had been added to each of four observation colonies. Wasps were added to each colony every other day.

**Behavioural observations and analysis.** Two colonies were observed per day on days alternating with worker introductions. At each colony scan samples were taken at 10 min intervals for 1 h in the morning and 1 h in the afternoon. The behaviour of all marked workers visible in or on the nest was recorded by an observer using an ethogram similar to that developed by Jeanne *et al.* (1988) (Table 1). During scan sampling, and for an additional contiguous period of 30 min in the morning, all occurrences of marked forager arrivals at the nest were recorded. Each colony was observed on 11 days over a 21-day period. At the end of observations each colony was collected and the workers preserved in fixative or frozen.

**Table 1.** Behavioural acts recorded in scan samples of *P. occidentalis* workers, grouped into behavioural categories. Acts that comprised less than 0.1% of the total sample were excluded from the table.

1. In-nest	5. Nest maintenance
Bail water	Build nest
Present on comb	Chew nest
Carry debris	Carry wood pulp
Drop debris	Drop water
Inspect cell	Fan
	Inspect nest
2. Transition	Suck water from nest
Exit nest	Take wood pulp
Enter nest	Take water
3. Nest exterior, non-task	6. Defence
Groomed by nestmate	Alert posture
Groom nestmate	Wing-buzzing run
Groom self	
Inactive	7. Foraging
Raise wings	Arrive: nectar, wood
Solicit nectar	pulp, prey, water, or
Solicited by nestmate	unknown
Trophallaxis	Give to nest wasp:
Walk	nectar, wood pulp, or
	water
4. Material handling	Leave nest
Take nectar from forager	

Because many behavioural acts were recorded at low frequency, acts were pooled into categories based on location of performance and functional similarity (Table 1). The seven categories we defined (location of performance in parentheses) were: in-nest (inside nest); transition (moving into or out of nest); non-task (exterior nest surface); nest maintenance (nest surface); material handling (nest surface); defence (nest surface); and foraging (away from nest).

In the behavioural experiments the response variable we analysed was worker age at first observed performance of acts in a given behavioural category. The distributions of age of first performance were analysed using the lifetable method of survival analysis. We used the Wilcoxon rank test to examine the effects of methoprene treatments on age of first performance of the seven behavioural categories separately. Workers that were still alive at the end of the study but had not yet performed acts in a given category were censored in the analysis of that category (see SAS Institute, 1985, for a discussion of censoring in survival analysis).

## Results

### *Effects of methoprene on longevity*

Topically applied methoprene had strong effects on longevity of workers maintained in the laboratory (Wilcoxon,  $\chi^2 = 64.5$ ,  $df = 5$ ,  $P < 0.001$ ). Longevity of acetone treated and no treatment control individuals did not differ (Wilcoxon,  $\chi^2 = 0.09$ ,  $df = 1$ ,  $P > 0.75$ ). The lowest dose

applied (1  $\mu\text{g}$ ) increased longevity relative to controls in the laboratory; increasing doses caused decreased longevity (Fig. 1). Methoprene doses of greater than 25  $\mu\text{g}$  were lethal, with all workers dying within 24 h of application; workers from these treatment groups were not included in our analysis.

#### Age polyethism in controls

As noted in earlier studies (Jeanne *et al.*, 1988), the control *P.occidentalis* workers passed through a series of behavioural roles as they aged, as indicated by differences in the distributions of age at first performance of the behavioural categories (Fig. 2; Wilcoxon,  $\chi^2 = 811.6$ ,  $df = 6$ ,  $P < 0.001$ ). As is the common pattern of age polyethism in eusocial insects (Wilson, 1971), behavioural acts performed in the nest were generally performed earliest, followed by movement onto the nest surface, non-task acts performed on the nest exterior, and finally acts associated with nest maintenance, material handling, foraging, and defence (Fig. 2). Although the latter four categories were not well-differentiated by age of first occurrence, they are functionally distinct so we treated them separately.

#### Effect of methoprene on age polyethism

For each of the behavioural categories acetone-treated controls and non-treated controls did not differ in age of first performance (Wilcoxon,  $\chi^2 < 0.7$ ,  $df = 1$ ,  $P > 0.25$  in all cases). Henceforth these two groups will be pooled as 'control' workers.

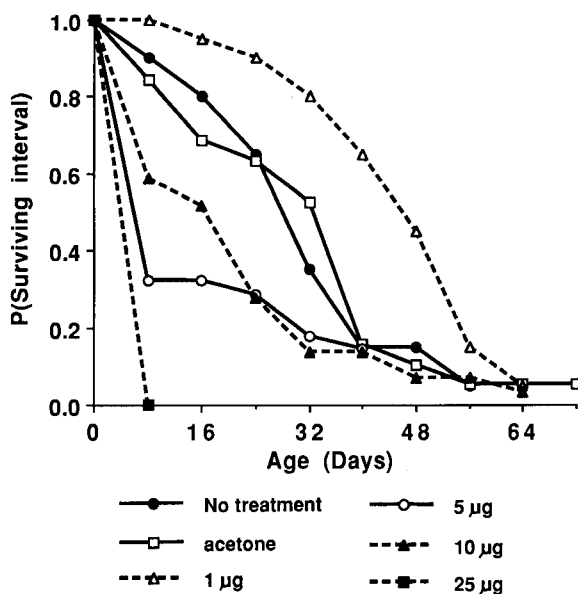


Fig. 1. Survival distribution function of laboratory-reared *P.occidentalis* workers treated with varying doses of methoprene ( $\mu\text{g}$  methoprene applied, shown in legend) and controls.

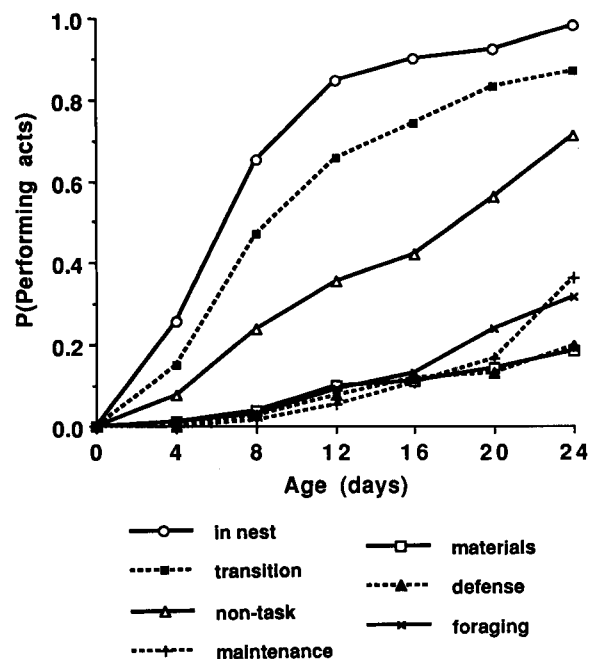
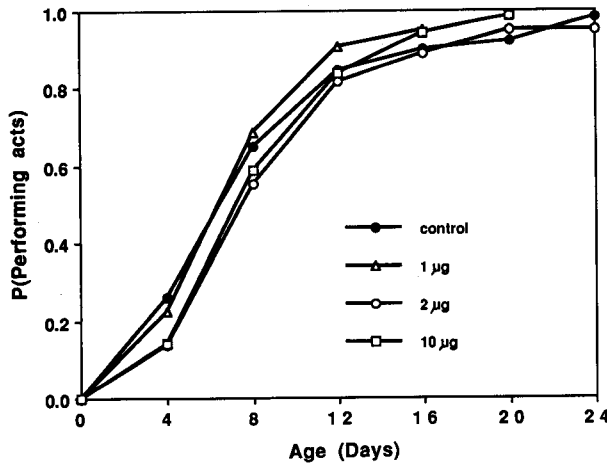


Fig. 2. Cumulative proportion of workers performing acts as a function of age of first performance of acts in seven categories by *P.occidentalis* control workers.

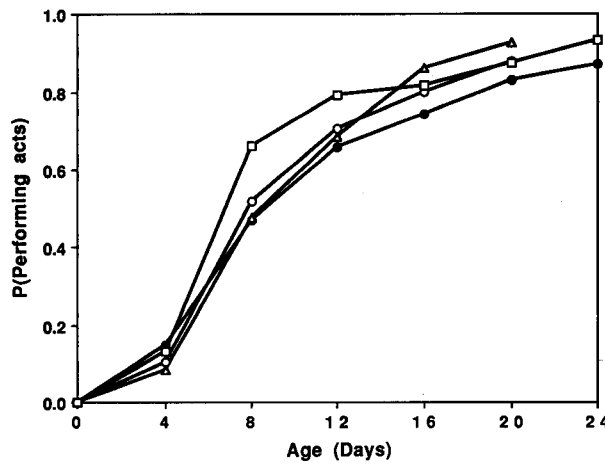
For some behavioural categories sample sizes from the individual colonies were small. In order to increase statistical power and to simplify presentation, data from the four colonies were pooled in our analyses. Examination of the distributions of age of first performance from the individual colonies revealed little variation among colonies. Typically applied methoprene had significant effects in decreasing the age of first performance of acts in the seven behavioural categories (Figs 3–9; Wilcoxon,  $\chi^2 > 9.7$ ,  $df = 3$ ,  $P < 0.05$  in all cases). When the four categories occurring later in life (defence, foraging, material handling, and nest maintenance) were pooled into a single category there was a dose-dependent methoprene treatment effect on age of first performance (Wilcoxon,  $\chi^2 = 42.1$ ,  $df = 3$ ,  $P < 0.001$ ). Differences in age of first performance due to treatment were less striking, but significant, in earlier categories (Figs 3 and 4) but were more pronounced in categories performed later in life (Figs 5–9).

#### Discussion

Our results suggest that JH plays a role in regulating age polyethism in *P.occidentalis*. Higher doses of methoprene caused an increased rate of behavioural development in a dose-dependent manner. Treated *P.occidentalis* workers began performing risky tasks associated with reduced opportunities for personal reproduction — more 'worker-like' as opposed to 'queen-like' activities (West-Eberhard, 1981) — at an earlier age. Shifts in the timing of performance of more altruistic but riskier acts are especially



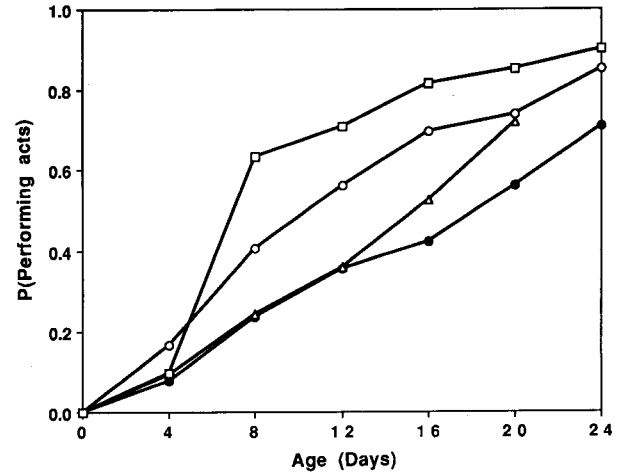
**Fig. 3.** Cumulative proportion of workers performing acts in a behavioural category as a function of age, showing the effect of varying doses of methoprene ( $\mu\text{g}$  methoprene applied, shown in legend) on age of first performance of in-nest acts.



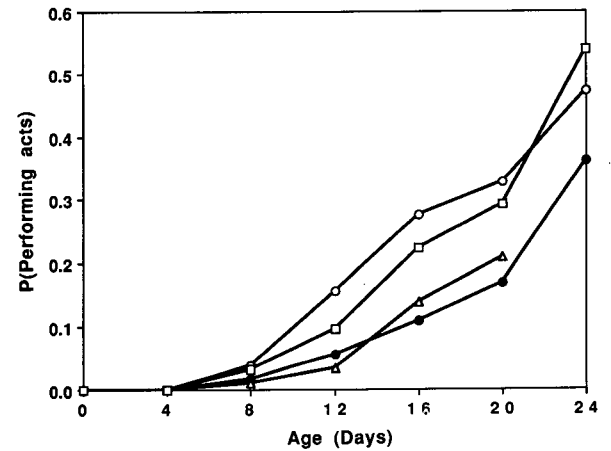
**Fig. 4.** Effect of methoprene treatments on age of first performance of transition from in-nest to on-nest.

important in polistine social wasps where reproductive caste is probably determined after adult eclosion (West-Eberhard, 1977; but see Gadagkar *et al.*, 1988). By engaging in worker-like behaviour, females may sacrifice opportunities for personal reproduction.

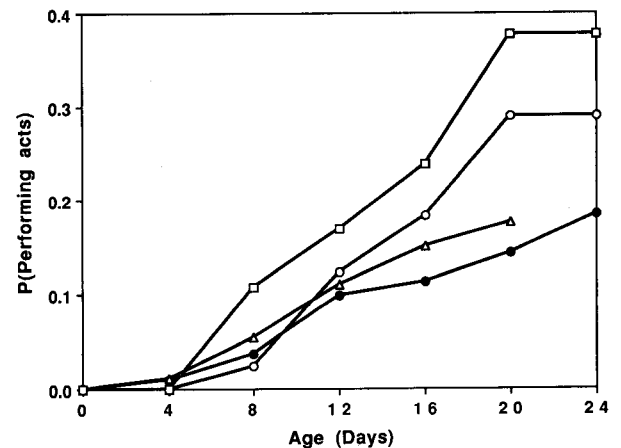
JH regulates reproduction in adult female insects of many species, with higher titres or higher doses of JH and JH analogues generally corresponding to increased reproductive potency (reviewed by Koeppe *et al.*, 1985). This is the case in primitively eusocial Hymenoptera (Barth *et al.*, 1975; Röseler & Röseler, 1978; Röseler *et al.*, 1985; Cameron & Robinson, 1990). By contrast, JH analogue applications accelerate age polyethism in workers of *Apis mellifera*, a highly eusocial insect with complex social structure and well-developed worker age polyethism (Robinson, 1987). Topical methoprene applications had similar effects on *P. occidentalis* workers.



**Fig. 5.** Effect of methoprene treatments on age of first performance of nest exterior non-task acts.



**Fig. 6.** Effect of methoprene treatments on age of first performance of nest maintenance acts.



**Fig. 7.** Effect of methoprene treatments on age of first performance of material handling acts.

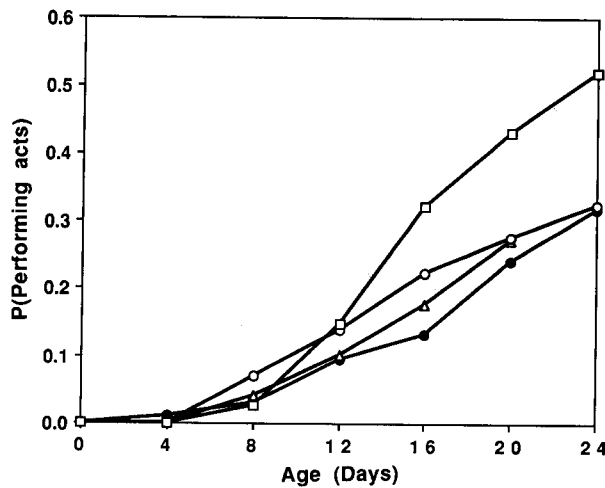


Fig. 8. Effect of methoprene treatments on age of first performance of foraging acts.

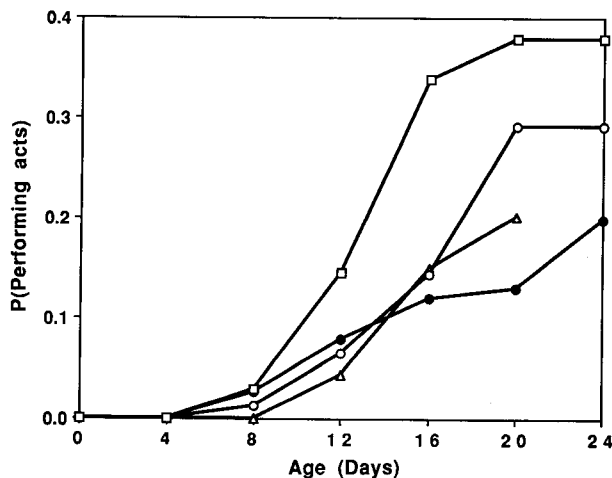


Fig. 9. Effect of methoprene treatments on age of first performance of defence acts (primarily against parasitoids).

The acceleration of performance of more worker-like behavioural acts in honeybees and *P. occidentalis* runs counter to predictions of the hypothesis that JH augments female reproductive potency in insects (Koeppel *et al.*, 1985). It appears instead that JH plays a role in regulating age polyethism in workers of highly eusocial Hymenoptera. The behavioural responses of workers to methoprene applications are consistent among highly eusocial bees and wasps, but not among primitively eusocial closer relatives, suggesting that JH regulation of age polyethism has evolved independently in the advanced species of different hymenopteran families.

Critical confirmation of the role of JH in age polyethism for swarm-founding wasps awaits measurements of JH titres. If JH regulates age polyethism we predict: a general increase in JH titre with age corresponding to the general task progression in age polyethism; and JH titres correlated

with the behavioural role, rather than the age, of exceptional (precocious or slowly ageing) workers. These relationships have been documented in *Apis mellifera* (Robinson *et al.*, 1989). The variability in rate of behavioural development among *P. occidentalis* workers in unmanipulated colonies (O'Donnell & Jeanne, 1992b) makes this species particularly suitable for such studies.

### Acknowledgments

Our thanks to Cindy Butler, Michael Evans, Chris d'Orgeix and Robinne Weiss for assistance in the field, and to Susan Bulova for assistance and advice on experimental design. Don Windsor and Stan Rand provided guidance and logistical support in Panama. David Cerf of Sandoz Crop Protection, Inc., generously donated the s-methoprene used in our experiments. Gene Robinson contributed valuable insights from his experience with social insect physiology. Comments from two anonymous reviewers helped improve the manuscript. This work was funded by NSF Grant BNS-8517519 to R.L.J., the University of Wisconsin Graduate School, a Smithsonian Tropical Research Institute Short Term Fellowship to S.O'D., and an Organization for Tropical Studies/Pew Charitable Trust Tropical Fellowship to S.O'D.

### References

- Barth, R.H., Lester, L.J., Sroka, P., Kessler, T. & Hearn, R. (1975) Juvenile hormone promotes dominance behavior and ovarian development in social wasps (*Polistes annularis*). *Experientia*, **31**, 691–692.
- Bohm, M.K. (1972) Effects of environment and juvenile hormone on ovaries of the wasp, *Polistes metricus*. *Journal of Insect Physiology*, **18**, 1875–1883.
- Cameron, S.A. & Robinson, G.E. (1990) Juvenile hormone does not affect division of labor in bumble bee colonies (Hymenoptera: Apidae). *Annals of the Entomological Society of America*, **83**, 626–631.
- Gadagkar, R., Vinutha, C., Shanubhogue, A. & Gore, A.P. (1988) Pre-imaginal biasing of caste in a primitively eusocial insect. *Proceedings of the Royal Society*, **233**, 175–189.
- Jeanne, R.L. (1991) Polyethism. *The Social Biology of Wasps* (ed. by K. G. Ross and R. W. Matthews), pp. 389–425. Comstock Publishing Associates, Ithaca, New York.
- Jeanne, R.L., Downing, H.A. & Post, D.C. (1988) Age polyethism and individual variation in *Polybia occidentalis*, an advanced eusocial wasp. *Intra-individual Behavioral Variability in Social Insects* (ed. by R. L. Jeanne), pp. 323–357. Westview Press, Boulder, Colorado.
- Koeppel, J.K., Fuchs, M., Chen, T.T., Hunt, L.-M., Kovalick, G.E. & Briers, T. (1985) The role of juvenile hormone in reproduction. *Comprehensive Insect Physiology, Biochemistry, and Pharmacology* (ed. by G. A. Kerkut and L. I. Gilbert), Volume 8, *Endocrinology II*, pp. 165–203. Pergamon Press, New York.
- O'Donnell, S. & Jeanne, R.L. (1992a) The effects of colony characteristics on life span and foraging behavior of individual wasps (*Polybia occidentalis*, Hymenoptera: Vespidae). *Insectes Sociaux*, **39**, 73–80.

- O'Donnell, S. & Jeanne, R.L. (1992b) Lifelong patterns of forager behaviour in a tropical swarm-founding wasp: effects of specialization and activity level on longevity. *Animal Behaviour*, in press.
- Robinson, G.E. (1987) Regulation of honey bee age polyethism by juvenile hormone. *Behavioral Ecology and Sociobiology*, **20**, 329–338.
- Robinson, G.E., Page, R.E., Strambi, C. & Strambi, A. (1989) Hormonal and genetic control of behavioral integration in honeybee colonies. *Science*, **246**, 109–112.
- Röseler, P.F. & Röseler, I. (1978) Studies on the regulation of the juvenile hormone titer in bumblebee workers, *Bombus terrestris*. *Journal of Insect Physiology*, **24**, 707–713.
- Röseler, P.F., Röseler, I. & Strambi, A. (1985) Role of ovaries and ecdysteroids in dominance hierarchy establishment among foundresses of the primitively social wasp, *Polistes gallicus*. *Behavioral Ecology and Sociobiology*, **18**, 9–13.
- Roubik, D.W. (1989) *Ecology and Natural History of Tropical Bees*. Cambridge University Press.
- SAS Institute (1985) *SAS User's Guide: STATISTICS*, Version 5 Edition. Cary, North Carolina.
- West-Eberhard, M.J. (1977) The establishment of reproductive dominance in social wasp colonies. *Proceedings of the VIIIth Congress of the International Union for the Study of Social Insects*, pp. 223–227.
- West-Eberhard, M.J. (1981) Intragroup selection and the evolution of insect societies. *Natural Selection and Social Behavior* (ed. by R. D. Alexander and H. Tinkle), pp. 3–17. Chiron Press, New York.
- Wilson, E.O. (1971) *The Insect Societies*. Belknap Press, Cambridge, Mass.

Accepted 1 September 1992