Journal of Insect Behavior, Vol. 10, No. 3, 1997

Factors Affecting the Acceptance of Alien Conspecifics on Nests of the Primitively Eusocial Wasp, *Ropalidia marginata* (Hymenoptera: Vespidae)

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Accepted November 12, 1996; revised February 25, 1997

In the primitively eusocial wasp, Ropalidia marginata, *individual females are known to drift from one newly founded nest to another. In the laboratory, young (<6- to 8-day-old) alien wasps are accepted onto unrelated colonies, while older (>6- to 8-day-old) wasps are not. Here we have investigated the factors that could influence the acceptance of foreign conspecifics onto unrelated nests. Individually marked wasps of different ages, isolated immediately after eclosion from the natal nest and from each other, were introduced onto unrelated recipient nests. Considered separately, both age and ovarian condition seemed to influence the probability of acceptance as well as the levels of aggression and tolerance received by the introduced wasps. However, partial correlation analysis and multiple regression analysis indicated that only age had a direct influence and that the ovarian condition acts only through age, a variable with which it is highly correlated. The observed acceptance of young aliens and rejection of old aliens are less likely to be due to the perception of older wasps as a reproductive threat rather than some age-related factor, other than ovarian condition, for example, the relative ease with which younger wasps can be molded into desired roles.*

KEY WORDS: conspecific acceptance; primitively eusocial wasps; *Ropalidia marginata*; Hymenoptera; Vespidae.

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0892-7553/97/0500-0343$12.50/0

INTRODUCTION

A fundamental feature of social insect colonies is the discrimination between nestmates and nonnestmates so that colonies can readily accept returning nestmates and keep away nonnestmates, thus maintaining colony integrity (Gadagkar, 1985; Gamboa *et al.*, 1986). The first few days of adult life, referred to as the "sensitive period," in social insects may be crucial for individuals to integrate into their colony. Such early experiences also appear to explain natural associations between species of ants (Jaisson, 1991; Errard, 1994). Young individuals in social insects are known to experience an imprinting-like phenomenon which may also allow them to be adopted into alien conspecific nests (Carlin and Hölldobbler, 1986; Morel *et al.*, 1988; Stuart, 1992; Carlin *et al.*, 1993) or even into allospecific ones (Errard, 1994).

Studies on the primitively eusocial wasp *Ropalidia marginata* (Lep.) (Hymenoptera: Vespidae) indicate that although females of this species can discriminate nestmates from nonnestmates even outside the context of their nests (Venkataraman *et al.*, 1988; Venkataraman and Gadagkar, 1992; Gadagkar, 1995), there is considerable movement of wasps between nests during the pre-emergence phase (Shakarad and Gadagkar, 1995). Venkataraman and Gadagkar (1995) observed that young ($<$8-day-old) wasps had a finite chance of being accepted into unrelated colonies but older ($\geq$8-day) wasps had no chance at all of being accepted. The ultimate (evolutionary) explanation for the acceptance of young wasps and rejection of older ones may have to do with (1) the perceived reproductive threat from the older wasps, which are known to have well-developed ovaries, or (2) the relative ease of molding younger individuals into desired roles or some such other factor related to age per se but unrelated to their ovarian condition. Here we attempt to discriminate between these hypotheses and demonstrate that age per se is more important than ovarian condition in influencing the probability of acceptance of alien conspecific wasps onto unrelated nests.

We also take this opportunity to overcome potential limitations of the previous study by Venkataraman and Gadagkar (1995), who used plastic containers to hold the wasps until the day of introduction. Hence, there remained in that study the possibility that the wasps acquired some smell from plastic boxes. Indeed, the older the wasp, the longer it was in the plastic box, and hence, the stronger may have been its smell of the plastic box. To show that the age-specific decrease in the probability of acceptance of alien wasps is not related to any odor they may have acquired from the plastic boxes, we used clean, ventilated glass beakers to hold the wasps.

MATERIALS AND METHODS

Twelve nests of *R. marginata* with about 10–15 pupae each were selected to serve as recipient nests. These were either naturally initiated or transplanted and maintained for several weeks in a vespiary at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore. The vespiary is a room measuring 9.3 × 6 × 4.8 m, covered with a wire mesh of dimensions 0.75 × 0.75 cm, which prevents the major predator, *Vespa tropica*, from entering but allows *R. marginata* to fly in and out freely. Donor nests with 20 to 30 pupae were collected for the purpose of obtaining unrelated wasps for introduction onto the recipient nests. Adults and larvae were removed and the nests with only pupae were maintained at room temperature and monitored daily for eclosion of adults. All adult wasps on the recipient nests were marked using quick-drying paints for individual identification. Nests collected for obtaining wasps for introduction were at least 10 km away from the site of collection or natural initiation of the recipient nests.

Wasps eclosing from the donor nests were removed immediately upon eclosion and isolated in 500-ml glass beakers which had holes in them for ventilation. These wasps were provided with ad libitum honey and water and were kept in the beakers till the day of introduction. The brood composition, number of resident wasps, and number of alien wasps introduced onto the 12 nests are given in Table I. The recipient nests were always located in open cages, which made it possible for the introduced wasps to leave if they chose to do so. Behavioral observations commenced immediately after the introduction of the alien wasps, in order to record behavioral interactions received by the introduced wasps from the resident wasps. All observations were done in the blind; i.e., the observer was not aware of the ages of the introduced wasps. Each observation session lasted for 5 min, with a 1-min interval between. Ten hours of such postintroduction observations (100 5-min sessions) was conducted, starting from the time of introduction of the alien wasps. Wasps that remained

Table I. Characteristics of the Nests Used in the Experiment

Nest No.	Number of cells	Total brood (egg + larva + pupa)	Number of adults	Number of wasps introduced
N 340	158	156	18	8
N 343	127	122	34	8
V 111	116	113	26	8
V 101	85	83	20	8
V 104	108	104	21	9
N 356	201	198	9	16
V 114	213	210	11	12
N 327	103	101	15	10
N 355	80	84	16	12
L 108	173	101	24	15
N 357	149	149	18	13
N 361	112	112	22	13

on the alien nest for the entire period of observation (10 h) are termed accepted. Rejected wasps were those that left the nest following high levels of aggression they elicited from the resident wasps; this happened within 20 min to 2 h after introduction. For the purpose of dissection, to ascertain their ovarian condition, accepted wasps were collected at the end of the 10 h of observation and rejected wasps were collected as they left the nest.

Six input measures of ovarian condition, namely, length of the largest oocyte, width of the largest oocyte, mean length of the proximal oocyte, mean width of the proximal oocyte, number of mature eggs, and total number of oocytes, were each converted to z-scores for normalization and were then used as input variables in a principal-components analysis to obtain an index of the ovarian condition. The first principal component, which explained 67.6% of the variance, is used as the ovarian index.

RESULTS

In all, 57 of 132 introduced wasps were accepted. We know from another similar experiment that young accepted wasps remain in their foster colonies, become well integrated, and may go on to become foragers and even replacement queens (Arathi *et al.*, 1997). All 10 1-day-old wasps were accepted, and thereafter, the probability of acceptance decreased with the age of the introduced wasps until age 6 days, after which none were accepted (Fig. 1A). Similarly, the proportion of introduced wasps that were accepted decreased with increasing ovarian index of the introduced wasps (Fig. 1B). Logistic regression analysis considering age and ovarian index separately indicated that both age and ovarian index significantly influenced the probability of acceptance (Table II). Since wasps >6 days of age were not accepted at all and some, but not all, wasps ≤ 6 days were accepted, we repeated the logistic regression analysis only for wasps ≤ 6 days old. The results were completely identical in that both age and the ovarian index had a significant influence on the probability of acceptance (Table II).

Our result that the probability of acceptance was significantly influenced both by age and by ovarian index when the two were considered separately is entirely mirrored in the pattern of behavioral interactions shown by the resident wasps toward the introduced wasps. Rejected wasps (old, with relatively well-developed ovaries) received high levels of aggression (Behaviors 1 to 9; Table III), while accepted wasps (young, with relatively poorly developed ovaries) received high levels of tolerant behaviors (Behaviors 11 to 13; Table III) from resident wasps. The minimum time that any alien wasp spent on the recipient nest before she was rejected was 20 min. Therefore, in the analysis of the behavioral data, only behavioral interactions seen during the first 20 min after introduction were considered for both the accepted and the rejected wasps.

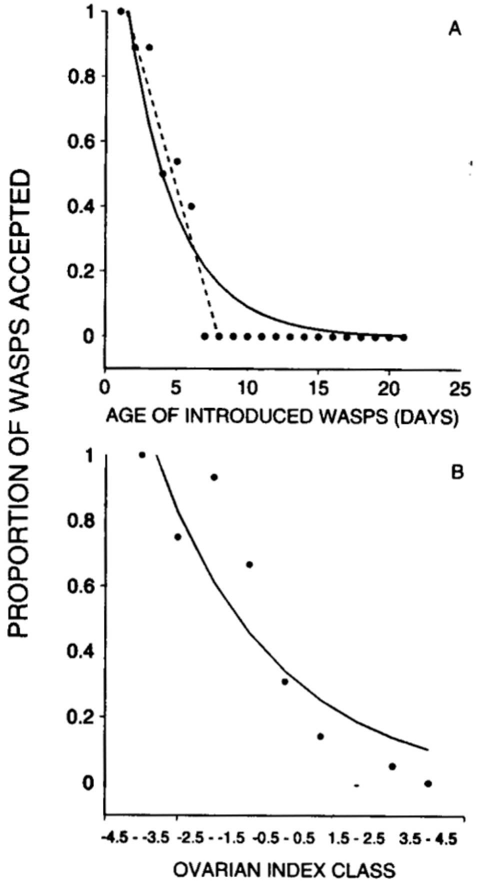

Fig. 1. (A) The proportion of alien wasps of different ages accepted on the recipient nests decreased with increasing age. Beyond 6 days none of the introduced wasps were accepted ($Y = 1.52 * e^{(-0.28X)}$; $r^2 = 0.91$; solid line). Linear fit for wasps 1 to 8 days only: ($Y = 1.22 - 0.15X$; $r^2 = 0.93$; dashed line). (B) The proportion of introduced wasps belonging to different ovarian index classes accepted onto the recipient nests decreased with increasing ovarian index values ($Y = 0.33 * e^{(-0.29X)}$; $r^2 = 0.84$).

Simple linear regression models considering age and ovarian index, one at a time, show that both the rates at which introduced wasps receive aggressive behaviors from the resident wasps and the number of wasps directing aggression toward the introduced wasps increased significantly with age and with the ovar-

Table II. Regression (Logistic and Linear) to Examine the Possible Effects of Age and Ovarian Index (Considered Separately) of Introduced Wasps on Their Probability of Acceptance and the Behaviors of Resident Wasps

Dependent variable	Independent variable	Estimate	SE	t_s	P
Probability of acceptance[a,b]	Age	−0.95	0.17	−5.53	<0.01
Probability of acceptance[a,b]	Ovarian index	−1.68	0.20	−5.83	<0.01
Probability of acceptance[a,c]	Age	0.73	0.23	−3.12	<0.01
Probability of acceptance[a,c]	Ovarian index	−0.71	0.24	−2.88	<0.01
Rates of aggressive acts received	Age	7.91	1.16	6.83	<0.01
Rates of aggressive acts received	Ovarian index	17.19	2.79	6.16	<0.01
Number of residents showing aggression	Age	0.63	0.13	5.00	<0.01
Number of residents showing aggression	Ovarian index	1.81	0.28	6.41	<0.01
Rates of tolerant acts received	Age	−0.06	0.01	−5.45	<0.01
Rates of tolerant acts received	Ovarian index	−0.15	0.02	−6.01	<0.01
Number of residents showing tolerance	Age	−0.07	0.01	−6.43	<0.01
Number of residents showing tolerance	Ovarian index	−0.17	0.03	−6.33	<0.01

[a] Logistic regression.
[b] All introduced wasps included in the analysis.
[c] Only wasps ≤6 days old.

Table III. Behaviors Exhibited by Resident Wasps Toward Introduced Wasps

Serial number in increasing order of tolerance	Behavior
1	Aggressive bite
2	Attack
3	Falling fight
4	Sit on
5	Hold in mouth
6	Nibble
7	Peck
8	Chase
9	Avoid
10	Solicit
11	Approach
12	Antennate
13	Allogrooming

ian index of the introduced wasps. Conversely, both the rates at which introduced wasps received tolerant behaviors and the numbers of resident wasps directing tolerant behaviors toward the introduced wasps decreased significantly with age and with the ovarian index of the introduced wasps (Table II). That both age and ovarian index have similar effects is not surprising because there is a significant positive correlation between age and ovarian index of the wasps (Fig. 2).

However, to see if both age and ovarian index act independently or whether one acts only through the other, we performed partial correlation analyses keeping either age or ovarian index constant. In all ages, the partial correlations were significant when the ovarian index was held constant and not significant when age was held constant, indicating that age has the primary effect and that ovarian index acts only through its correlation with age (Table IV).

Similarly, we also checked the independence or otherwise of the effects of age and ovarian index with multiple (logistic and linear) regression models using both age and ovarian index simultaneously. In all cases the results mirrored the partial correlation analysis, in that age but not the ovarian index significantly influenced the dependent variable being considered, directly (Table V). The fact that both partial correlation analysis and multiple regression analysis show that age has the primary effect and that the ovarian index acts only through its correlation with age is somewhat surprising but is not impossible because not all the variance in age is explained by the ovarian index ($r^2 = 0.63$). To rule out the unlikely possibility that the ovarian index somehow did not reflect the true ovarian condition, we also repeated the logistic regression analysis using

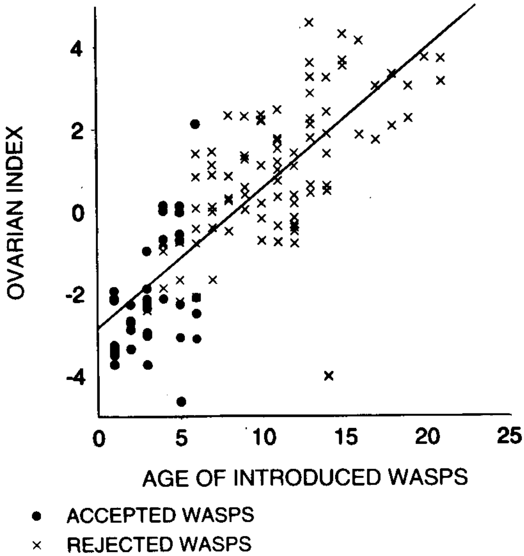

Fig. 2. Regression of the age of introduced wasps on their respective ovarian index values indicated that age and ovarian index are significantly positively correlated ($Y = -2.86 + 0.34X$; $r^2 = 0.64$; $n = 132$; slope significantly different from zero, $P < 0.01$). Filled circles refer to accepted wasps and crosses refer to rejected wasps.

Table IV. Partial Correlation to Examine the Relation Between Age and Ovarian Index of Introduced Wasps and Behaviors of Resident Wasps

Variables[a]	Partial correlation coefficient	t	P
$r_{c,a.b}$	0.25	3.02	<0.01
$r_{c,b.a}$	0.12	1.33	>0.05
$r_{d,a.b}$	0.31	3.69	<0.01
$r_{d,b.a}$	0.03	0.31	>0.05
$r_{e,a.b}$	0.23	2.65	<0.01
$r_{e,b.a}$	0.12	1.34	>0.05
$r_{f,a.b}$	0.20	2.34	<0.01
$r_{f,b.a}$	0.14	1.65	>0.05

[a] $r_{c,a.b}$ is to be read as "partial correlation between age of the introduced wasps and rates of aggressive acts received, with ovarian index being kept constant." Note that all partial correlations with a.b are significant, while all those with b.a are not significant, indicating that any influence of ovarian index on the dependent variable is only mediated through age. Subscripts: a, age of the introduced wasps; b, ovarian index of the introduced wasps; c, rates of aggressive acts received by the introduced wasps; d, number of residents showing aggression toward the introduced wasps; e, rates of tolerance acts received by the introduced wasps; f, number of residents showing tolerance towards the introduced wasps.

Table V. Multiple Regression (Logistic and Linear) to Examine the Possible Effects of Age and Ovarian Index (Considered Simultaneously) of Introduced Wasps on Their Probability of Acceptance and the Behaviors of Resident Wasps

Dependent variable	Independent variable	Estimate	SE	t_s	*P*
Probability of acceptance[a,b]	Age	−0.79	0.19	−4.01	<0.01
	Ovarian index	−0.40	0.26	−1.51	>0.05
Probability of acceptance[a,c]	Age	−0.57	0.26	−2.17	<0.01
	Ovarian index	−0.37	0.27	−1.36	>0.05
Rates of aggressive acts received	Age	5.69	1.91	2.98	<0.01
	Ovarian index	6.54	4.48	1.46	>0.05
Number of residents showing aggression	Age	1.72	0.47	3.67	<0.01
	Ovarian index	0.05	0.24	0.24	>0.05
Rates of tolerant acts received	Age	−0.11	0.04	−2.61	<0.01
	Ovarian index	−0.02	0.02	−1.29	>0.05
Number of residents showing tolerance	Age	−0.04	0.02	−2.29	<0.01
	Ovarian index	−0.09	0.05	−1.68	>0.05

[a] Logistic regression.
[b] All introduced wasps included in the analysis.
[c] Only wasps ≤6 days old.

each of the individual measurements of the ovaries (used to obtain the ovarian index) but the results did not change (data not shown).

DISCUSSION

By introducing 132 alien wasps ranging in age from 1 to 20 days onto 12 unrelated recipient nests, we have shown that age is the primary factor that significantly influences the probability of acceptance and the nature of behavioral interactions between resident and introduced wasps. Ovarian condition of the introduced wasps also exerted an influence but only through its correlation with age. Ideally one would have preferred to introduce all the alien wasps (or at least a number sufficient for statistical analysis) into a single recipient nest at the same point in time, to avoid the potential problem of variation between the recipient nests in their response to the introduced wasps. However, most nests of *R. marginata* are small, and introducing any more than 10 to 15 foreign wasps would create perturbation of a magnitude that would certainly be unrealistic. We have therefore sacrificed some statistical rigor for biological realism using 12 recipient nests. However, we suspect that variation between recipient nests (which was beyond our control) is unlikely to be a major determinant of our results because multiple regression analysis showed that the proportion of introduced wasps that were accepted in different nests was not significantly influenced by their number of resident wasps, total brood, and total cell number.

The proximate explanation for the decreasing probability of acceptance, increasing aggression, and decreasing tolerance shown by the resident wasps toward the introduced wasps may have to do with the gradual development of recognition labels by the introduced wasps (Gadagkar, 1985; Gamboa *et al.*, 1986; Waldman, 1988; Singer and Espelie, 1992). The fact that their ovarian condition does not appear to influence directly their probability of acceptance suggests that the developing ovaries are not the sole source of the recognition template. Age-dependent variation in the cuticular hydrocarbon profile as demonstrated in *Camponotus floridanus* (Morel *et al.*, 1988) and *Drosophila virilis* (Jackson and Bartlet, 1986) may also be responsible in *R. marginata.*

Concerning the possible ultimate (evolutionary) explanations for the results obtained here, the fact that age but not ovarian condition directly influences the probability of acceptance of alien wasps argues against the idea that the probability of acceptance is entirely related to the perceived reproductive threat from the introduced wasps. On the other hand, some factor other than the ovarian condition, associated with age, for example, the ease of molding younger wasps into desired roles, may be more important in deciding the response of the resident wasps toward the introduced wasps.

ACKNOWLEDGMENTS

This work was supported by the Department of Science and Technology and Ministry of Environment and Forests, Government of India.

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