A Sensitive and Reliable Assay for Queen Discrimination Ability in Laboratory-Reared Workers of the Ant Camponotus japonicus

Author: Hara, Kenji

Source: Zoological Science, 19(9) : 1019-1025

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.19.1019

ZOOLOGICAL SCIENCE **19**: 1019–1025 (2002)

A Sensitive and Reliable Assay for Queen Discrimination Ability in Laboratory-Reared Workers of the Ant *Camponotus japonicus*

Kenji Hara*

Biology, Tokyo Gakugei University, Koganei-shi, Tokyo 184-8501, Japan.

ABSTRACT—The queen discrimination abilities of laboratory-reared *Camponotus japonicus* workers were examined individually by allowing them to carry their nestmate larvae toward either the mother queen or an alien queen. Source colonies had been reared under controlled conditions from founding queens and maintained at small size (≦10 workers each). Fifty-two of fifty-four workers raised in these eight different colonies carried nestmate larvae to the mother queen, and never carried them to the alien queen. Most of them attended nestmate larvae but never alien larvae. These results clearly demonstrate that the tested workers discriminate the nestmate queen and larvae from non-nestmate conspecifics. The assay used in this study is novel and sensitive, and may be suitable for neuroethological and molecular studies of social discrimination mechanisms.

Key words: queen discrimination, behavioral assay, Formicidae, carpenter ants, *Camponotus japonicus*

INTRODUCTION

Discrimination behavior is a fundamental feature of social insects, and the discrimination ability allows the individual to be integrated into the colony. Such highly sophisticated intraspecific communication of social insects is generally mediated by chemical cues that constitute a ‘colony odor’ common to all colony members, and may have genetic and environmental components. The colony odor resides in differences in cuticular hydrocarbon profiles among colonies, both between and within species, and is learned by colony members (for reviews, see Breed and Bennet 1987; Hölldobler and Wilson, 1990). Therefore, an ability to process the sensory information produced by blends of chemical components is likely to be important for discrimination behavior.

The results of recent studies of odor learning in a large number of invertebrate species have already revealed the anatomical and physiological characteristics of the olfactory/antennal lobes, and some indications of how they may optimize their learning capacity for olfactory signals composed of several odorant components (Hammer and Menzel, 1995; Hildebrand and Shepherd, 1997). In the antennal lobe of the ant *Camponotus rufipes*, however, pheromonal and non-pheromonal processing are functionally indistinguishable (Galiza *et al*., 1999), indicating that the colony odor discrimination may be a task of structures upstream from the antennal lobe, such as the mushroom body or the lateral protocerebrum. It is still unclear how sensory information can be processed in an elaborate central nervous system and generate appropriately modified behavioral output.

Neuroethology is concerned with the causal relationship and interconnection between the nervous system and behavior of animals. To address the functional significance of particular brain regions for behavior, it should be an efficient approach to assess the effects of experimental manipulations such as surgical operations, injection of chemicals into the brain, and control of gene expression. Studies of behavioral mutants in flies (*Drosophila melanogaster*) have revealed molecular and neuronal mechanisms associated with these behaviors (Yin *et al*., 1994; Grotewiel *et al*., 1998; Zars *et al*., 2000). Similarly, molecular-behavioral studies in social insects may lead to the understanding of the brain functions associated with colony odor discrimination.

The social system of carpenter ants (*Camponotus*) has been well studied, and the experimental trainings can affect the odor learning and memory required for nestmate recognition in a predictable way (Carlin and Hölldobler, 1983, 1986, 1987, 1988; Carlin *et al*., 1987; Morel *et al*., 1988; Carlin and Schwartz, 1989). In most previous studies, the behavioral assay for nestmate recognition has depended on the aggressive response to non-nestmate workers (e.g. Carlin and Hölldobler, 1986, 1988). For neuroethological studies of colony odor recognition, it is necessary to test the behav-

---

* Corresponding author: Tel. +81-42-329-7522;
FAX. +81-42-329-7522.
E-mail: khara@u-gakugei.ac.jp

ior of ants reared in environmentally homogeneous laboratory conditions. Workers of the ant *C. japonicus*, when reared in laboratory conditions, show reduced aggression and greater tolerance to alien workers; this is particularly true for callows (unpubl. obs.). It seems to be generally true that laboratory-reared workers are less aggressive toward aliens (cf. Obin, 1986), because the aggressive response of workers is necessary for learning not only colony odor but also non-nestmate and environmental cues (cf. Hölldobler and Wilson, 1990). Therefore, the aggressive response to non-nestmate workers is not a suitable behavioral assay for use with laboratory colonies.

The discrimination of the mother queen by workers has been documented in *Camponotus* species. Queen discriminators, transferred to and learned by workers, provide the dominant cues for aggressive response of workers to non-nestmates when colonies are small (Carlin and Hölldobler, 1986). Therefore, queen discrimination is potentially valuable as an assay for the ability of *Camponotus* workers to discriminate their own colony odor. The purpose of this study is first to report a new and sensitive assay for the ability of workers to discriminate between their mother queen and alien queens, and secondly to present the results of this assay with the laboratory-reared ant *Camponotus japonicus*.

MATERIALS AND METHODS

Insects

Founding queens of *Camponotus japonicus* were collected in Tokyo, Japan. They were housed in plaster boxes (257×190× 87 mm), and maintained under controlled conditions (25°C, relative humidity 60%, and dark). Colonies were fed honey-water and both adult and larval flies twice weekly. In *Camponotus* spp., the influence of the queen on worker discrimination cues is strong in a newly founded nest (containing few workers) and declines as the colony grows (Carlin and Hölldobler, 1986). Therefore, the eight experimental colonies (A to H) were maintained at 'founding' size (≦10 workers each). The workers were marked individually shortly after eclosion with cloth threads of different colors tied between the petiole and gaster. Fifty-four workers were tested in the experiment,

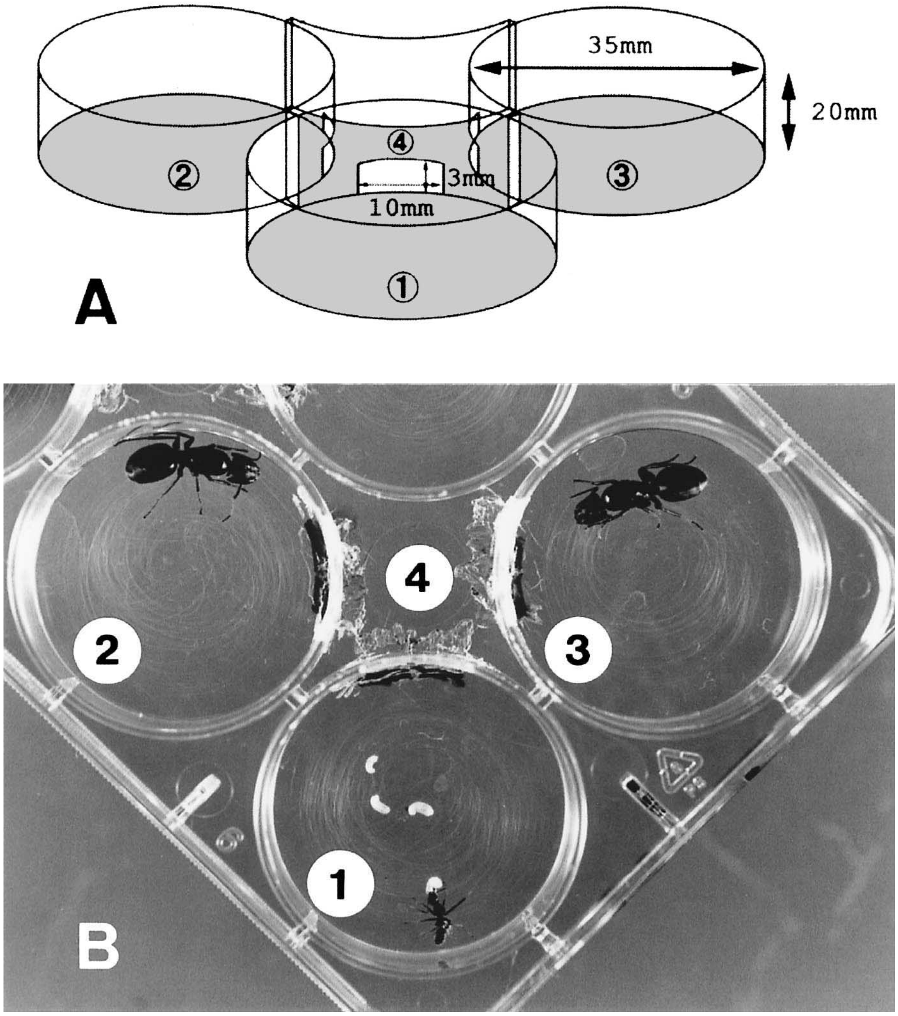

Fig. 1. Test for the queen discrimination ability of the worker ant. A) A diagram of the experimental apparatus (see Materials and Methods). B) Queen discrimination test by allowing the worker to carry nestmate larvae. Four nestmate larvae were in room ①, the experimental worker licked one of them. A larva had already been carried to mother queen in room ② by her (hidden under the queen). An alien queen was in the room ③. The number indicates each room.

Table 1. Number and locations of nestmate larvae carried by the workers in laboratory-reared colonies of *C. japonicus*.

tested	number and location of the larvae [(a)]			
worker	(A, B, n)	(A, C, n)	(A, D, n)	p [(b)]
a1	(5, 0, 10)	(4, 0, 11)	(4, 0, 11)	0.925
a2	(9, 0, 6)	(15, 0, 0)	(8, 0, 7)	0.263
a3	(13, 0, 2)	(8, 0, 7)	(5, 0, 10)	0.153
a4	(7, 0, 8)	(4, 0, 11)	(7, 0, 8)	0.606
a5	(14, 0, 1)	(7, 0, 8)	(6, 0, 9)	0.121
a6	(11, 0, 4)	(5, 0, 10)	(9, 0, 6)	0.327
a7	(8, 0, 7)	(15, 0, 0)	(13, 0, 2)	0.338
a8	(4, 0, 11)	(8, 0, 7)	(8, 0, 7)	0.466
a9	(3, 0, 12)	(6, 0, 9)	(8, 0, 7)	0.341
a10	(9, 0, 6)	(6, 0, 9)	(12, 0, 3)	0.367

tested	number and location of the larvae [(a)]			
worke	(B, A, n)	(B, C, n)	(B, D, n)	p [(b)]
b1	(9, 0, 6)	(9, 0, 6)	(13, 0, 2)	0.600
b2	(10, 0, 5)	(15, 0, 0)	(9, 0, 6)	0.403
b3	(13, 0, 2)	(6, 0, 9)	(15, 0, 0)	0.140
b4	(13, 0, 2)	(5, 0, 10)	(15, 0, 0)	0.078
b5	(14, 0, 1)	(8, 0, 7)	(12, 0, 3)	0.449
b6	(14, 0, 1)	(7, 0, 8)	(14, 0, 1)	0.247

tested	number and location of the larvae [(a)]			
worker	(C, A, n)	(C, B, n)	(C, D, n)	p [(b)]
c1	(12, 0, 3)	(4, 0, 11)	(7, 0, 8)	0.121
c2	(2, 2, 11)	(3, 4, 8)	(1, 3, 11)	0.607
c3	(6, 0, 9)	(5, 0, 10)	(10, 0, 5)	0.368
c4	(15, 0, 0)	(13, 0, 2)	(6, 0, 9)	0.140
c5	(4, 0, 11)	(9, 0, 6)	(5, 0, 10)	0.311

tested	number and location of the larvae [(a)]			
worker	(D, A, n)	(D, B, n)	(D, C, n)	p [(b)]
d1	(8, 0, 7)	(15, 0, 0)	(13, 0, 2)	0.338
d2	(14, 0, 1)	(6, 0, 9)	(6, 0, 9)	0.100
d3	(12, 0, 3)	(11, 0, 4)	(8, 0, 7)	0.661

tested	number and location of the larvae [(a)]			
worker	(E, F, n)	(E, G, n)	(E, H, n)	p [(b)]
e1	(5, 0, 10)	(7, 0, 8)	(11, 0, 4)	0.300
e2	(8, 0, 7)	(5, 0, 10)	(7, 0, 8)	0.708
e3	(11, 0, 4)	(13, 0, 2)	(6, 0, 9)	0.273
e4	(10, 0, 5)	(10, 0, 5)	(9, 0, 6)	0.967
e5	(6, 0, 9)	(10, 0, 5)	(7, 0, 8)	0.569
e6	(9, 0, 6)	(7, 0, 8)	(7, 0, 8)	0.870
e7	(12, 0, 3)	(9, 0, 6)	(6, 0, 9)	0.368
e8	(12, 0, 3)	(5, 0, 10)	(13, 0, 2)	0.150
e9	(8, 0, 7)	(7, 0, 8)	(9, 0, 6)	0.882

tested	number and location of the larvae [(a)]			
worker	(F, E, n)	(F, G, n)	(F, H, n)	p [(b)]
f1	(9, 0, 6)	(4, 0, 11)	(8, 0, 7)	0.870
f2	(10, 0, 5)	(6, 0, 9)	(8, 0, 7)	0.607
f3	(6, 5, 4)	(10, 4, 1)	(9, 1, 5)	0.596
f4	(5, 0, 10)	(8, 0, 7)	(13, 0, 2)	0.153
f5	(8, 0, 7)	(13, 0, 2)	(6, 0, 9)	0.236
f6	(11, 0, 4)	(7, 0, 8)	(7, 0, 8)	0.529
f7	(7, 0, 8)	(9, 0, 6)	(3, 0, 12)	0.230

tested	number and location of the larvae [(a)]			
worker	(G, E, n)	(G, F, n)	(G, H, n)	p [(b)]
g1	(5, 0, 10)	(15, 0, 0)	(14, 0, 1)	0.070
g2	(5, 0, 10)	(12, 0, 3)	(10, 0, 5)	0.236

tested	number and location of the larvae [(a)]			
worker	(H, E, n)	(H, F, n)	(H, G, n)	p [(b)]
h1	(6, 0, 9)	(10, 0, 5)	(11, 0, 4)	0.459
h2	(9, 0, 6)	(7, 0, 8)	(10, 0, 5)	0.765
h3	(15, 0, 0)	(5, 0, 10)	(14, 0, 1)	0.070
h4	(10, 0, 5)	(10, 0, 5)	(6, 0, 9)	0.542
h5	(8, 0, 7)	(15, 0, 0)	(8, 0, 7)	0.207
h6	(9, 0, 6)	(8, 0, 7)	(5, 0, 10)	0.555
h7	(8, 0, 7)	(5, 0, 10)	(4, 0, 11)	0.467
h8	(6, 0, 9)	(9, 0, 6)	(7, 0, 8)	0.729
h9	(10, 0, 5)	(13, 0, 2)	(5, 0, 10)	0.174
h10	(13, 0, 2)	(14, 0, 1)	(8, 0, 7)	0.414
h11	(10, 0, 5)	(11, 0, 4)	(6, 0, 9)	0.459
h12	(6, 0, 9)	(9, 0, 6)	(4, 0, 11)	0.370

[(a)]; The results of three trials using the same alien queen were combined and compiled for each location; mother queen, alien queen (shown by the colony name), or both spaces ① and ④ in the test box (n).

[(b)]; Difference for the numbers of larvae carried to the mother queen by each worker among the sets of three trials with different alien queens (χ^2 test).

Table 2. Number and locations of alien larvae carried by the workers in lab-reared colonies of *C. japonicus*.

tested	number and location of the larvae (a)		
worker	(A, B, n)	(A, C, n)	(A, D, n)
a1	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
a2	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
a3	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
a4	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
a5	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
a6	(0, 0, 15)	(1, 0, 14)	(0, 0, 15)
a7	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
a8	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
a9	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
a10	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)

tested	number and location of the larvae (a)		
worker	(B, A, n)	(B, C, n)	(B, D, n)
b1	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
b2	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
b3	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
b4	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
b5	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
b6	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)

tested	number and location of the larvae (a)		
worker	(C, A, n)	(C, B, n)	(C, D, n)
c1	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
c2	(2, 3, 10)	(3, 1, 11)	(4, 3, 8)
c3	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
c4	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
c5	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)

tested	number and location of the larvae (a)		
worker	(D, A, n)	(D, B, n)	(D, C, n)
d1	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
d2	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
d3	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)

tested	number and location of the larvae (a)		
worker	(E, F, n)	(E, G, n)	(E, H, n)
e1	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
e2	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
e3	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
e4	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
e5	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
e6	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
e7	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
e8	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
e9	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)

tested	number and location of the larvae (a)		
worker	(F, E, n)	(F, G, n)	(F, H, n)
f1	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
f2	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
f3	(3, 2, 10)	(2, 4, 9)	(4, 3, 8)
f4	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
f5	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
f6	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
f7	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)

tested	number and location of the larvae (a)		
worker	(G, E, n)	(G, F, n)	(G, H, n)
g1	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
g2	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)

tested	number and location of the larvae (a)		
worker	(H, E, n)	(H, F, n)	(H, G, n)
h1	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
h2	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
h3	(0, 0, 15)	(1, 0, 14)	(0, 0, 15)
h4	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
h5	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
h6	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
h7	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
h8	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
h9	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
h10	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
h11	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)
h12	(0, 0, 15)	(0, 0, 15)	(0, 0, 15)

(a); The results of three trials using the same alien queen were combined and compiled for each location; mother queen, alien queen (shown by the colony name), or both spaces ① and ④ in the test box (n).

at 10–20 days posteclosion: 10 from colony A (identified as a1 to a10), 6 from B (b1 to b6), 5 from C (c1 to c5), 3 from D (d1 to d3), 9 from E (e1 to e9), 7 from F (f1 to f7), 2 from G (g1 and g2), and 12 from H (h1 to h12; a total of 12 workers could be tested after all, since some workers died after finishing the tests). All were minor workers, and mean body length from head to the tip of the gaster was 9.85±1.25 mm (mean±SE).

Experimental apparatus

An acrylic box was used for the queen discrimination test (Fig. 1). The test box consisted of three rooms, ①, ②, and ③, and a central space ④ (Fig. 1A). Each room (35 mm in diameter, 20 mm in depth) was connected to the space by a doorway (3×10 mm) that prevented queens from leaving the rooms but allowed the workers free access to all rooms (Fig. 1A).

Discrimination test

To examine the ability of workers to discriminate the mother queen from alien queens, the nursing behavior of assembling larvae around the mother queen was observed in the box. The queen from the natal colony of the testing worker (the mother queen) was put in room ② or ③ of the test box, and another queen from a different colony (the alien queen) was put in the other room, ③ or ②, respectively. Then a test worker and five larvae were introduced into room ① (time 0). Each trial lasted one hour. At the end of a trial, the result was recorded as follows: a = number of larvae carried to mother queen, b = number of larvae carried to alien queen, c = the number of larvae carried to neither queen but remaining in room ① or/and the central space ④.

A total of eighteen trials were done with each worker. In the first nine trials the worker was given nestmate brood and a choice between the mother queen and an alien queen. Three different alien queens were used, in three trials each. The second set of nine trials was done in the same way, but with alien brood instead of nestmate brood. For each worker, the three trials using the same alien queen were done consecutively.

All trials were performed in dim light at 25°C. The test box was changed for every trial to exclude any effects of chemical odors on the worker's behavior. The positions of the mother queen and the alien queen were interchanged between trials. The used boxes were wiped with 70% ethanol, washed with detergent, and thoroughly dried before being used again.

RESULTS

Queen discrimination

The typical behavior of the experimental workers during the test was as follows: after the worker and nestmate larvae were introduced to room ①, the worker walked around and searched in the room. Frequently it would visit and lick the larvae. After a while, the worker holding a larva would leave the room and visit the queens in room ② and ③. Workers were not aggressive toward alien queens but rarely left larvae with them. Some workers left larvae with the mother queen and returned to room ① to nurse other larvae (Fig. 1B), whereas others remained with the queen, often grooming her.

The results show clear and highly reliable discrimination of mother queen from alien queens (Table 1). Fifty-two of fifty-four workers carried nestmate larvae only to the mother queen in all trials, and in no trial were nestmate larvae carried only to the alien queen. There was no significant difference for the numbers of larvae carried to the mother queen by each worker among the sets of three trials with different alien queens (*p*>0.05, χ^2 test), indicating that their brood-retrieval activities were stable across trials and were not affected by the particular alien queens used.

Two workers, c2 and f3, carried larvae to both the mother queen and each alien queen in trials. The χ^2 test showed that c2 did not discriminate between the mother queen C and the alien queens A, B, and D *(p*>0.05). Worker f3 carried significantly more larvae to the mother queen F than to each alien queen (*p*<0.01, χ^2 test).

Response to alien larvae

Typically the experimental workers either avoided the alien larvae entirely or antennated but never licked them. The worker would leave the room without licking any larvae and would visit one or both queens. Workers visiting the alien queen did not show strong aggression, but never groomed the alien queen and left the room quickly. Workers visiting the mother queen groomed her and remained in the room with her for the duration of the trial.

Fifty of fifty-four workers neither licked the alien larvae nor carried them out from room ① (Table 2). Four workers (a6, c2, f3, and h3) were observed to lick and hold alien larvae. Workers c2 and f3 carried larvae to not only the mother queen but also to alien queens (*p*>0.05, χ^2 test). Neither queen C nor F was aggressive toward the alien larvae brought to them by workers c2 and f3, respectively.

DISCUSSION

Experimental design

For neuroethological and molecular-behavioral studies of colony odor discrimination in ants, it is necessary first to establish a stable and objective assay that can be used in the laboratory. Then the functional significance of particular brain regions for the ability can be tested with experimental manipulations.

Agonistic (or tolerance) behavior has been used commonly in nestmate recognition research on ants (e.g. Obin and Vander Meer, 1988; Stuart, 1992; Errard, 1994). Carlin and Hölldobler (1986) recorded 7 different aggression behavior of adult workers toward non-nestmates in *Camponotus* spps., and ranked them in increasing aggression from 0 to 6 in an ordinal scale. They gathered behavioral data on a sample of individuals, then calculated a mean aggression score for various classes of interactants based on the most aggressive behavioral interaction recorded. A decision about behavior of a population or species was made by statistical inference. A potential difficulty with using agonistic (or tolerance) behavior as an assay for use with laboratory colonies is how observation bias could be avoided (cf. Gamboa *et al*., 1991). For example, one might assume that biting would be usually aggressive and such objective behavior could not be easily misidentified. In many cases of field-collected foragers, such assumption is seems to be justifiable. However, in our experience with assaying discrimination in the laboratory-reared ants, especially callows, we frequently find it difficult to distinguish biting from such tolerance behaviors as mouth-to-mouth contact, grooming, food exchange, or lunging with an open mouth. Since behavioral observations are inherently subjective, an assay that involves behavioral observations is susceptible to observation bias that can undermine the validity of the conclusions.

One way to avoid the observation bias is to offer the workers a choice among nestmates and non-nestmates. The brood discrimination ability of workers has previously been tested in this way (e.g. Jaisson and Fresneau, 1978; Meudec 1978; Lenoir 1981). The nursing behaviors directed toward nestmate and non-nestmate broods were recorded and the number of licks or the speed of brood retrieval was analyzed by statistical methods. It appears that workers in the genus *Camponotus* will accept conspecific alien cocoons, though they tend to prefer nestmate to non-nestmate conspecific cocoons (Carlin *et al*., 1987; Carlin and Schwartz, 1989). For demonstrations of cause and effect in neuroethological studies, there must be a close correspondence between the brain and behavior of individuals. Therefore, assays that depend on statistical analysis for detecting the discrimination ability should also be avoided, indicating that brood discrimination is not a suitable assay for neuroethological study.

In small *Camponotus* colonies, queen-derived cues are major components of colony-specific recognition label (Carlin and Hölldobler, 1986). Therefore, queen discrimination by the workers in small colonies is a biologically meaningful behavior as an assay for the ability to discriminate their own colony odor. In this study, a choice between the mother queen and an alien queen was used as the assay for queen discrimination ability of laboratory-reared workers. The interpretation of results on the basis of brood-retrieval ensured the objectivity in this assay. Most workers chose unambiguously to carry brood only to the mother queen. Statistics are not needed to determine the results in such cases. These results clearly demonstrate the suitability of the assay for neuroethological and molecular studies.

Queen and larval discrimination of *C. japonicus* workers in small colonies

Most workers in this study carried nestmate larvae to their own mother queen but not at all to alien queens, indicating that the workers in the laboratory-reared colonies of *C. japonicus* have the ability to distinguish their mother queen from others. Workers c2 and f3 carried larvae to both the mother queen and the alien queen in each combination, showing that there may be some interindividual variation in queen discrimination ability even among workers in the same colony. This natural variation may represent an opportunity for mechanistic studies. In the genus *Camponotus,* queen labels have a great influence in founding colonies (less than 10 workers) (Carlin and Hölldobler, 1986), but in larger colonies (~190 workers) the queen label is less effective and worker cues become more important (Carlin and Hölldobler, 1987). The assay described here is based on discrimination among queens by workers and it was hypothesized that responses on the bioassay would be more clear and reliable in small colonies. This reasoning seems to be confirmed by the very clear results. By the same reasoning, more variation among individuals might be expected in larger colonies. If that should prove to be the case, then comparisons of nestmate discrimination in small vs large colonies might reveal behavioral variation among workers that could open up additional avenues for mechanistic experiments.

Recognition of nestmate brood has been shown for several ant species. In discriminating against alien brood, worker ants in large colonies do not have an ‘all or nothing’ response but rather exhibit a preferential choice behavior (cf. Carlin, 1988). In this study, the discrimination between nestmate and alien larvae was not shown directly, since the workers did not experience both groups of larvae at the same time. However, a nearly perfect discrimination ability and a preference for nestmate larvae are very strongly suggested by the responses of the workers. The response could be mediated by two brood pheromones derived from several cue sources, those that attract both nestmate and non-nestmate workers and those that mediate recognition of nestmate brood by workers (cf. Hölldobler and Wilson, 1990). The simplest hypothesis would be that there is a hierarchy of importance of cues in determining nestmate brood recognition, that this is different in small colonies than in large colonies, and that an influence of queen-derived cues on brood recognition by workers is greater in small colonies. The results obtained with small colonies may be relevant to *Camponotus* populations in the field, because kin recognition would play an important role in the success of founding nests. The assay used in this study might provide a good opportunity to examine this hypothesis.

ACKNOWLEDGMENTS

I am grateful to Dr. S.N. Beshers, Univ. of Illinois at Urbana-Champaign, for valuable discussions and critical reading of the manuscript. I also thank Dr. M. Mizunami, Tohoku Univ., for valuable comments.

REFERENCES

Breed MD, Bennet B (1987) Nestmate recognition in highly social insects. “In: Kin Recognition in Animals”, Eds. By Fletcher DJC, Michener CD, Wiley, New York, pp 243–286

Carlin NF, Halpern R, Hölldobler B, Schwartz P (1987) Early learning and the recognition of conspecific cocoons by carpenter ants (*Camponotus* spp.). Ethology 75: 306–316

Carlin NF, Hölldobler B (1983) Nestmate and kin recognition in interspecific mixed colonies of ants. Science 222: 1027–1029

Carlin NF, Hölldobler B (1986) The kin recognition system of carpenter ants (*Camponotus* spp.),I: Hierarchical cues in small colonies. Behav Ecol Sociobiol 19: 123–134

Carlin NF, Hölldobler B (1987) The kin recognition system of carpenter ants (*Camponotus* spp.), II: Larger colonies. Behav Ecol Sociobiol 20: 209–217

Carlin NF, Hölldobler B (1988) Influence of virgin queens on kin recognition in the carpenter ant *Camponotus floridanus* (Hymenoptera: Formicidae). Insectes soc 35: 191–197

Carlin NF, Schwartz PH (1989) Pre-imaginal experience and nestmate brood recognition in the carpenter ant, *Camponotus floridanus*. Anim Behav 38: 89–95

Errard C (1994) Development of interspecific recognition behavior in the ant *Manica rubida* and *Formica selysi* (Hymenoptera: Formicidae) reared in mixed-species groups. J Insec Behav 7: 83–99

Galizia CG, Menzel R, Hölldobler B (1999) Optical imaging of odor-evoked glomerular activity patterns in the antennal lobes of the ant *Camponotus rufipes*. Naturwissenschaften 86: 533–537

Gamboa GJ, Reeve HK, Holmes WG (1991) Conceptual issues and methodology in kin-recognition research: a critical discussion. Ethology 88: 109–127

Grotewiel MS, Beck CDO, Wu KH, Zhu XR, Davis RL (1998) Integrin-mediated short-term memory in *Drosophila*. Nature 391: 455–460

Hammer M, Menzel R (1995) Learning and memory in the honeybee. J Neurosci 15: 1617–1630

Hildebrand JG, Shepherd GM (1997) Mechanisms of olfactory discrimination: Converging evidence for common principles across phyla. Annu Rev Neurosci 20: 595–631

Hölldobler B, Wilson EO (1990) The Ants. The Belknap Press of Harvard University Press, Cambridge, Mass 732 pp

Jaisson P, Fresneau D (1978) The sensitivity and responsiveness of ants to their cocoons in relation to age and methods of measurement. Anim Behav 26: 1064–1071

Lenoir A (1981) Brood retrieving in the ant, *Lasius niger L*. Sociobiol 6: 153–178

Meudec M (1978) Response to and transport of brood by workers of *Tapinoma erraticum* (Formicidae; Dolichoderinae) during nest disturbance. Behav Proc 3: 199–209

Morel L, Vander Meer RK, Lavine BK (1988) Ontogeny of nestmate recognition cues in the red capenter ant (*Camponotus floridanus*). Behav Ecol Sociobiol 22:175–183

Obin MS (1986) Nestmate recognition cues in laboratory and field colonies of *Solenopsis invicta* BUREN (Hymenoptera: Formicidae). J Chem Ecol 12:1965–1975

Obin MS, Vander Meer RK (1988) Sources of nestmate recognition cues in the imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Anim Behav 36: 1361–1370

Stuart RJ (1992) Nestmate recognition and the ontogeny of acceptability in the ant, *Leptothorax curvispinosus*. Behav Ecol Sociobiol 30: 403–408

Yin JCP, Wallach JS, Vecchio MD, Wilder, EL, Zhou H, Quinn WG, Tully T (1994) Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. Cell 79: 49–58

Zars T, Fischer M, Schulz R, Heisenberg M (2000) Location of a short-term memory in *Drosophila*. Science 288: 672–675

(Received February 25, 2002 / Accepted July 2, 2002)