20 μM TTX in order to block sodium channels completely, the depolarization was larger than in Ringer with choline, but half to one-quarter of that in normal Ringer solution. Hence, photostimulation may be attributed to the formation of transient, light-induced, and probably nonspecific channels. However, these channels are small because they exclude choline.

The light pulse in the presence of these photostimulation probes often causes some irreversible photochemical damage. Only a limited number of repetitions (1 to 20, usually more than 3) of the photostimulation were possible. However, the fast recovery of the membrane resistance (Fig. 1F) indicates that the effect of light and the photostimulation probe on the membrane is different from the well-known photodynamic damage (from which recovery is slow or absent) observed with other dyes (*7*). The experiments described below indicate that the photochemical damage does not limit the usefulness of the technique, because even one or two photostimulations are sufficient to discover unknown synaptic connections.

Established neuronal pathways in the leech ganglion were confirmed by photostimulation. Figure 2A shows that a photostimulated medial sensory P cell was indeed synaptically connected to the NUT (cell 251). The same experiment was repeated 25 times, and in 22 experiments a microbeam pulse on the P cell evoked the inhibitory response. More than ten other established synaptic connections, both chemical and electrical, were each confirmed three to ten times by photostimulation.

Further studies of the medial P to NUT connection suggested that it was polysynaptic (*11*). We therefore searched for the unknown interneuron in the medial P to NUT synaptic pathway by using photostimulation. After scanning about 50 cells on the ventral side (*12*) of the ganglion, we discovered a 10-μm-diameter cell that forms an inhibitory synapse with the NUT (Fig. 2B). We have named this cell the PN neuron. It is found in the cluster of small cells posterior to the P cell corresponding in position to cells 51 to 54 (*2*).

While the use of a single electrode and the microbeam was far easier than the use of a pair of microelectrodes, photostimulation is still equivalent only to a stimulating electrode that cannot record. Because precise latencies are unknown, it is more difficult to determine whether monosynaptic or polysynaptic connections are detected. Nevertheless, after fast optical detection of connectivity (as in locating the cell body of a presynaptic neuron), the synaptic connections can be studied in detail with conventional electrophysiological techniques.

We confirmed that the PN neuron is indeed an interneuron in the P to NUT pathway by using pairs of microelectrodes to record synaptic activity between P to PN and PN to NUT (Fig. 2, C and D). In these experiments the PN interneuron was also injected with Lucifer yellow in order to unambiguously identify it by its characteristic morphology (Fig. 2E). Furthermore, when the PN neuron was killed with a 30-second laser pulse the P cell could no longer evoke a synaptic potential in the NUT, but the P to AE motoneuron connection remained functional.

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8. With some leech neurons we observed an increased rate of spontaneous firing by T cells but not P or N sensory cells. This phenomenon has been eliminated with the new probe, RH-500 (I. C. Farber *et al.*, unpublished results).
9. Theoretically, the laser microbeam offers excellent spatial resolution in three dimensions: practically, the in-plane resolution is about 2 μm and the out-of-plane resolution is 4 to 8 μm. Thus small neurons can be selectively stimulated without difficulty. However, when very small cells are stimulated, one should control for the possibility of stimulation of neuropil processes or cells in deeper layers. (Excessive staining may lead to a deterioration of the resolution by a factor of ~ 2.)
10. Laser light can modulate the electrical characteristics of the microelectrode tip. To eliminate artifacts it was necessary to minimize the gate leakage current of the recording electrometer.
11. The following observations suggest a polysynaptic pathway: (i) occasional variability in a relatively long (5- to 6-msec) latency of onset, (ii) occasional failure of single action potentials to elicit an inhibitory postsynaptic potential (IPSP), (iii) reversible blocking of the IPSP solution containing 15 mM Ca^{2+} and 20 mM Mg^{2+}, (iv) occasional stimulation of the P-evoked excitatory response (1 to 2 mV) in the NUT, and (v) failure of the injection of 3 M tetraethylammonium ion into the P cell to affect the IPSP recorded from the NUT. Some of these observations differ from those for *Macrobdella decora* [A. L. Kleinhaus and S. Brand, *Comp. Biochem. Physiol.* **70A**, 37 (1981)].
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Nestmate and Kin Recognition in Interspecific Mixed Colonies of Ants

Abstract. *Recognition of nestmates and discrimination against aliens is the rule in the social insects. The principal mechanism of nestmate recognition in carpenter ants (*Camponotus*) appears to be odor labels or "discriminators" that originate from the queen and are distributed among, and learned by, all adult colony members. The acquired odor labels are sufficiently powerful to produce indiscriminate acceptance among workers of different species raised together in artificially mixed colonies and rejection of genetic sisters reared by different heterospecific queens.*

A fundamental proposition of sociobiology, that altruistic behavior can evolve by natural selection only if the beneficiaries of the behavior are related to the donors, requires that helpers somehow direct their aid preferentially toward kin (*1*). Insect societies exhibit an extreme form of altruism in which most colony members do not reproduce at all but instead help others in their reproductive effort. The means by which social insects attempt to distinguish kin from nonkin have recently been subjected to intensive experimental and theoretical analysis (*2–4*). Two principal recognition mechanisms seem likely to occur in highly eusocial ants and bees: each colony member may produce genetically determined odor labels or "discriminators" that are distributed among all nestmates to form a collective colony odor; or the queen may produce discriminators that are distributed to all of her workers (*5*). We here report experimental evidence suggesting that queen discriminators serve as recognition cues in monogynous ants of the genus *Camponotus*. These acquired odor labels are sufficiently

Table 1. Interspecific and intraspecific interactions in six mixed colonies. Antennation, allogrooming, and regurgitation are summed. Interspecific interactions are listed by the species which initiated the act. Numbers of workers in each colony are given in parentheses after the species name; the queen is of the species listed first. Expected values (weighted for numbers of each species per colony) are given in parentheses following the observed interactions (*8*). Colonies with a single adoptee worker have no intraspecific adoptee interactions (–). Significance of preferences was calculated by χ_1^2 test; nonsignificant preferences have $P > 0.35$.

Species	Interactions		Preference (*P*)
	Intraspecific	Interspecific	
1.*C. noveboracensis* (2)	111 (118.0)	361 (354.0)	N.S.
+*C. pennsylvanicus* (3)	181 (178.5)	176 (178.5)	N.S.
2.*C. noveboracensis* (7)	512 (508.0)	504 (508.0)	N.S.
+*C. pennsylvanicus* (6)	232 (250.4)	369 (350.6)	N.S.
3.*C. pennsylvanicus* (3)	220 (228.7)	123 (114.3)	N.S.
+*C. noveboracensis* (1)	–	227 (227.0)	–
4.*C. pennsylvanicus* (4)	265 (242.7)	463 (485.3)	N.S.
+*C. ferrugineus* (6)	375 (369.4)	290 (295.6)	N.S.
5.*C. pennsylvanicus* (10)	520 (685.5)	394 (228.5)	< 0.001 interspecific
+*C. americanus* (3)	160 (95.5)	413 (477.5)	< 0.001 intraspecific
6.*C. pennsylvanicus* (6)	506 (561.7)	168 (112.3)	< 0.02 interspecific
+*C. americanus* (1)	–	265 (265.0)	–

powerful to produce indiscriminate acceptance among workers of different species that were raised together in artificially mixed colonies, and rejection of genetic sisters reared by heterospecific queens.

Colonies consisting of a queen and two worker groups, the queen's daughters plus workers from a heterospecific colony, were created to test certain predictions of the two proposed recognition mechanisms. If each individual bears a mixture or "gestalt" (*3*) of discriminators produced by itself, sisters, and heterospecific nestmates, all equally concentrated, transferable, and effective, then the odor gestalt of a colony containing a large proportion of one group will be similar to that of a pure colony of sisters of that group. As the proportion of a group of sisters in a mixed colony decreases, its odor gestalt should become progressively less recognizable to sisters that are not nestmates (Fig. 1, line b). However, if queen discriminators label all workers equally, then adoptees will be completely unrecognizable to their non-nestmate sisters, no matter what their proportion in the mixed colony (Fig. 1, line a).

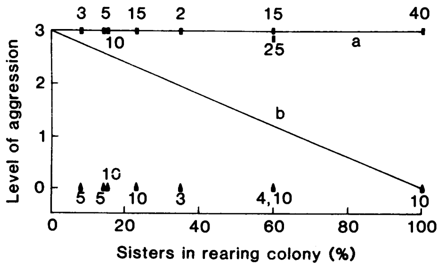

Fig. 1. Aggressive responses of workers reared in interspecific mixed colonies containing varying proportions of sisters. (Rectangles) Tests with non-nestmate genetic sisters reared in stock colonies. (Triangles) Control tests with nestmate sisters. (Line a) Expected results for a distributed queen discriminator model (see text). (Line b) Expected results for a gestalt model. Numbers of trials shown next to each point. Data pooled from mixed colonies of different species composition. Aggression scale defined in text.

Founding queens and mature colonies of five *Camponotus* species were collected in the field and housed in clean laboratory nests; all received the same diet to preclude environmental odor differences (*6*). When the foundresses began egg-laying, small heterospecific larvae were introduced into their nests in varying numbers. Most were adopted, groomed, and fed and grew to adulthood. All cross-fostered larvae given to any one queen were sisters from the same stock colony, and their heterospecific nestmates were also all sisters, daughters of the queen. The queen's own brood was removed from one nest, to produce a colony of all-adoptee workers. Adoptees could easily be identified by the experimenter, as the species used differ in color and size.

The colonies had reached an average size of 9.3 ± 3.2 workers (5.9 ± 3.8 queen daughters, 3.4 ± 2.6 adoptees) when tested (*7*). To assess discrimination within mixed nests, each of six colonies was observed for 16 hours, during which 6835 intra- and interspecific worker interactions—antennations, allogrooming, and food exchanges (Fig. 2A)—were cataloged. The ratios of inter- to intraspecific behavior expected from random interaction, given the number of workers of each species in each nest, were calculated and compared with the observed results by χ_1^2 test (*8*). In four colonies, the distributions of worker interactions did not differ significantly from random (Table 1). In the two colonies containing *C. pennsylvanicus* and *C. americanus* workers, both species solicited significantly more regurgitated food from *americanus*. Almost all foraging in these colonies was performed by *americanus* workers, while the *C. pennsylvanicus* remained within the nest, the former thus serving as food donors to both species (*9*). No overall pattern of preference for kin or rejection of heterospecific nestmates emerged in any colony.

The strong aggressive response of these ants to adult non-nestmates was used as a measure of mutual recognizability of genetic sisters reared in mixed and pure colonies. An adoptee worker was placed in a glass dish (9 cm in diameter), while another worker, from the same stock colony from which the adoptee was taken as a larva, was removed to a similar dish. After about 1 minute, when both became calm, the stock colony sister was placed with the adoptee. Aggression was scored on an arbitrary scale of 0 to 3: (0) acceptance, huddling, and grooming; (1) avoidance, mutual threat; (2) weak attack, nipping, or light leg pulling; (3) full attack, locking together, biting, or spraying formic acid. Level 3 aggression was often lethal for the smaller ant, unless the two were separated (*10*). Adoptees from eight mixed colonies were each tested against five non-nestmate sisters (fewer if the

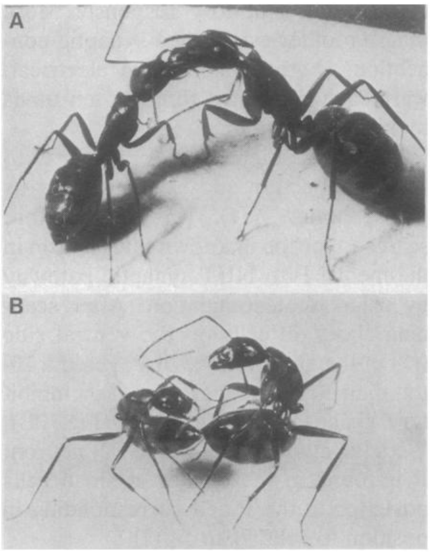

Fig. 2. (A) Food exchange between heterospecific workers reared together in a mixed colony. [*Camponotus ferrugineus* (right) and *C. pennsylvanicus*.] (B) Strong aggressive reaction between an adoptee *C. americanus* worker (left), raised by a *C. pennsylvanicus* queen, and her genetic sister, raised in the colony of their mutual mother queen. Abdomens forward, the sisters spray one another with formic acid.

stock colony had fewer surviving sisters or if adoptees were killed in tests), for a total of up to 25 trials per colony (40 in the all-adoptee colony); 115 trials in all.

The results confirmed the prediction of the queen discriminator hypothesis (Fig. 1). All non-nestmate sisters attacked each other violently, regardless of the proportion of adopted sisters in the mixed colony (Fig. 2B). Stock colony workers found that their sisters from the all-adoptee colony, containing a heterospecific queen but no nonsister workers, were just as unrecognizable as a single sister reared in a colony of heterospecific workers. Control reintroductions of mixed colony sisters to one another, immediately after their attacks on stock colony sisters, always resulted in acceptance (Fig. 1). In ten out of ten coded (blind) tests, workers of unknown origin could be assigned correctly to stock or mixed colonies on the basis of their reaction to stock colony sisters (*11*).

In the extensively studied small colonies of the primitively social bee, *Lasioglossum zephyrum*, the discriminators of individual nestmates are learned separately. Discriminators are not shared or transferred among nestmates; an unfamiliar bee is accepted if its odor is similar to that of any one nestmate (*3, 12*). In the very large colonies of highly eusocial ants and bees, it is unlikely that workers could learn every individual nestmate's odors. Some blend of discriminators must form a homogeneous label acquired and learned by all, as is demonstrated by the indiscriminate mutual acceptance of members of interspecific mixed colonies. Either a shared odor gestalt or transferable queen discriminators would serve this function; our data support the latter model (*13*). We do not suggest that workers lack discriminators, but that these are dominated by those of the queen, possibly to be "uncovered" on her removal or death. As a kin recognition system this mechanism is somewhat error-prone, vulnerable to incursion by slave-making ants and myrmecophiles (*14*). Normally, however, any individual enclosing in a colony is an offspring of its queen and is correctly so labeled by the acquisition of her discriminators; and any other ant sharing this label is usually a relative. Our results might also explain the apparently reduced efficiency of recognition in polygynous ants, if learning multiple queens' discriminators gives workers broader tolerances (*5*).

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6. Colonies of *Camponotus americanus*, *C. ferrugineus*, *C. noveboracensis*, *C. pennsylvanicus*, and *C.* (*Mytmentoma*) *nearcticus* were collected in eastern Massachusetts. (Voucher specimens deposited in the Museum of Comparative Zoology, Harvard University.) Queens were housed in new test tubes (2.2 cm inner diameter, 15 cm long) containing water trapped at the bottom behind a cotton plug, placed in thoroughly washed plastic boxes (19.5 by 14 by 7.5 cm); mature colonies were given several test tubes in a larger arena. All were fed synthetic ant diet [A. Bhatkar and W. H. Whitcomb, *Fla. Entomol.* **53**, 229 (1970)], honey-water, chopped cockroaches, and *Tenebrio* larvae.
7. Kept at 30°C, these ants remain active through winter, but no new adults are produced. Evidence suggests that colony odors are learned or acquired (or both) by callow adults shortly after eclosion (*3*). Our colonies were tested 4 months after the summer-produced workers had passed this period.
8. A worker in a colony containing n_c conspecifics (including itself) and n_h heterospecifics may interact with $n_c - 1$ of the former and all n_h of the latter. If there is no preference for interacting with either, then for a total of A acts observed in a given species, the expected number of intraspecific and interspecific interactions are $e_c = A(n_c - 1)/(n_c - 1 + n_h)$ and $e_h = A(n_h)/(n_c - 1 + n_h)$, respectively. The significance of deviation of observed intraspecific and interspecific acts, A_c and A_h, from e_c and e_h was calculated from a χ^2 statistic with one degree of freedom.
9. This "pseudocaste" phenomenon is one in which members of mixed colonies show species-specific behavior differences (unpublished data). Data on antennation, allogrooming, and regurgitation are pooled in Table 1, but their significance is the same analyzed separately. In one of the two *pennsylvanicus-americanus* colonies, both species preferred to antennate and solicit regurgitation from *americanus*, while in the other *americanus* was solicited but not antennated preferentially. Allogrooming was indiscriminate among and between species in both colonies.
10. Aggressive behavior patterns varied in different species. For example, at level 3, *C. americanus* sprayed formic acid, while *pennsylvanicus* and *ferrugineus* grappled and bit but rarely sprayed. *C.* (*Mytmentoma*) *nearcticus* workers were nonaggressive, always avoiding other ants in 9-cm glass dishes. When forced to encounter each other by being confined in glass vials (0.8 cm in diameter, 5.5 cm long), these ants did attack non-nestmates.
11. We also observed level 3 aggression in each of six tests between genetic *C. americanus* sisters adopted by different *C. pennsylvanicus* queens. This excludes the possibility that species-specific, rather than colony-specific, discriminators are derived from the queen, since the workers had conspecific queens.
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Sex Differences in Mathematical Reasoning Ability: More Facts

Abstract. *Almost 40,000 selected seventh-grade students from the Middle Atlantic region of the United States took the College Board Scholastic Aptitude Test as part of the Johns Hopkins regional talent search in 1980, 1981, and 1982. A separate nationwide talent search was conducted in which any student under age 13 who was willing to take the test was eligible. The results obtained by both procedures establish that by age 13 a large sex difference in mathematical reasoning ability exists and that it is especially pronounced at the high end of the distribution: among students who scored ≥ 700, boys outnumbered girls 13 to 1. Some hypothesized explanations of such differences were not supported by the data.*

In 1980 we reported large sex differences in mean scores on a test of mathematical reasoning ability for 9927 mathematically talented seventh and eighth graders who entered the Johns Hopkins regional talent search from 1972 through 1979 (*1, 2*). One prediction from those results was that there would be a preponderance of males at the high end of the distribution of mathematical reasoning ability. In this report we investigate sex differences at the highest levels of that ability. New groups of students under age 13 with exceptional mathematical aptitude were identified by means of two separate procedures. In the first, the Johns Hopkins regional talent searches in 1980, 1981, and 1982 (*3*), 39,820 seventh graders from the Middle Atlantic region of the United States who were selected for high intellectual ability were given the College Board Scholastic Aptitude Test (SAT). In the second, a nationwide talent search was conducted for which any student under 13 years of age who was willing to take the SAT was eligible. The results of both procedures substantiated our prediction that before age 13 far more males than females would score extremely high on SAT–M, the mathematical part of SAT.

The test items of SAT–M require numerical judgment, relational thinking, or insightful and logical reasoning. This test