The kin recognition system of carpenter ants (Camponotus spp.)

III. Within-colony discrimination

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Received July 1, 1986 / Accepted October 4, 1986

Summary. Hamilton's (1964) hypothesis linking haplodiploidy and eusociality in the Hymenoptera could be reconciled with the occurrence of polygyny and multiple insemination if workers are able to distinguish full (3/4 related) sisters from other familiar matriline and patriline within the colony, and direct altruistic behavior toward them preferentially. We examined this possibility in small genetically mixed nests of the carpenter ant Camponotus floridanus, formed by the transfer of worker pupae from two unrelated source colonies. In 120 h of observation on 12 queenright and 12 queenless nests, more than 15,000 behavioral interactions were recorded. Workers antennated familiar non-kin significantly more frequently than familiar sisters. However, they failed to discriminate consistently between kin and non-kin in food exchanges and grooming. Aggressive behavior was occasionally observed in some queenless nests, but almost never in the presence of a queen. When aggression did occur, it was directed significantly more often toward non-kin. Though related adult workers did not cooperate preferentially, the biases in antennation and aggression do indicate an ability to discriminate familiar kin from familiar non-kin, which may be employed in other contexts such as the rearing of reproductive brood.

Introduction

In his original presentation of the possible connection between inclusive fitness, haplodiploidy and eusociality in the Hymenoptera, Hamilton (1964) detailed the problems posed for his hypothesis by the frequent occurrence of multiple queens per colony (polygyny) and multiple inseminations per queen (so-called "polyandry"). He suggested two remedies: Inbreeding might restore high levels of relatedness between workers and reproductive sisters. Or, multiple mating and polygyny might be more recent adaptations, arising long after queen specialization and control of reproduction prohibited "sexualized" mutant workers from successfully competing. The latter explanation has never satisfied students of kin selection, largely because it seems untestable, while the former has been falsified by a number of electrophoretic studies measuring quite low levels of within-colony relatedness (e.g., Craig and Crozier 1979; Crozier et al. 1984; Lester and Selander 1981; Metcalf and Whitt 1977; Page and Metcalf 1982; Pamilo 1982; Pearson 1983; but see also Crozier 1977; Ward 1983).

Recently, a number of investigators have attempted to rescue Hamilton's model from these difficulties by invoking kin recognition, now well known in a wide variety of animals. If eusociality in the contemporary Hymenoptera is maintained by kin selection, and 3/4-related full sisters are distinguishable from half sisters within colonies, then preferential cooperation is expected among full-sister cohorts (reviewed by Gadagkar 1985). However, most studies have focused on recognition between unfamiliar individuals (of varying degrees of relatedness) and/or between colonies (Breed et al. 1985; Buckle and Greenberg 1981; Carlin and Hölldobler 1983, 1986; Getz and Smith 1983; Mintzer 1982; Stuart 1985), rather than within colonies. The advantages of between-colony discrimination, providing territorial exclusivity and protection from robbers and parasites, accrue equally to...
all matrilineal and patrilineal kin in the nest. Thus, though highly adaptive, the ability to recognize nestmates and reject non-nestmates is not directly pertinent to the haplodiploidy hypothesis. Inclusive fitness advantages certainly do arise from the recognition of unfamiliar kin; e.g., queens may prefer to confound with relatives (Gamboa et al. 1986), or potentially reproductive foreigners may be admitted on the basis of kinship (Greenberg 1979). However, neither of these contexts necessarily bears on the problem of sterile workers.

An experimental demonstration that workers can distinguish familiar kin from familiar non-kin, and familiar full sisters from familiar half sisters, could reconcile polygyny and multiple mating with inclusive fitness theory. Three studies of honey bees have addressed this question to date: Getz et al. (1982) investigated segregation of worker patriline during swarming; Evers and Seeley (1986) reported aggressive discrimination between patriline; and Frumhoff and Schneiders (in press) examined cooperative interactions biased toward full sisters. In all of these experiments, artificial insemination was used to produce patrilineal cohorts recognizable to the experimenter by different color phenotypes. However, the genes determining color markers could also affect the patriline’s odor cues or discriminators (Hölldobler and Michener 1980). Cordovan bees might discriminate between cordovan full sisters and black half sisters, but be unable to distinguish half-sisters fathered by an unrelated cordovan male; other behaviors such as the timing of swarm departure (Getz et al. 1982) might also be altered.

In this paper, we present evidence for discrimination between familiar kin and familiar non-kin in *Camponotus floridanus*, a monogynous, and probably multiply inseminated, carpenter ant. The number of matings has not been documented for this genus, but it does exhibit the characteristic “male aggregation” mating syndrome often associated with multiple insemination (Hölldobler and Bartz 1985). As queens have been shown to play a major role in rejection of non-nestmates by these ants (Carlin and Hölldobler 1983, 1986, 1987), we also examined their influence on within-colony recognition. Though *C. floridanus* workers adopted into small colonies did prove able to recognize adult kin cohorts, preferential altruistic interactions among sisters were not observed. However, the “haplodiploidy plus kin recognition” model of eusociality could be supported by kin-directed altruism in other contexts than those tested here, such as the rearing of reproductive brood.

### Methods

**Experimental colonies**

Founding queens and incipient colonies of *Camponotus floridanus* were collected in Tallahassee and on Shark, Sugarloaf and the Saddlebunch Keys and Key West, Florida. Voucher specimens are deposited in the Museum of Comparative Zoology, Harvard University. This species remains active year round in the laboratory, facilitating the formation of experimental colonies and observation of behavior. Colonies were housed and maintained as described in Carlin and Hölldobler (1986); all were fed synthetic ant diet (Bhatkar and Whitcomb 1970), dilute honey and chopped cockroaches (*Nauphoeta cinerea*).

Two large stock colonies, reared from foundresses collected in Tallahassee and on Shark Key, served as sources of pupae for mixed nests. Twelve queens were collected from founding chambers, most with eggs and larvae, and each was provided with pupae nearly ready to eclose, taken from a stock colony. On emergence, the workers were permanently marked with loops of colored wire, tied around the petiole (Dobrzański 1966), and pupae from the other stock colony were added. By the conventions used in Carlin and Hölldobler (1986), each colony contained *(A1)* and *(A2)* adoptees, with an *(A3)* queen unrelated to either. (The letter “A” indicates that queens and workers are of the same species; different number subscripts indicate different colonies of origin.) The queens’ own eggs and larvae were allowed to accumulate, but were removed on pupation.

Twelve queenless groups were also created. The first several workers of each group were manually removed from their cocoons, using fine forceps to tear the silk and pupal skin. Those *(A1)* workers that survived for 24 h were wire-marked and given additional sister *(A1)* pupae. After eclosion these too were marked, and *(A2)* pupae were added. No eggs or larvae were added to the queenless groups, to prevent possible transfer of recognition cues borne by brood from other nests, and no ovipositing workers were observed; these groups were therefore without brood.

In some queenless and queenright mixed groups, mortality among the first-eclosed *(A1)* group was high. The emerging *(A2)* workers in these nests were marked with wire of another color and further *(A1)* pupae were added. In others the majority of the *(A1)* workers survived, and only one kin group was wire-marked. Thus the 24 experimental nests each contained two groups of sisters in varied proportions; their compositions at the time of behavioral observation are given in Tables 1 and 2. If stock colony queens were multiply inseminated, each kin group also contained two cohorts, of full sisters and half sisters, in unknown proportions. In collecting data on interactions within and between kin groups, we hypothesize that workers capable of within-colony discrimination should prefer interacting with both full and half sisters to interacting with familiar non-kin.

**Data collection**

Queenright colonies were observed beginning 7 months after the last stock pupae were added, when each contained an average of 13.1 ± 3.1 (5 ± SD) workers (range 11 to 18). Observations of queenless mixed groups, with 13.2 ± 4.1 (range 5 to 19) workers each, began 2 months after pupae were added, as previous experience suggested that high mortality would occur in queenless groups by 7 months. Data were not collected at a specific time of day; however under laboratory conditions these small colonies did not exhibit marked circadian variation in behavior. Each experimental nest was observed for 5 h,