

## Genotypic Effects on Forager Behavior in the Neotropical Stingless Bee *Partamona bilineata* (Hymenoptera: Meliponidae)

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Specialized performance of tasks by workers is a common phenomenon in eusocial insects, particularly in species with adult colony populations in excess of several hundred individuals. In a number of taxa nestmates that are genetically more similar tend to specialize on the same tasks. In stingless bees (Meliponinae) foraging workers specialize by collecting one of the several materials needed by the colony. This study tested whether nestmates of the stingless bee *Partamona bilineata* that forage for the same material are genetically more similar than they are to foragers collecting other materials. We found that the degree of sharing of random amplified polymorphic DNA marker bands was higher among foragers for the same material in each of three subject colonies. These data suggest that division of labor among stingless bee workers includes a genetic component, as it does in honeybees and in other eusocial insects.

Behavioral specialization occurs at several levels in insect colonies. Reproductive division of labor refers to the specialization by certain individuals (queens and drones) on producing offspring and by others (workers) on nonreproductive task performance. Workers may further specialize on

one or a few nonreproductive tasks. Division of labor among workers is called polyethism. In many eusocial insect species, the age of the worker influences polyethism, such that workers progress through several task sets as they age [1]. Age-based polyethism is a separate phenomenon from task specialization, which occurs within a given suite of age-specific tasks. Task specialization by workers in social insect colonies can lead to more efficient division of labor and therefore greater reproductive fitness [2]. Increasing attention has been paid to the mechanistic bases for task specialization [3]. Much of this research has focused on honeybees, *Apis mellifera* in particular, and genetic differences among workers within colonies have been shown to affect task specialization [3–5]. Recently, genetic effects on task specialization have been demonstrated in a diversity of eusocial insects where colonies have multiple queens (polygyny), or where queens mate with several males (polyandry) [6–10]. Eusocial insect species with large colony sizes and genotypically diverse worker forces can employ genetic effects on task specialization as a component of division of labor among workers. Genetic effects may enhance efficiency and/or reliability of task performance at the individual level [11].

While task specialization occurs in both honeybees and stingless bees (Meliponinae), the genetic basis of

specialization has not been investigated in stingless bees. Stingless bee workers specialize when foragers collect one or a subset of the various materials used by the colony [12, 13]. Some stingless bee species resemble *Apis mellifera* in colony size and structure [14]. Stingless bees are highly eusocial (queens differ morphologically from workers) and colonies of some species contain more than 10,000 individuals [15]. Colonies of most meliponines are thought to be monogynous with singly mated queens [15] (J. Strassmann, personal communication). However, behavioral observations suggest that multiple mating can occur [16]. Furthermore, allozyme electrophoresis has provided evidence for either polygyny or polyandry in *Partamona helleri* [17], suggesting that genetic diversity can be elevated (relative to colonies with a single, once-mated queen) within colonies of stingless bees in the genus *Partamona*.

We asked whether forager behavior corresponds to genotypic similarity among nestmates in a stingless bee species, *Partamona bilineata*. Nests of *P. bilineata* are found in fallen logs along roadsides and in bolls of trees in premontane habitats in Costa Rica. Fieldwork was conducted at Monteverde, Costa Rica (10°18'N, 84°49'W, elevation 1300 m). On 12 May 1994 workers from three colonies of *P. bilineata* were collected at their nest entrances as they returned from foraging trips. Approximately 30 foragers were collected from each colony over a 20- to 30-min period. After collection bees were sorted according to the contents of the pollen baskets on their hind legs (corbiculae). Individuals were scored as carrying either pollen (colony B only), resin, or nothing (empty corbiculae). A later study showed that workers with empty corbiculae usually carried nectar, and that those carrying pollen usually had empty crops (S. Ranger, K. Ober, and E. Smith, unpublished data). After collection, bees were stored in 100% ethanol and frozen at -20°C.

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Genomic DNA was extracted from individual bees using a standard phenol:chloroform protocol [18]. DNA samples were diluted to 3 ng/ $\mu$ ml in TE buffer and refrigerated at 4°C until used in the polymerase chain reaction. Random amplified polymorphic DNA (RAPD) markers were generated from the genomic DNA of each forager (for detailed protocols see [9, 19]) using 16 different ten-base DNA primers (Operon Technologies) that had been screened for variability within the study population. PCR products were separated on gels of 1% agarose/0.7% Synergel (Diversified Biotech) exposed to a current of 100 V for 5 h. Following electrophoresis DNA in the gels was stained with ethidium bromide. The gels were then exposed to UV light on a transilluminator and photographed. RAPD markers were scored from photographs. Protocols for all laboratory procedures are available from the corresponding author upon request. All RAPD markers were scored as presence/absence polymorphisms. Only presence/absence marker bands that were scored identically by two independent observers were used in the study. RAPD marker phenotypes were checked for repeatability on 5–15% of the individuals (range across colonies) in the study. Between 20 and 31 informative (variable) marker loci were scored for each colony. Scoring was performed blind to worker identity and foraging classification. RAPD loci that vary as presence/absence polymorphisms produce dominant marker alleles; heterozygous individuals cannot be distinguished from those homozygous for the present allele [20]. Although true genotypes cannot always be distinguished at a given locus, sharing of presence/absence RAPD markers provides information on genetic similarity among individuals [9, 21–24]. Statistical analyses tested whether individuals that were genetically more similar were also more similar in foraging behavior. Matrices of RAPD marker band phenotypes for individuals from each colony were subjected to hierarchical cluster analyses using Ward's method and Euclidean distance (SPSS 6.1; we chose this clustering algorithm be-

cause it was employed in several earlier studies to analyze genotypic effects on worker specialization [10, 21–23]). Results of the cluster analysis are represented as dendrograms (Fig. 1). The dendrograms indicate the degree of genotypic similarity among nestmates. Three or four clusters of genetically similar workers were delimited by selecting dendrogram branches between 50% and

75% of the total genetic distance for each colony. Cluster demarcation was performed blind to forager identity. The likelihood ratio  $\chi^2$  test was used to determine whether the frequency of forager types differed among the clusters. This test is less sensitive to the bias of a  $\chi^2$  test when expected values within some cells of the contingency table are small [25].

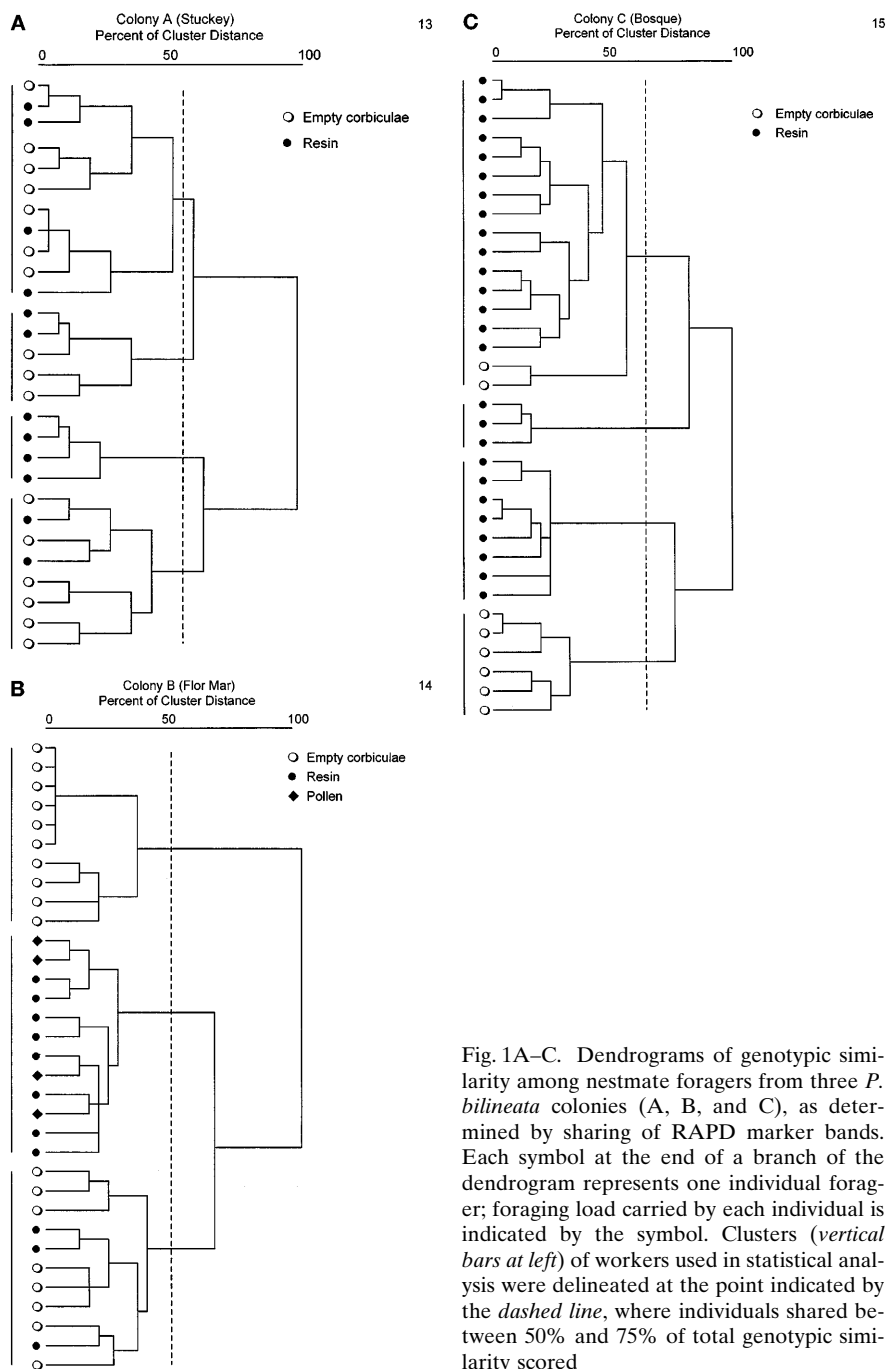


Fig. 1A–C. Dendrograms of genotypic similarity among nestmate foragers from three *P. bilineata* colonies (A, B, and C), as determined by sharing of RAPD marker bands. Each symbol at the end of a branch of the dendrogram represents one individual forager; foraging load carried by each individual is indicated by the symbol. Clusters (vertical bars at left) of workers used in statistical analysis were delineated at the point indicated by the dashed line, where individuals shared between 50% and 75% of total genotypic similarity scored

DNA was extracted from 95 foragers (colony A:  $n=28$ ; colony B:  $n=33$ ; colony C:  $n=34$ ). Of these, 49 bees carried resin in their corbiculae, 4 carried pollen (all from colony B) and 42 had empty corbiculae. Forager type frequencies within clusters of genotypically similar individuals were significantly nonrandom, i.e., forager types tended to cluster together (Fig. 1; likelihood ratio test: colony A:  $\chi^2=8.10$ ,  $df=3$ ,  $P<0.05$ ; colony B:  $\chi^2=34.71$ ,  $df=4$ ,  $P<0.001$ ; colony C:  $\chi^2=24.79$ ,  $df=3$ ,  $P<0.001$ ). Using Fisher's exact test (a more conservative contingency test), similar results were obtained in colonies B and C ( $P<0.001$ ), but the association was not significant in colony A ( $P=0.10$ ). These results indicate that *P. bilineata* nestmates foraging for the same material were genetically more similar to one another than to those foraging for other materials. This association of foraging behavior with genotypic similarity suggests that task specialization includes a genetically determined component in *P. bilineata*.

Task specialization by workers in the form of foraging specialization on materials occurs in many taxa of social insects and is well-documented in stingless bees [8–10, 13, 22]. Studies of stingless bees and other swarm-founding eusocial insects [26] demonstrate that foragers often specialize by collecting a single material, and that forager specialization does not change sequentially with age. For example, Inoue et al. [12] found that some foragers of a Sumatran stingless bee, *Trigona minangkabau*, specialize on resin collection, while other individuals switch between pollen and nectar collection, and that switching does not follow a regular temporal sequence. Sommeijer et al. [27] noted most foragers (75%) of *Melipona favosa* specializing on either nectar or pollen, with little switching throughout their foraging careers. Our finding of genetic effects on task specialization in stingless bees adds to the growing evidence for genotypic effects on task specialization in social insect taxa.

One would expect to find task specialization among workers in stingless bee colonies because of their large populations [28]. Polygyny, polyan-

dry, or genetic recombination of alleles in heterozygous queens represent possible sources of variation in genetic similarity among workers within colonies. Although the sources of genetic variability in *P. bilineata* colonies were not investigated in this study, studies of a Brazilian *Partamona* spp. suggest that *P. bilineata* can be either polygynous or polyandrous [17]. Furthermore, high rates of recombination of the mother queen's genome can contribute to genotypic diversity among her offspring workers and thereby influence division of labor [19]. Recombination rates are very high in honeybees [19], and if recombination rates are also high in stingless bees, genetic differences among workers would be enhanced even in monogynous/monandrous colonies.

The evolutionary relationships among eusocial bees, including that between Meliponinae (stingless bees) and Apinae (honeybees) is unresolved [29]. In particular, it is uncertain whether advanced eusociality arose once in the family Apidae (in a common ancestor of honey bees and stingless bees), or whether it evolved twice from a primitively eusocial ancestor. Our data suggest that genetic effects on task specialization occur in two of four subfamilies of apid bees. If these subfamilies are sister taxa, as suggested by Prentice [14] and Roig-Alsina and Michener [30], parsimony would suggest a single origin of genetic effects on polyethism within the Apidae.

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## Carbon Dioxide Is a Close-Range Oviposition Attractant in the Queensland Fruit Fly *Bactrocera tryoni*

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The skin of a fruit forms a barrier against penetration by the ovipositors of parasites such as tephritid flies. After alighting on an apple, gravid females of *B. tryoni* strongly prefer to oviposit into an existing small lesion in the skin rather than creating a new one. A lesion facilitates the escape of volatile substances from the inside of a fruit, including respiratory carbon dioxide. It is found that there is a behavioural correlate to the known presence of CO<sub>2</sub>-receptor neurons in *B. tryoni*: A localized CO<sub>2</sub> source simulating a lesion is sufficient by itself and at ecologically relevant concentrations to attract female flies and to elicit oviposition behaviour. A source concentration of 100 ppm above am-

bient (360 ppm) evokes a detectable response, and between 100 and 3000 ppm the concentration-response function is logarithmic. It is concluded that CO<sub>2</sub> stimuli contribute to close-range oviposition site identification by *B. tryoni*.

In common with many other land arthropods (Stange 1996), and across a range of environments, dipteran insects carry receptor neurons that sense CO<sub>2</sub>. In a confined environment the concentration of CO<sub>2</sub> can be high, and oriented behavioural responses to CO<sub>2</sub> have been observed in the larvae of the carrot fly *Psila rosae* (Städler 1971, 1972; Jones and Coaker 1977) and other soil dwellers. In the open atmosphere, large con-

centrations occur in plumes of air exhaled by warm-blooded animals and contribute to host orientation in haematophages such as tsetse flies (Willemse and Takken 1994) and mosquitoes (Bowen 1991). Another source of fluctuations in open-air CO<sub>2</sub> concentration, albeit much smaller, consists of the metabolic activity of the plant hosts of herbivores. Electrophysiological experiments show that CO<sub>2</sub>-receptor neurons in adults of the corn earworm moth *Helicoverpa armigera* can detect fluctuations by 0.14% of a 360 ppm background (Stange 1992). In the vicinity of plants, detectable fluctuations occur at close range, and larvae of *H. armigera* are strongly attracted by CO<sub>2</sub> contrasts that are likely to occur in the boundary layer around growing plant organs (Rasch and Rembold 1994). In the moth *Cactoblastis cactorum* exposure of host plants to artificial CO<sub>2</sub> fluctuations (noise) suppresses oviposition, attributable to disruption of close-range orientation (Stange 1997).

Tephritid flies are fruit parasites of considerable economic importance. In *B. tryoni* high concentrations of CO<sub>2</sub> evoke arousal responses (Rice 1989); recently, antennal sensilla that bear the hallmark of CO<sub>2</sub> receptors (lamellated dendrites in wall-pore