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Preferential Brood Care in Artificially Mixed *Acromyrmex* Colonies

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Novelty Statement — We tested the hypothesis of Holmes and Sherman (1983) that an individual that emerges in a mixed colony integrates the chemical signals of the social environment

We concluded that *A. subterraneus molestans* and *A. subterraneus brunneus* workers tend to prefer larvae of their own species, regardless of whether or not they are concolonial, thus confirming the hypothesis of Holmes and Sherman.

Abstract — **Here we tested the hypothesis of Holmes and Sherman (1983) that an individual that emerges in a mixed colony integrates the chemical signals of the social environment (allo- and conspecific) and acquires a mixed memory for future comparisons, a topic still unknown for *Acromyrmex* species. For this purpose, the interactions of workers with concolonial, conspecific and allospecific larvae were recorded over a period of 1 hour in two experimental groups of two *Acromyrmex* species (*A. subterraneus brunneus* and *A. subterraneus molestans*), with 10 replications in each experimental group, totaling 40 hours of recording. Five behaviours related to offspring care performed by *A. subterraneus molestans* and *A. subterraneus brunneus* workers were recorded: licking the larval body, carrying larvae into fungus sponge, ingesting faecal liquid, inserting hyphae on body of larvae, and scraping the larval mouthparts. The results indicate different levels of acceptance and rejection of concolonial, conspecific and allospecific larvae in *A. subterraneus brunneus* and *A. subterraneus molestans* workers. In conclusion, the data suggest that *A. subterraneus molestans* and *A. subterraneus brunneus* workers tend to prefer larvae of their own species, regardless of whether or not they are concolonial, thus confirming the hypothesis of Holmes and Sherman.**

Keywords — **Hymenoptera, Social insects, Leaf-cutting ants**

I. INTRODUCTION

In terms of relative abundance, ants account for about one-third of the insect biomass in the humid tropical forests of South America (Fittkau and Klinge, 1973). Ant species can have populous colonies, as is the case of the genus *Acromyrmex* whose colonies are composed of hundreds of individuals that must behave in order to maintain colony integrity, protecting the colony against predators, microorganisms and parasitism and ensuring its development and reproduction. This objective is achieved through a chemical recognition system that promotes the cohesion, integration and internal communication among members of a colony.

Cuticular hydrocarbons are the most common chemical compounds found on the cuticle of social insects and are composed for environmental odours. Although cuticular hydrocarbons protect insects against desiccation (Lockey, 1988) and invasion of microorganisms or toxins (Howard, 1993; Lorenzi et al. 1996), studies suggest these compounds to play a role in the recognition system, modifying the acceptance of other individuals as nestmates (Bonavita - Congourdan et al. 1987; Morel et al. 1988, Henderson et al. 1990; Lahav et al. 1999; Suarez et al. 2002). In addition to cleaning the bodies of nestmates by grooming, workers apply and reorganize chemical substances (Wilson, 1971; Jander, 1976; Kovac and Mashwitz, 1989), suggesting the participation of this behavioural act in the recognition system. The chemical recognition system may also be related to the tendency of individuals to search for and to keep social contacts with nestmates (Boulay and Lenior, 2001), permitting the perception, maintenance and transmission of chemical signals. The exchange of cuticular hydrocarbons between colony members enables odour homogenization inside the nest, mixing individual odours which leads to the formation of the specific odour of a colony.

The capacity of individuals to recognize their colonies of origin has been demonstrated for *Cataglyphis*, *Camponotus*, *Tetramorium* and *Solenopsis*, although some authors suggest that individuals of different species that are not attacked must present similar odours (Mintzer and Vinson, 1985; Crossland, 1989). The formation of artificially mixed colonies with different species shows alterations in the specific odour that cohabit without aggression (Errard and Jallon, 1987; Bagnères et al. 1991; Soroker et al. 1994; Errard, 1994; Errard and Hefetz, 1997).

In this study, we tested the hypothesis of Holmes and Sherman (1983) that an individual that emerges in a mixed colony integrates the chemical signals of the social environment (allo- and conspecific) and acquires a mixed memory for future comparisons, a topic still unknown for *Acromyrmex* species.

II. MATERIAL AND METHODS

Three *Acromyrmex subterraneus brunneus* (Asb) colonies were collected in the park of ESALQ/USP, Piracicaba, São Paulo, Brazil, and three *Acromyrmex subterraneus molestans* (Asm) colonies on the campus of UFJF, Juiz de Fora, Minas Gerais, Brazil. The colonies were collected by excavation for removal of the fungus and of the largest number of adults, larvae and pupae possible. The collected material was stored in 1-liter plastic pots. The bottom was covered with a 1-cm plaster layer to maintain humidity for fungal growth. The colonies were transported to the Laboratory of Mirmecology, Universidade Federal de Juiz de Fora. The ambient temperature was kept at approximately 24 ± 2°C, with a relative humidity of 70 ± 20%. The colonies were fed *Ligustrum* spp. and *Acalypha* spp. throughout the experiment.

In the laboratory, each colony was reared in a transparent glass box covered with plaster. Each glass box containing one colony was connected through transparent plastic tubes to two containers: one container on one side to provide leaves and the other on the other side for waste material. These containers consisted of cylindric plastic flasks with a lid that contained a central orifice covered with a wire screen for ventilation. And *A. subterraneus brunneus* (Asb) were used, from where had been selected and removed with a clamp two larvae of each colony (12 larvae to all). The larvae were fed fungal hyphae previously immersed in distilled water containing 10% glucose P.A. (w/v) and 2% of a food dye (w/v). The individuals were marked with different colours according to the colony to which they belonged (Lopes et al. 2005).

After 24 hours, groups of 12 larvae were transferred to an experimental glass arena (15 x 15 cm) for observation. We only used last instar larvae that can be distinguished by their darkened gut and the absence of a hyphal blanket covering their bodies. Marked larvae were then placed on small (approximately 5 cm in diameter and 1 cm in height) pieces of mature fungus garden together with a group of 12 workers removed from either colony (Asm or Asb). The larvae were unable to feed themselves upon the fungus because they were placed ventral side up and were immobile (Weber, 1972). The fungal pieces never showed any signs of stress or contaminants during the short durations of the experiments. Therefore, the observations refer to the interaction of workers with concolonial, conspecific and allospecific larvae in the two experimental groups (Asm and Asb). The frequency of the behavioural acts was recorded over a period of 1 hour, with 10 replications in each experimental group (Asm and Asb), totaling 40 hours of recording. The difference in the frequency of the behaviours between the different types of interactions was analyzed separately for each experimental group (Asm or Asb) by the Kruskal-Wallis test, adopting a level of significance of 5%.

III. RESULTS

Five behavioural acts related to brood care performed by *A. subterraneus molestans* and *A. subterraneus brunneus* workers were recorded: licking the larval body, transporting larvae around the fungus garden, ingesting faecal liquid excreted by larvae, planting fungal hyphae on the larval body, and scraping the mouthparts of larvae. The observed behavioural acts were the same as those reported by Lopes et al. (2005), except for feeding the larvae fungal hyphae. The lack of observation of this behavioural act might be related to the non-starvation status of larvae in the present study.

Licking the larval body was the most frequently recorded behaviour in the two experimental groups (Asm and Asb) and there was no significant difference in the acceptance of concolonial and conspecific larvae (Asm: H=56.27; p>0.05; Asb: H=37.38; p=0.1366). For allospecific larvae of the species tested, the frequency of this behavioural act was significantly lower in *A. subterraneus brunneus* (H=56.28; p<0.05) (Fig. 1A).

The same result was observed for transporting larvae, with no significant difference in the mean frequency of transporting concolonial and conspecific larvae (H=15.23; p>0.05) in the Asm group. A significantly lower frequency was observed for transporting allospecific larvae (H=15.24; p>0.05) in the Asm group (Fig. 1B). There were no differences in transporting concolonial, conspecific and allospecific larvae in the Asb group (H=8.49; p=0.1315) (Fig. 2B).

Although the frequency of ingestion of larval faecal liquid by workers from the same colony was slightly higher, no preference for any group of larvae was observed (H=6.48; p=0.26) (Fig. 1C) in the Asm group. In the Asb group, significant differences in this behaviour were observed between concolonial, conspecific and allospecific larvae (H=19.33; p=0.0017) (Fig. 2C).

No preferences were observed in either group for planting fungal hyphae on the larval body (Asm: H=0.84; p=0.98; Asb: H=8.56; p=0.1280) (Figs. 1D and 2D) or scraping the mouthparts of larvae (Asm: H=1.93; p=0.86; Asb: H=8.28; p=0.1417) (Figs. 1E and 2E).

IV. DISCUSSION

The results corroborate the hypothesis that an individual that emerges in a mixed colony integrates the chemical signals of the social environment (allo- and conspecific) and acquires a mixed memory for future comparisons (Holmes and Sherman, 1983). Our results indicate different levels of acceptance and rejection of concolonial, conspecific and allospecific larvae in *A. subterraneus molestans* and *A. subterraneus brunneus* workers. Workers exhibited preferential care of conspecific brood, especially concolonial larvae. This preferential care suggests greater acceptance of conspecific larvae and that workers are skillful in distinguishing larvae of different species. The opposite behaviour is observed for *A. laticeps nigrosetosus* workers that do not discriminate larvae and pupae of different colonies from their own (Souza et al. 2003). In the discrimination process against alien brood, worker ants do not exhibit an "all or nothing" response, but rather a preferential behaviour for the brood of their own colony (Hölldobler and Wilson 1990).

The constant licking of the larval body suggests that the brood emits attractive and stimulating chemicals, establishing a communication system between larvae and workers (Lopes et al., 2005), since larvae are entirely dependent on worker care and are unable to survive without them (Wilson, 1971). It is possible that workers obtain information about the origin of larvae during the act of licking their body

The comparison of behavioural acts related to brood care to verify the occurrence of nepotism indicates the existence of a mechanism of colony-brood recognition based on chemical cues. These cues seem to be acquired mainly during larval life and persist through metamorphosis into the adult stage (Isingrini et al. 1985).

In conclusion, the data suggest that *A. subterraneus molestans* and *A. subterraneus brunneus* workers tend to prefer larvae of their own species, regardless of whether or not they are concolonial, thus confirming the hypothesis of Holmes and Sherman.

V. CONCLUSION

We concluded that *A. subterraneus molestans* and *A. subterraneus brunneus* workers tend to prefer larvae of their own species, regardless of whether or not they are concolonial, thus confirming the hypothesis of Holmes and Sherman.

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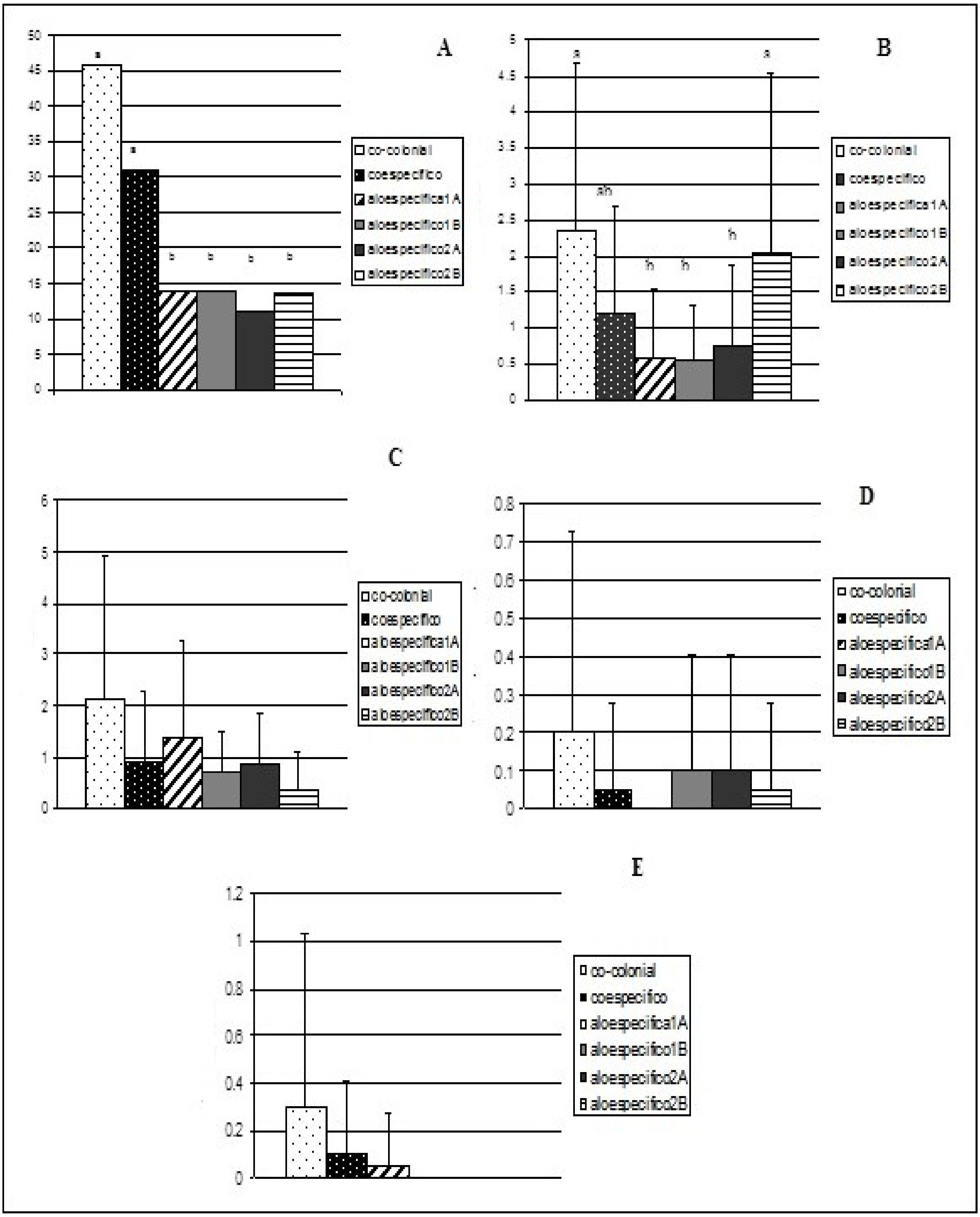

Fig. 1. Mean frequency of behavioural acts related to brood care performed by *Acromyrmex subterraneus molestans* workers of different origins. (A) Licking larval body; (B) transporting larvae around the fungus garden; (C) ingesting faecal liquid excreted by larvae; (D) planting fungal hyphae on the larval body; (E) scraping the mouthparts of larvae. Different letters indicate significant differences according to the Kruskal-Wallis test (5% level of significance).

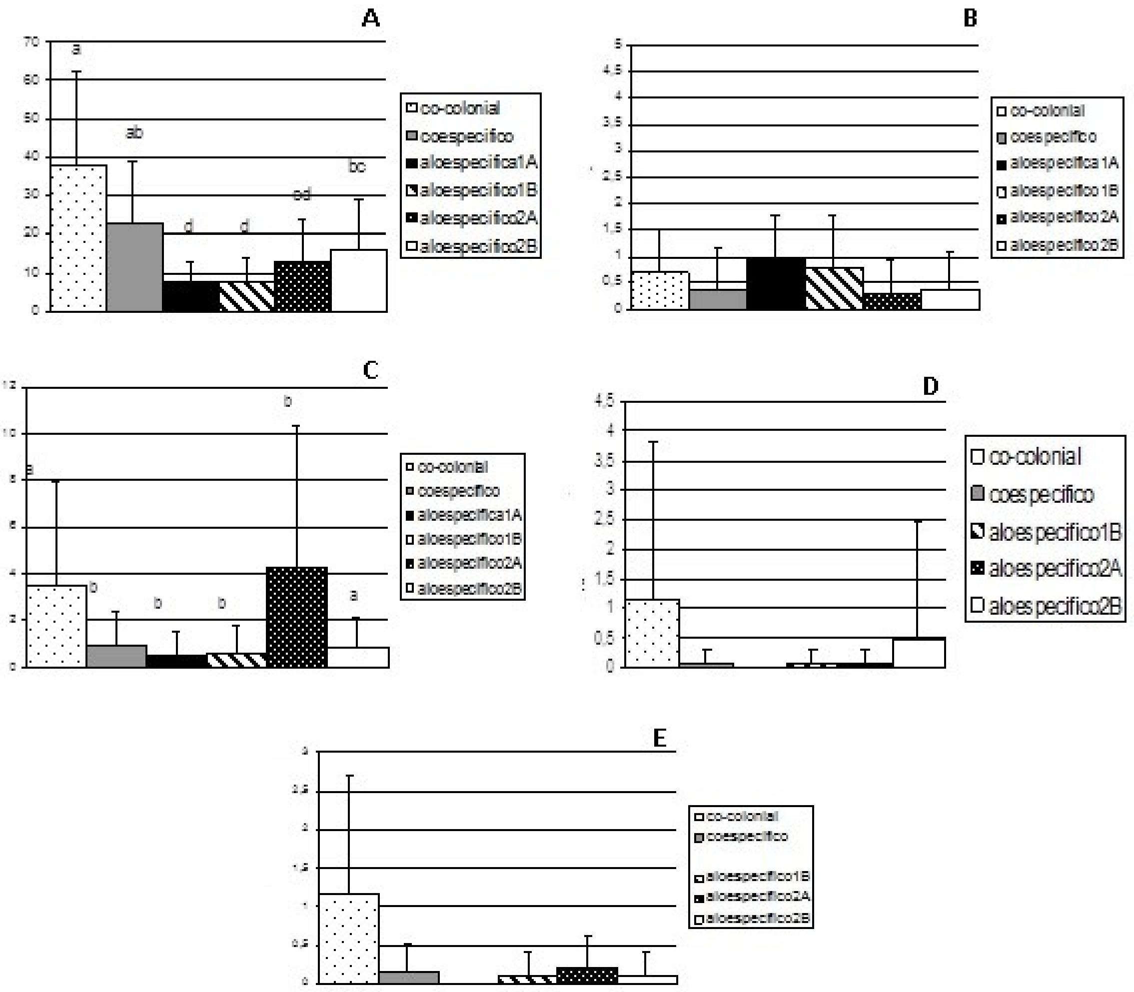

Fig. 2. Mean frequency of behavioural acts related to brood care performed by *Acromyrmex subterraneus brunneus* workers of different origins. (A) Licking larval body; (B) transporting larvae around the fungus garden; (C) ingesting faecal liquid excreted by larvae; (D) planting fungal hyphae on the larval body; (E) scraping the mouthparts of larvae. Different letters indicate significant differences according to the Kruskal-Wallis test (5% level of significance).