Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 8545–8547, December 1985
Developmental Biology

Preimaginal learning as a basis of colony-brood recognition in the ant *Cataglyphis cursor*

(insect sociobiology/kin recognition)

MICHEL ISINGRINI*†, ALAIN LENOIR*, AND PIERRE JAISSON†

*Laboratoire d'Ethologie et de Psychophysiologie, Faculté des Sciences, Parc de Grandmont 37200 Tours, France; and †Laboratoire d'Ethologie et Sociobiologie, U.A. Centre National de la Recherche Scientifique No. 667, Université Paris Nord, 93430 Villetaneuse, France

Communicated by Edward O. Wilson, August 12, 1985

ABSTRACT **In most circumstances, social insects recognize their nestmates. They can discriminate against alien adults and also against alien larvae. Results presented here indicate that the mechanism of colony-brood recognition is acquired in large part during larval life and persists through the metamorphosis into the adult stage. During the first days after emergence of the adult, a weaker form of learning can also occur. These phenomena are discussed in relation to kinship theory. It appears that kin recognition is determined not so much by genetic relatedness as by spatial proximity of the individuals during the early stages of life.**

Most social insects can discriminate nestmates from nonnestmates (1). The hostility shown to adults of alien colonies is evidence of colony recognition. Recent data have revealed that intraspecific brood discrimination also exists in some ant species (2–5) and wasps; such discrimination manifests itself in differential brood-nursing activities (5–7). For the honey bee, comparable discrimination is not clearly established, as contradictory results have recently been published (8, 9).

In *Cataglyphis cursor* Fonscolombe, brood-nursing ants prefer larvae from their own colony to those from an alien colony, which they give only half as much care as their own brood (unpublished data). We report here results that indicate an important role for learning such discrimination very early in life, during the larval stage.

METHODS AND RESULTS

Experiment 1: Effect of isolation during the first 10 days of adult life

Young *C. cursor* workers were taken from their colony less than 8 hr after eclosion and were placed for 10 days in one of three situations.

In one group (G1; 98 ants) each ant was completely isolated until age 10 days. The workers were then formed into subgroups of seven workers each, and 15 min later they were tested by being offered a choice among three nestmate larvae (NL) and three alien larvae (AL). The larvae in this and other experiments were identified by sticking colored plastic spots on them. Each instance of licking or carrying of a larva for the next 15 min was recorded.

In another group (G2), each of 82 young workers was familiarized with members of an alien colony, here called the "familiar colony," by being kept with two adults and two larvae (FL) from that colony for 10 days. These ants too were then formed into groups, of about seven each, and 15 min later were tested, in this case for their choice among three NL and three FL.

The 98 newly hatched ants of the third group (G3) were kept for 10 days in groups of seven nestmates each and then were tested for choice among three NL and three AL.

In G1 and G3, NL were clearly preferred to AL (Fig. 1) ($P < 0.01$) (except where noted otherwise, Wilcoxon tests were used). Early isolation as adults (G1) did not reverse the ants' preference for their NL, although this preference was weaker than in the group kept with nestmates (G3) ($P < 0.05$, Mann–Whitney U test).

The ants of G2 (familiarized with individuals from an alien colony) still slightly preferred their NL to the FL; this was not significant for the nursing activities as a whole, but was significant for licking ($P < 0.05$). In this group, then, the preference for the sister brood was merely attenuated, not reversed, by their post-hatching experience. Although the results do not rule out an effect of adult experience in the first 8 hr after hatching, they are at least consistent with the hypothesis that colony-brood recognition could be learned during preimaginal life.

Experiment 2: Effect of living in an alien colony during the entire larval stage

C. cursor eggs were transferred from their parent colony into one of three recipient colonies. The larvae produced from these eggs spent their whole larval stage in the alien colony. The recipient colonies, again called the "familiar colonies," were divided into two parts by a barrier: in one part we left the queen and her brood, and into the second part we introduced the experimental eggs. The workers of the recipient colony were divided between the two compartments. In preliminary tests we verified that the workers remained influenced by the inhibitory activity of the queen and did not lay; this was necessary to be sure that all the larvae were indeed adopted. Before the transfers it was also verified in a control test that the workers of the familiar colony were able to discriminate between the broods of their own and the other colony.

Soon after pupation, the cocoons of the experimental larvae were transferred back into their parent colony, where they spent on average 15 days before eclosing. The workers that emerged were tested at age 5 days by being given a choice among three of their own nestmate larvae (NL) and three larvae from the familiar colony (FL). For this test, the callow ants were placed in 12 groups of two to five each, with the six larvae, and were tested for 30 min (the total number of ants was 48). Fig. 2 clearly shows that the ants preferred the larvae of the familiar (alien host) colony, FL ($P < 0.01$). These results demonstrate that learning took place during the larval stage.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NL, nestmate larvae; FL, familiar larvae; AL, alien larvae.

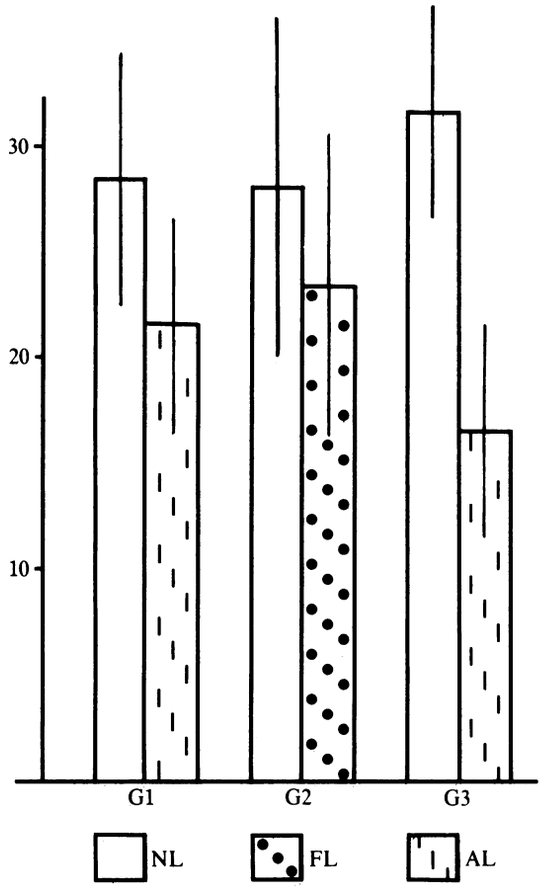

FIG. 1. Mean numbers (and confidence intervals) per adult ant of instances of brood-nursing behaviors (licking and carrying) directed towards each type of larva in experiment 1.

After these observations, the ants were reintroduced into their own nests and were tested again when they were 15 and 25 days old. At 15 days, the preference for FL was significant only as regards carrying behavior ($P < 0.02$), and at 25 days the preference had entirely disappeared: there was no significant preference for either FL or NL. Because the result for this part of the experiment was established with only 7 groups (at 15 days of age) and 4 groups (at 25 days of age), we consider it a preliminary finding that requires confirmation. Nevertheless, it seems to indicate that familiarization is possible during the early days of adult life, in this case with the odor of the parent colony. It suggests also that the effect of a preimaginal learned preference for alien larvae over nestmates may decline in adults exposed to nestmates for a time. But, again, this post-hatching familiarization did not actually reverse the learned preference.

Experiment 3: Effect of living in an alien colony during only part of the larval stage

The small and large larvae of two colonies were selected. For each brood, these small or large larvae were transferred as a group into a recipient colony (the "familiar colony") whose own brood had been eliminated. The large larvae spent 2–5 days in the familiar colony before pupation, and the small larvae, from 10 to 20 days; as a consequence, the larval size was inversely related to the duration of the pre-imaginal experience in the familiar colony. A total of 105 workers emerged from the group of large larvae (group G1) and 77 from the group of small larvae (G2). These adopted individuals were reared in the familiar colony until 3 to 7 days after their emergence into adult life. Within each group, the newly

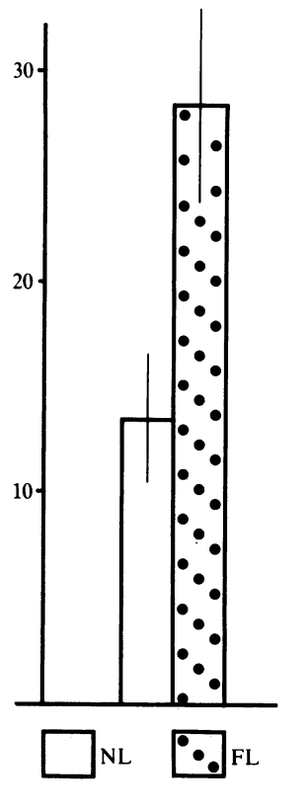

FIG. 2. Mean numbers (and confidence intervals) per adult ant of instances of brood-nursing behaviors (licking and carrying) directed toward each type of larva in experiment 2.

emerged workers were then divided into subgroups of seven individuals. Each of these subgroups was given three choice tests, lasting 10 min each, the choice being between four NL and four FL. As Fig. 3 shows, the workers that had been

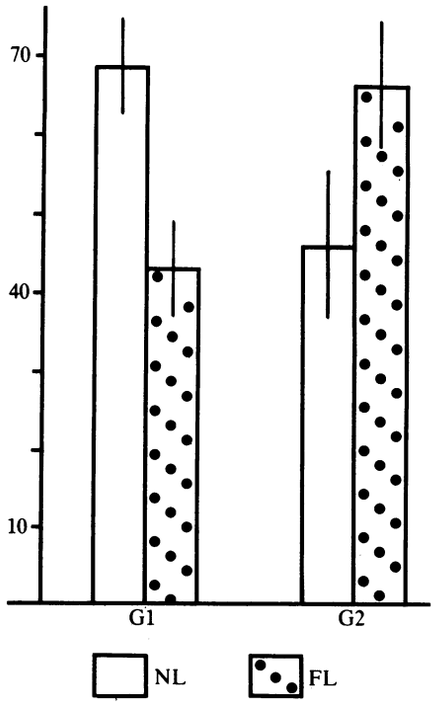

FIG. 3. Mean numbers (and confidence intervals) per adult ant of instances of brood-nursing behaviors (licking and carrying) directed towards each type of larva in experiment 3.

transferred as large larvae (G1) preferred their NL ($P < 0.01$), whereas workers that had been transferred as small larvae (G2) preferred the FL ($P < 0.01$). So adult ants that had spent most of their larval life in an alien colony preferred nursing the larvae of that colony to nursing their own sister larvae.

The results of experiment 3 show that colony-brood recognition, as reflected in the nursing behavior of workers, cannot be altered during the last instar of larval life. This might be attributable either to the existence of a more sensitive period in the first instars of larval development or the total duration of the exposure to the colony odor.

DISCUSSION

In the ant *C. cursor*, a certain familiarization during the first days after hatching can affect colony-brood recognition. This phenomenon confirms the existence of a high capacity for learning during this period as suggested by previous independent studies (10–23).

However, the main process affecting colony-brood recognition seems to occur during the larval stage: the experiments in which eggs or small larvae were transferred (experiments 2 and 3) allowed the normal preference for kin in the adult to be replaced with a preference for nonkin. Information acquired during the larval stage persisted through the metamorphosis into adulthood. Preimaginal learning has previously been reported only for feeding behavior in solitary insects (24–29).

Experiment 2 clearly showed that the significant stimuli acting on the larvae come mainly from the *adults* of the recipient colony; the larvae were evidently not sensitive to stimuli from their sisters. This suggests that what is learned is the colony odor originating from workers.

Experiment 3 demonstrated that larvae which are transferred to a recipient colony when they are already quite large do not develop a preference for the odor of the recipient colony. We therefore conclude that the first instars of larval life might be a more sensitive period. As was the case for the large larvae in experiment 3, the callow workers familiarized with the alien colony (experiment 1, G2) did not reverse their previous acquired preference for their kin; nurturant behavior towards the nonkin brood was increased only to nearly the same level as that towards the kin brood. Only habituation to an alien colony starting at the egg stage (experiment 2) or the first instar of larval life (experiment 3) produces a clear preference for the nonkin brood. This is probably because the individual has not yet been behaviorally fixed at this age. Additional observations showed that in *C. cursor* every conspecific brood is durably tended by adults, but the familiar one is nursed with the greater intensity and efficiency. Further experiments will be necessary to explore the hypothesis that there is a specific sensitive period involved in the preference for the brood of the familiar colony.

Preimaginal learning as a basis of brood recognition in ants was previously suggested by Jaisson (30). This important phenomenon is now demonstrated by the present data. We know of no other presentation of evidence of preimaginal learning involved in insect social behavior. This learning could be present in many other species, at least among social hymenopterans, and it suggests the merit of studying the mechanisms of kin recognition, which is fundamental to the sociobiology of altruism. The discovery that larval learning affects colony-brood recognition by adults suggests how the correlation between altruistic behavior and genetic relatedness, which is explained by kinship theory (31–33), might be established. As recently pointed out,‡ nestmate recognition that is a consequence of learning would be based much more directly on early spatial proximity than on kinship. These two phenomena are not necessarily related, as is shown by the interspecific altruistic behavior in dulotic species, where the ants "kidnapped" as brood tend the slavemakers later. The effect of proximity therefore seems to better account for the orientation of altruistic behavior, whose evolutionary significance is given by the concept of kinship. In nonparasitic species, the workers and the larvae of a colony are genetically related to each other. Thus, the ethogenesis of the individual during its larval stage will normally direct the altruistic behavior towards the kin, and this recognition will be reinforced by the worker's experience after its emergence.

‡Jaisson, P., *Proceedings of the Nineteenth International Ethological Conference*, August 1985, Université Paul Sabatier, Toulouse, France, pp. 87–90.

We thank Prof. E. O. Wilson for his helpful suggestions, which improved the manuscript.

1. Wilson, E. O. (1971) *The Insect Societies* (Harvard Univ. Press, Cambridge, MA).
2. Lenoir, A. (1981) *Sociobiology* **6,** 153–178.
3. Febway, G., Mallet, F. & Kermarrec, A. (1984) *Actes Coll. Insects Soc.* **1,** 79–86.
4. Lenoir, A. (1984) *Anim. Behav.* **32,** 942–944.
5. Lenoir, A., Isingrini, M. & Nowbahari, M. (1982) *La Communication chez les Sociétés d'Insectes* (Univ. Barcelona Press, Barcelona, Spain), pp. 107–114.
6. Klahn, J. E. & Gamboa, G. J. (1983) *Science* **221,** 482–484.
7. Errard, C. (1984) *Coloquio Intern. Etologia* (Univ. Barcelona Press, Barcelona, Spain), pp. 71–80.
8. Page, R. E. & Erickson, E. H., Jr. (1984) *Ann. Entomol. Soc. Am.* **77,** 578–580.
9. Breed, M. D., Velthuis, H. H. W. & Robinson, G. E. (1984) *Ann. Entomol. Soc. Am.* **77,** 737–739.
10. Fielde, A. (1903) *Biol. Bull. Marine Biol. Lab.* **5,** 320–325.
11. Jaisson, P. (1972) *C.R. Acad. Sci. (Paris) Sér. D* **275,** 2721–2723.
12. Bell, W. J. (1974) *J. Comp. Physiol.* **93,** 195–202.
13. Jaisson, P. (1975) *Behaviour* **52,** 1–37.
14. Kukuk, P. F., Breed, M. D., Sotbi, A. & Bell, W. J. (1977) *Behav. Ecol. Sociobiol.* **2,** 319–327.
15. Jaisson, P. (1980) *Biol. Ecol. Médit.* **7,** 163–166.
16. Jaisson, P. (1980) *Nature (London)* **286,** 388–389.
17. Le Roux, A. (1980) *Biol. Ecol. Médit.* **7,** 203–204.
18. Breed, M. D. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2635–2637.
19. Buckle, G. R. & Greenberg, L. (1981) *Anim. Behav.* **29,** 802–809.
20. Carlin, M. F. & Hölldobler, B. (1983) *Science* **22,** 1027–1029.
21. Morel, L. (1983) *C.R. Acad. Sci. (Paris) Sér.* III **296,** 449–452.
22. Pfenning, D. W., Reeve, H. K. & Shellman, J. S. (1983) *Anim. Behav.* **31,** 412–416.
23. Errard, C. (1984) *Insectes Soc.* **31,** 185–198.
24. Isingrini, M. & Lenoir, A. (1984) *Coloquio Intern. Etologia* (Univ. Barcelona Press, Barcelona, Spain), pp. 99–106.
25. Thorpe, W. H. & Jones, F. W. G. (1937) *Proc. R. Soc. London Ser. B* **124,** 56–81.
26. Thorpe, W. H. (1939) *Proc. R. Soc. London Ser. B* **127,** 424–433.
27. Cushing, J. E. (1941) *Proc. Natl. Acad. Sci. USA* **50,** 163–178.
28. Manning, A. (1967) *Nature (London)* **216,** 338–340.
29. Alloway, T. M. (1972) *Am. Zool.* **12,** 471–477.
30. Jaisson, P. (1974) *C.R. Acad. Sci. (Paris) Sér. D* **279,** 1205–1207.
31. Hamilton, W. D. (1964) *J. Theor. Biol.* **7,** 1–52.
32. Hamilton, W. D. (1972) *Annu. Rev. Ecol. Syst.* **3,** 193–232.
33. Wilson, E. O. (1975) *Sociobiology* (Harvard Univ. Press, Cambridge, MA).