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SHORT NOTE

Brood hiding test: a new bioassay for behavioral and neuroethological ant research

A Szczuka, B Symonowicz, J Korczyńska, A Wnuk, EJ Godzińska

Nencki Institute of Experimental Biology, Warsaw, Poland.

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Corresponding author
Ewa Joanna Godzińska
Laboratory of Ethology
Department of Neurophysiology
Nencki Institute of Experimental Biology
PAS, Pasteur St. 3, PL 02-093
Warsaw, Poland
E-Mail: e.godzinska@nencki.gov.pl

Abstract

We describe a new bioassay for behavioral and neuroethological ant research, the brood hiding test. A group of adult ants is taken out of the nest, confined together with brood and exposed to strong light. Ants may interact with brood, and, in particular, transport it to the provided shadowed area. The brood hiding test may be accompanied by administration of neuroactive compounds and/or by measurements of their levels in the brain and/or in specific brain structures. During pilot tests with workers of *Formica polyctena* the values of the score quantifying ant behavior were positively correlated with the group size.

Introduction

Adult workers of social Hymenoptera usually engage first in intranidal tasks and then switch to extranidal ones (Wilson, 1971; Hölldobler & Wilson, 2009). This transition is often called the transition nurse-forager (e.g., Heylen et al., 2008). However, older ant workers and/or foragers may retain the ability to engage in intranidal brood care (Lenoir, 1979; Sorensen et al., 1984; Seid & Traniello, 2006; Muscedere et al., 2009). Moreover, several studies revealed that ant foragers are more attracted to brood found outside the nest than nurses and show higher readiness to retrieve it to the nest (Weir, 1958; Walsh & Tschinkel, 1974; Lenoir, 1977; 1981). On the basis of these findings Lenoir (1977; 1981) proposed a bioassay acting as a reliable technique of identification of ant foragers: the brood-retrieving test. At the start of the test a simple artificial ant nest (a test tube equipped with a water reservoir and partly covered by a black paper tube to assure darkness in its humid zone) is inclined so that the brood falls on the dry cotton plug closing the other end of the tube. Workers inhabiting the nest may then retrieve brood back to the dark zone close to the water reservoir. This test was successfully applied to identify about 80% of foragers in small colonies of *Lasius niger* L. However, it is less suitable for experiments carried out to evaluate the effects of various experimental treatments, as the ants are tested in their home nests, and neither the number of workers, nor the quantity and quality of brood can be easily controlled. Moreover, it is difficult to disentangle ant responses to humidity and illumination. Therefore, we developeda new bioassay for behavioral and neuroethological ant research, the brood hiding test. During the test a group of adult ants is taken out of the nest and confined together with brood in a container exposed to strong light. The ants may interact with brood and, in particular, transport it to the provided shadowed area.

Experimental settings recommended for the application of that test to study the behavior of workers of the wood ant *Formica polyctena* Först (Table 1) were chosen on the basis of the results of 20 pilot tests during which workers taken from a laboratory colony fragment (1-20) were confined together with 5 homocolonial pupae in various types of containers exposed to strong white light (Fig. 1). Single workers did not

show brood hiding behavior and their interactions with brood were limited to antennal contacts. More advanced interactions with brood appeared as a function of increasing group size. The values of the score quantifying the outcome of each test (1-9) were highly significantly positively correlated with the number of workers tested together (Spearman rank correlation test: $r = 0.8536$, $P < 0.000001$).

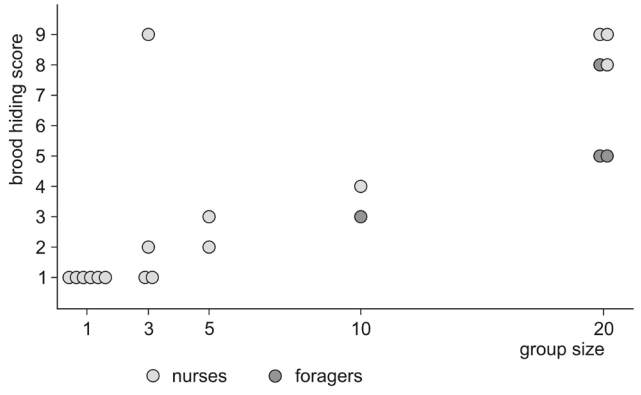

Fig 1. The results of 20 min pilot tests (n = 20) investigating the responses of workers of the red wood ant *Formica polyctena* to homocolonial brood (5 pupae) exposed to strong white light (5000 lx) in various types of containers containing a shadowed area. Ant behavior was quantified as a score (1-9) denoting the most advanced form of interactions with brood observed during the test: 1: antennal contacts; 2: seizing; 3: attempt at transport (a short episode of transport no longer than 2- 3 s); 4. transport; 5. hiding of 1 pupa in the hadowed area; 6. hiding of 2 pupae; 7. hiding of 3 pupae; 8. hiding of 4 pupae; 9. hiding of 5 pupae. Two classes of nestmate workers were tested: nurses (taken out of artificial nest chambers from among workers employed in intranidal brood care), and foragers (taken from the foraging area of the same artificial nest).

These findings provide a new example of the crucial role of group size in the mediation of the expression of specific behavior patterns in social Hymenoptera. The phenomenon of critical (threshold) group size necessary for the expression of a particular behavior trait was first described by Chauvin (1954) and then reported in many other studies [reviewed in Wilson (1971) and Szczuka & Godzińska (2004a)]. Particularly clear-cut effects of group size on the expression of a specific behavior pattern were documented in a series of experiments during which workers of *F. polyctena* were confronted with dead adult houseflies (*Musca domestica* L.) offered to them in the foraging areas of their nests (Szczuka & Godzińska 1997; 2000; 2004a; 2004b). The values of the score quantifying the responses of ants to prey increased as a function of increasing group size, and prey retrieval was observed only in groups counting at least 30-40 workers. Several studies also reported similar relationships between worker group size and the degree of escalation of ant aggressive behavior (Roulston et al., 2003; Tanner, 2006; 2008; Buczkowski & Bennett, 2008).

During our pilot tests brood hiding scores tended to be lower in the case of foragers than in the case of nurses (Fig. 1). This preliminary result was confirmed by another experiment in which brood hiding test was applied to compare the behavior of various subclasses of workers of *F. polyctena* (Szczuka et al. unpublished results). In that experiment foragers collected from the trails performed less well than workers from other experimental groups. As reported by Lenoir (1977; 1981), during the brood-retrieving tests brood is retrieved mostly by foragers. Responses to brood displayed by ants during the brood hiding test and the brood-retrieving test are thus mediated by at least partly different proximate mechanisms.

The brood hiding test may be used to investigate such questions as behavioral polymorphism within ant colonies, ontogeny of ant behavior, nestmate and species recognition, and behavioral differences between ants from various

Table 1. Experimental settings recommended for the application of the brood hiding test to study worker-brood interactions in the red wood ant *Formica polyctena* Först.

Element of experimental procedure	Description
Experimental arena	an open cylindrical glass container (5 cm high, inner diameter 10 cm) with the walls coated with Fluon®
Shadowed area	a small (25 mm x 30 mm x 5 mm) rectangular shelter made of aluminum foil with one of its longer side walls (facing the center of the arena) left open to allow the ants to enter the shadowed zone
Source of illumination	strong white light (5000 lx) produced by the lamp "Fotovita FV-10" (ULTRA-VIOL sp. j.).
Chemical cues left by nestmates	present (20 homocolonial ants are allowed to walk inside the container during 1 h preceding the first test carried out on a particular day)
Brood	5 homocolonial worker pupae
Time during which the ants are allowed to settle after the introduction into the test container and before the introduction of brood	30 min
Duration of the test	15 or 20 min
Recording of ant behavior	digital video camcorder
Analysis of behavioral recordings	software for the analysis of video recordings of behavior [for instance, "The Observer Video-Pro" (Noldus Information Technology)]

systematic groups related both to phylogenetic distance and differences in ecology. Groups of individuals tested together may be homogenous, but may also consist of individuals belonging to different castes and/or worker subclasses. Not only homocolonial, but also allocolonial and/or allospecific brood may be used, and the tested ants may be subjected to the situation of choice between various categories of brood. Behavior of the tested ants may be quantified by assigning a score to the outcome of each test, or by video recording the tests and analyzing the recordings by means of an appropriate software.

The brood hiding test may also be accompanied by the administration of neuroactive compounds delivered by various techniques including acute and chronic oral administration, injections, and topical (transcuticular) application. The variables quantifying ant behavior may also be analyzed as a function of levels of specific neuroactive compounds in the brain or specific brain structures. Such more complex versions of the brood hiding test may be applied to study neurobiological processes underlying such phenomena as task-related differences in responses to brood, effects of experience on expression of worker behavior, worker cooperation and communication, and inter-individual variability of behavior.

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