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SOCIAL AND BIOLOGICAL CORRELATES OF BEHAVIORAL DEVELOPMENT IN THE ANT NOVOMESSOR ALBISETOSUS (MAYR)

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SOCIAL AND BIOLOGICAL CORRELATES OF BEHAVIORAL DEVELOPMENT IN THE ANT *NOVOMESSOR ALBISETOSUS* (MAYR)

by

PHILIP McDONALD

A dissertation submitted to the Graduate Faculty
in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy,
The City University of New York.

1985

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

SOCIAL AND BIOLOGICAL CORRELATES OF BEHAVIORAL DEVELOPMENT IN THE ANT NOVOMESSOR ALBISETOSUS (MAYR)

by

Philip McDonald

Adviser: Professor Howard Topoff

In Part I, temporal polyethism, the division of labor according to age, is used as a gauge for behavioral development in an ant. By removing various age groups from the colony and changing the number and composition of brood, it was found that behavioral development could be accelerated and retarded relative to a baseline. When older workers were removed, young workers progressed through age related tasks in one third of the baseline time. Removal of young workers caused older workers, which had not been observed touching queen or brood for 4-6 months, to once more tend brood and queen. It is hypothesized that there are two types of stimuli coming from the larvae which affect behavioral development. Larval and eclosion stimulation are of particular importance in the social organization of the colony. Part II is a study of the development of protean behavior against army ants by laboratory and field colonies. Protean behavior includes escape with or without brood, and aggressive defense. All protean behaviors developed in ants raised in isolation. However, evidence is presented suggesting that experience with army ants can alter the course of this development. Part III deals with the development of ovaries, trail pheromone, and exoskeleton hardness in workers of selected ages. It was found that certain stages in the development of these structures correspond with important behavioral changes. Behavioral and structural changes are considered not as separately occurring phenomena, but as a dynamic complex of developmental processes.

Acknowledgements

I thank Dr. Howard Topoff, the principal sponsor of this dissertation, for introducing me to the study of behavioral development, for intellectual stimulation, and for his high level of humor and enthusiasm. These last, I think, are innate traits. Drs. Jim Cane, Deborah Gordon, Carol Simon, and Gerald Turkewitz also read this manuscript and made thoughtful suggestions and helpful criticisms. I owe a special debt to Jeff Cole for his indispensable help on many long, tedious, frustrating, exuberant nights in the desert.

This research was conducted at the American Museum of Natural History, New York City, and the Museum's Southwestern Research Station, Portal, Arizona. Work was supported by grants from the Theodore Roosevelt Memorial Fund and the Explorers Club, and N. I. M. H. Training Grant #4-41602; and by PSC-CUNY grant #14093 to Howard Topoff.

This dissertation is dedicated to an exceptional companion, Susan Puretz, first for suggesting that I study for a doctorate in biopsychology, and then for encouraging me every inch of the way.

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General Introduction

The capacity of organisms to change during ontogenesis has long been of central interest to students of behavioral science. The behavioral epigeneticist's chief concern is that of development--the process of interaction between the organism and its environment, both external and internal. The development of behavior has two fundamental problems: the sequence of development, and the origins of, or stimuli associated with, particular behaviors as they appear during ontogeny.

The epigenetic approach to animal behavior was being pursued (Kuo, 1967; Schneirla, 1971) without much fanfare during the rise of the European ethologists. It was not until the 1960's, however, when the differences between ethologists and animal psychologists seemed irreconcilable, that many animal behaviorists from these disciplines and others adopted behavioral epigenesis as a logical and explanatory framework for their studies.

> An epigenetic approach holds that all response systems are synthesized during ontogeny and that this synthesis involves the integrative influence of both intraorganic processes and extrinsic stimulative conditions. It considers gene effects to be contingent on environmental conditions and regards the genotype as capable of entering into different classes of relationships depending on the prevailing environmental context. In the epigeneticist's view, the environment is not benignly supportive,

> but actively implicated in determining the very structure and organization of each response system (Moltz, 1965, p. 44).

When designing experiments, a researcher may find an epigenetic approach to behavioral development unwieldy. If one's interest focuses on the developing physiology or structure of an organism, one must not forget their development may differ in another environment. Similarly, if one is interested in particular environmental influences on an organism's behavior, one must not forget that these influences may have different effects at another stage in the organism's development. If at times we must be somewhat reductionistic in the design of our research, we must not lose sight of the concomitant assumptions made or that behavioral ontogeny is a dynamically complex and continual interaction between the developing organism and its environment. For each species a complex picture of the ontogenetic process must be assembled from the fragmentary studies on certain phases of behavioral development.

Development of Social Behavior

Many organisms after birth or hatching are introduced not only to a new physical environment but to a social environment as well. Social regulation of behavioral development has been well documented in vertebrates. For example, if the male of a male dominated harem of cleaner fish (Labroides dimidiatus) dies, the largest and most dominant female transforms, first behaviorally and later structurally, into a male (Robertson, 1972). Mexican jays live in small flocks and do not reproduce until their third year. After a pair successfully mates, immature jays and adults unable to find mates help bring food to the nestlings (Brown, 1963, 1972). The dominant male in age-graded male troops of primates such as vervets and gorillas, may prevent additional males from assuming the role of full adult (Eisenberg, Muckenhirn, and Rudran, 1972). In their surrogate-mother experiments, the Harlows (1962) demonstrated that the social experiences of young primates are critical for later social development.

By contrast, social regulation of behavioral development in insects had been ignored for many years. Behavior was largely seen as developing according to a rigid, genetically determined plan, chained to biological maturation. However, some studies correlating behavioral development with physiology have been done. Temporal division of labor in honey bees has been correlated with the reduction of mandibular and wax glands that occurs concomitantly with the worker bees' progress through various age-related tasks both within and outside the nest (King, 1933). Free (1965) demonstrated social regulation of proportions of worker bees performing tasks under crowded conditions, and Lindauer (1961) has shown that the removal of younger age groups is correlated with a regeneration of wax glands and hypopharyngeal glands in older workers. These older workers then recommence comb building and nursing the brood.

Social Behavior and Temporal Polyethism

The behavioral sequence of temporal polyethism, the division of labor according to age, seems to be common to most ants. Temporal polyethism (also called age polyethism) has been observed in ants at least since the time of Forel (1874/1920). He wrote of young workers spending most of their time nursing brood within the nest, while older workers were engaged more often in work outside the nest. Documentation of temporal polyethism has been provided by a number of researchers (Buckingham, 1911; Heyde, 1924; Ledoux, 1950; Otto, 1958; Weir, 1958 a & b; Dobrzanska, 1959).

More recently, researchers have gone beyond describing the time course of polyethism in ants to the processes influencing temporal polyethism. Jaisson (1975) has demonstrated a critical period and possible imprinting in the ontogenesis of brood care in *Formica polyctena* Forst. Social manipulations, such as removing an age group from a colony of Tapinoma erraticum L., reveals that temporal division of labor can not be attributed entirely to age-dependent maturational processes (Lenoir, 1979). Other age groups, while not totally relinquishing their own labors, will take over the tasks of the missing age group. The role of learning in the development of behaviors outside the nest has been explored by Rosengren (1971) for foraging, and by Dobrzanski (1968) and Chauvin (1974) for manipulating loads: Rosengren showed that ants were capable of learned orientation to landmarks when following a route; Dobrzanski, using marked and dated ants, observed their increased efficiency with practice in repairing holes made in their nest; Chauvin demonstrated that ants may learn what objects can or cannot be successfully moved.

The division of labor according to age implies the existence of temporal castes. Temporal castes have been difficult to define to the satisfaction of all. The one presented here is the working definition for this dissertation: A temporal caste is an age group distinguished by its behavior, specifically one specializing in a particular task or cluster of tasks.

Wilson (1976 a) has documented a discretized caste system in *Pheidole dentata*, whereby three temporal castes perform three different clusters of tasks with relatively little overlap. Mirenda and Vinson (1981) have found a continuous caste system in colonies of *Solenopsis invicta*, the red imported fire ant; Calabi, Traniello, and Werner (1984) have found the same for *Pheidole hortensis*. In both these last there is an overlap in the frequency distribution of age classes performing the various tasks.

Both Wilson (1976 a) and Seeley (1982) claim that spatial efficiency is the basis for performing clusters of tasks: for example, efficiency is enhanced if an age group which grooms larvae, regurgitates to them as well. Spatial factors cannot be the only consideration however. Topoff (1972) has shown that callows of army ants of the genus *Neivamyrmex* (1972 a) and the genus *Eciton* (1972 b) tested under the same conditions as older workers, are capable of following scent trails but are reluctant to do so. Heyde (1924) found that callows of *Manica rubida* are able to bite but do not actively attack as do older workers. Similarly, Otto (1958) for *Formica* and Weir (1958) for *Myrmica* found behavioral abilities present in callows that they were not inclined to use. Hence motivational as well as spatial factors are pertinent to the consideration of temporal castes.

Regardless of the problems in precisely defining temporal castes, the phenomenon of workers moving through various tasks as they age does exist. Whether we can alter the sequence of behavioral development, or whether certain natural factors alter it for some workers, can only help us to understand why the pattern involved occurs as often as it does in so many workers. In the first part of this dissertation, temporal polyethism is used as a guage for behavioral development in the arid land ant Novomessor albisetosus (Mayr). In the second part, a number of defensive behaviors which are an intrinsic part of temporal polyethism are studied. Part III treats of some structural developments which are concomitant with, and perhaps influential in, temporal polyethism as manifested by the development of behaviors treated in Parts I and II.

Part I

Introduction

N. albisetosus is of the myrmicine subfamily and inhabits arid uplands and mountainsides (1,000 - 2,000 m) from southwest Texas, New Mexico, and Arizona into northern Mexico (Wheeler and Creighton, 1934; Creighton, 1950, 1955). Though once considered a harvester ant, it is actually omnivorous and forages according to seasonal availability of resources (Wheeler and Creighton, 1934; Chew, 1977; Whitford, Depree, and Johnson, 1980). Slow and deliberate in movement, it forages mostly during cool night temperatures, early mornings, and overcast days. While obviously flourishing in the arid Southwest, it is not well suited anatomically to desert life as are other xerophilous species (Wheeler and Creighton, 1934): For example, the body and gular hair of desert dwelling Pogonomyrmex spp may serve to convey heat from the body and protect delicate mouthparts from blowing sand, respectively.

N. albisetosus is, however, ideally suited to studies of behavioral development. It exhibits temporal polyethism, cannot sting, does not bite hard, adapts readily to artificial laboratory nests, and consumes a variety of easily gotten and easily stored foods. Within a short period one can witness the development of these relatively docile creatures from the egg to the mature adult organism.

Though it describes some of these changes, this dissertation aims to be more than just a descriptive study about changes in the behavior of ants maturing in the colony environment. Questions about the causes of behavior which involve processes acting during the development of experimental individuals can be partly answered by various social manipulations: for instance, manipulating brood proportions, age group proportions, the inclusion or exclusion of the queen. The important question, "What social factor has the greatest effect on the behavioral development of a worker?", can be answered by these manipulations. Heretofore, researchers have had little concern for the possibility that social regulation is involved in developmental change in insects. This part of the dissertation is aimed at determining the role of social factors in the behavioral development of the arid land ant, Novomessor albisetosus (Mayr).

General Method

Subjects

Colonies of N. albisetosus were collected near Portal, Arizona (Cochise County) at an elevation of about 1,600 m, and maintained in the laboratory for 3 months prior to experimentation. The colonies were collected by releasing hundreds of army ants, Neivamyrmex nigrescens, into the openings to the nests. This caused an alarm called protean behavior whereby the workers rush out over the incoming army ants while carrying the brood and queen with them. Virtually the entire colony can then be collected. Numbers and compositions of castes and brood are given for each experiment.

Apparatus

The Lucite housing apparatus consisted of three transparent nest boxes (15 cm diameter x 4 cm high) with dental stone substrates, connected in a line by 13-mm diameter tubing to a foraging arena (61 cm x 61 cm x 30.5 cm) containing fine-grained sand. In order to simulate environmental features of a natural nest that enable adult ants to regulate brood development, a heating pad was placed under the nest box farthest from the arena, This box was kept dry and on a cycle of 11 hrs at 32°C and 13 hrs at room temperature (22-24°C), a condition simulating those chambers of a natural nest nearest the sun-heated desert surface. The center box was kept at room temperature and humidified by periodically moistening the dental stone, in order to simulate chambers deeper in the earth. The box nearest the arena was left dry and at ambient temperature, as a transitional zone between the first two.

In order to facilitate night observation, the apparatus was kept under constant light. An infrared beam counter monitoring ant traffic between the nest and foraging arena revealed no significant difference in traffic as a function of time of day.

Experiment 1

Procedure

Baseline. Baseline data were taken to determine how long it would take a group of recently eclosed callows to proceed through age-related tasks within the nest and eventually spend as much time outside the nest as mature adult workers. Twenty-five callows were dated and identified as they eclosed by marking their thoraces and gasters with enamal paints. Twenty-five mature adult workers (defined as darkly pigmented ants foraging at least 60 cm from the nest entrance in the foraging arena) had been similarly marked 1 month previously. They were often seen feeding, drinking, foraging, and digging in the arena. Their ages were unknown because Novomessor are virtually indistinguishable in color after 5 weeks of age. However, those marked as mature adults would have been at least 2 months old at the beginning of the experiment. (As both callows and older workers are technically adults, morphologically, I refer to them as <u>domestics</u> and <u>outside workers</u>, respectively. Both terms connote developmental and behavioral distinctions, because callows typically engage in domestic work and older adults typically work outside the nest.) Marked ants were returned to that part of the nest or arena from which they had been collected. The colony consisted of about 400 workers of all ages, 2 egg clusters, 95 larvae, 55 white pupae, and 1 queen. The proportions of brood and workers reflected the actual numbers in the colony at the time observations began.

Each of the 50 marked ants was observed for 5 min daily. Outside workers and domestics were observed for time spent in the following behaviors both inside and outside the nest: walking, grooming, feeding, debris removal, and inactivity. Because food (ad-lib honey water, crickets, mealworms, and cereal) and water were provided only in the arena, observations of time spent foraging and drinking were confined to ants outside the nest. Observations of time spent in behaviors occurring exclusively within the nest included those of tending the brood and queen.

Marked outside workers and domestics were observed every day until statistical analysis by *t* tests showed no significant differences between the two groups in time spent inside and outside the nest. It was further required that the domestics, when inside the nest, have little or no contact with the brood and queen, because pilot observations found this to be the behavior of outside workers. This requirement to some extent would assure that the domestics were not in a transitional phase of development and that their behaviors would be more similar to those of the baseline outside workers. When these criteria were met for a period of 4 consecutive days, the baseline time for maturation from eclosion to outside worker was considered completed.

Outside-worker removal. In order to determine whether the absence of outside workers can influence the rate of the domestics' behavioral development, all ants were removed except the queen, 2 egg clusters, 95 larvae, 55 white pupae, 30 deeply pigmented pupae (used for marked callows upon eclosion, they were all the dark pupae available), and 3 older callows to help with the eclosion of the first few callows. The older callows were removed after 3 new callows were seen helping with eclosion, within 5 days into the manipulation (all callows eclosed within 5 days). The 30 marked and dated callows were considered the domestic group.

Observations of their behaviors and the statistical analysis were done the same as in the baseline condition. Observations were terminated when callows met the criteria set for baseline outside workers.

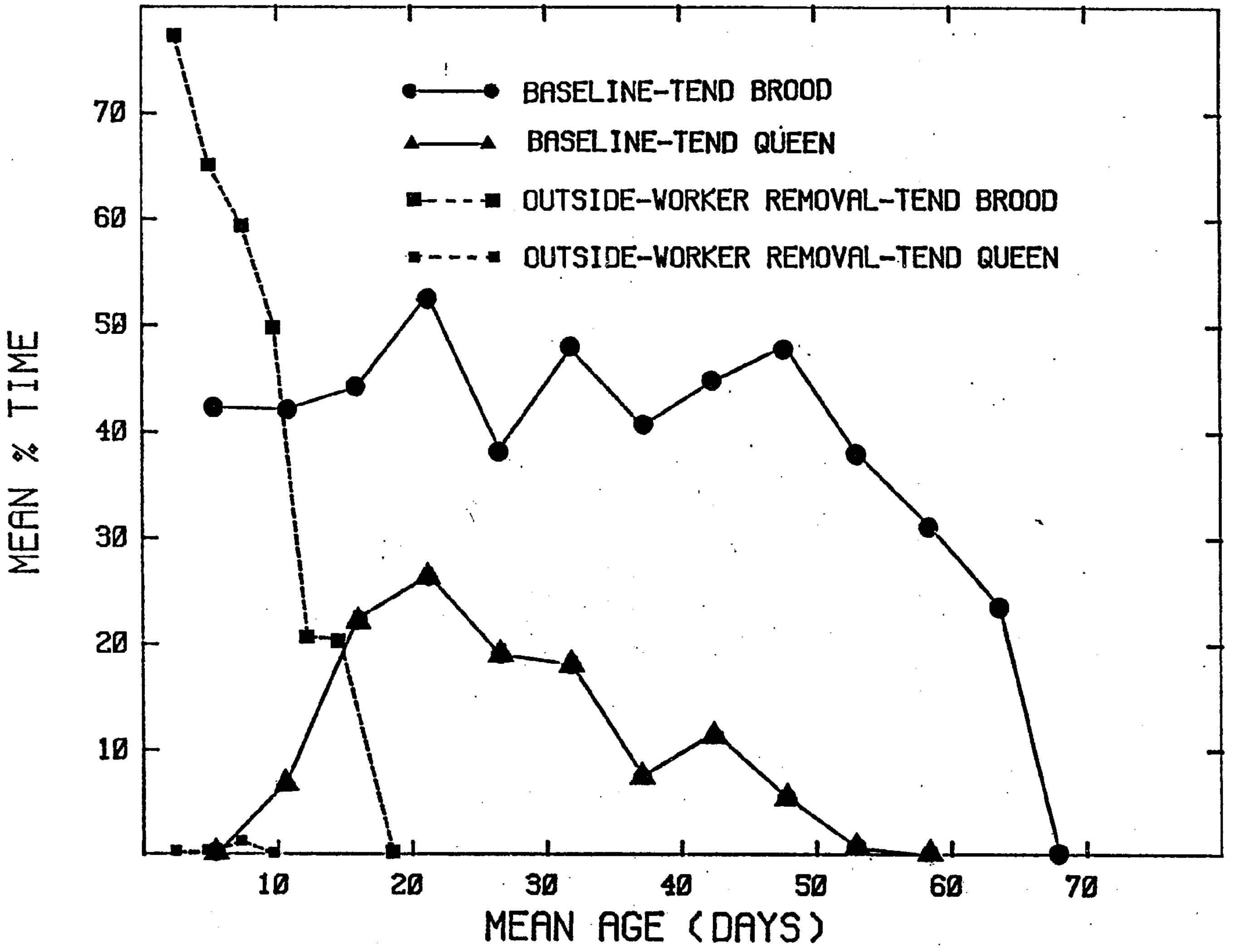
Results

Baseline. At the end of baseline observations, the worker population rose to approximately 470, and brood had increased to about 120 larvae, 67 pupae, and 3 egg clusters. I decided it best to not keep the colony culled to its original numbers. Any intrusion caused considerable disturbance: further, I would have had no way of knowing what age groups were culled.

Twenty of the marked domestics in baseline survived: they eventually exhibited behaviors not significantly different from those of outside workers. The average age for this group was 68 days (range, 51-82 days). At the end of this period, the domestics were spending 55.5% of their time outside the nest, compared with 55.7% for the outside workers over the 68 days of observation.

Figure 1 shows the development of tending brood and queen for approximately 5 day periods from the time of eclosion to time of meeting outside worker criteria. Over the 68 days of observation, contact with the brood and queen

Figure 1. Time spent by domestics tending the brood and queen in baseline and outside-worker removal manipulation.

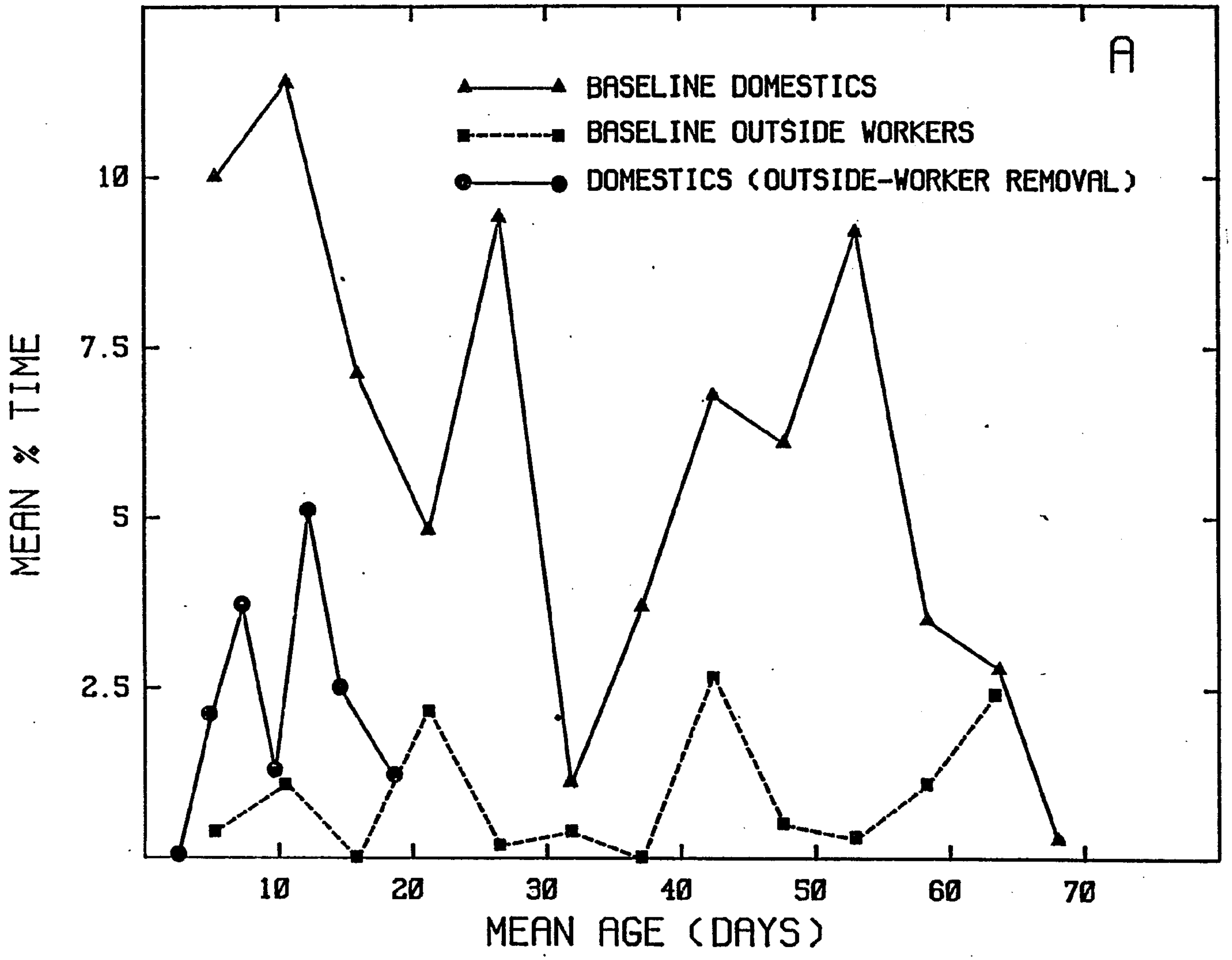

BASELINE-TEND BROOD
BASELINE-TEND QUEEN
OUTSIDE-WORKER REMOVAL-TEND BROOD
OUTSIDE-WORKER REMOVAL-TEND QUEEN
MEAN % TIME
70
60
50
40
30
20
10
10
20
30
40
50
60
70
MEAN AGE (DAYS)

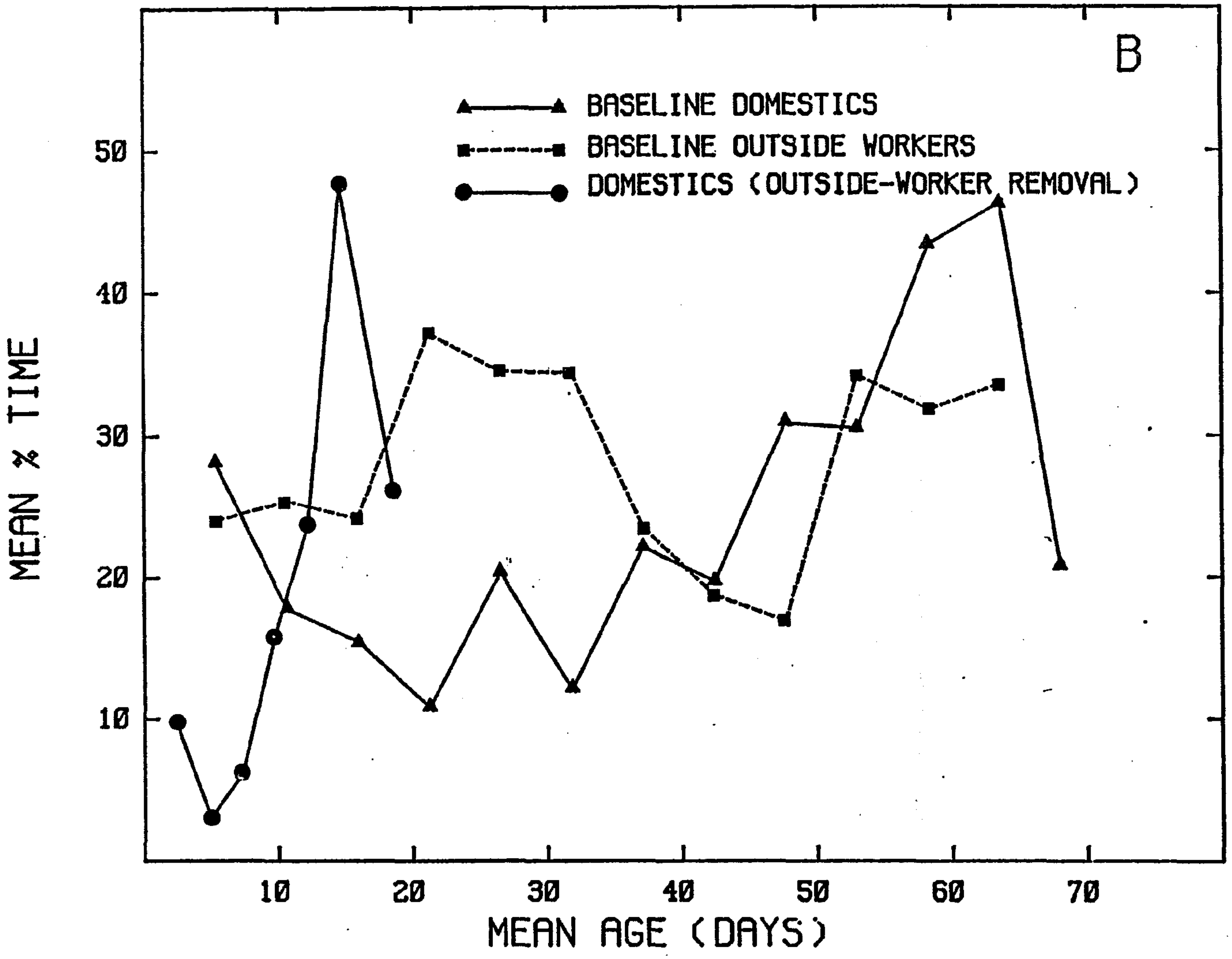
dropped from highs of 52.8% and 26.5%, respectively, to 0%. Outside workers did not contact the brood or queen during this time. The outcomes depicted in Figure 1 represent two of the criteria necessary for being judged outside workers; the figure also shows the temporal shift from the 10th through the 30th day of the domestics' lives. Whereas all domestics tended the brood, 7 of the 20 domestics were never observed in contact with the queen.

Figure 2A shows a significant drop in feeding in the nest by domestics from a high of 11.4% during the early stages of their lives to a low of less than 1% by the time they are outside workers (time spent feeding in the nest as a function of age: $r = -.63$, $p < .02$). The low is within the range of percentage of time feeding within the nest by outside workers. As with the outside workers, much of the domestics' feeding after they have become outside workers takes place outside the nest.

As the domestics mature and spend less time tending the brood and queen, their time spent in inactivity significantly increases ($r = -.92$, $p < .01$). Figure 2B shows that, with the exception of the first stage of the domestics' lives after eclosion, the percentage of time inactive increases from approximately 17% to 46%. The outside workers

Figure 2. Time spent feeding (A) and inactive (B) within the nest by baseline outside workers and domestics, and by domestics in the outside-worker removal manipulation. (For baseline outside workers, the x-axis simply represents days of observation.)


A
BASELINE DOMESTICS
BASELINE OUTSIDE WORKERS
DOMESTICS (OUTSIDE-WORKER REMOVAL)
MEAN % TIME
10
7.5
5
2.5
10
20
30
40
50
60
70
MEAN AGE (DAYS)


B
BASELINE DOMESTICS
BASELINE OUTSIDE WORKERS
DOMESTICS (OUTSIDE-WORKER REMOVAL)
MEAN % TIME
10
20
30
40
50
10
20
30
40
50
60
70
MEAN AGE (DAYS)

exhibited no such trend in within nest activity over the same period of time. The abrupt decrease in the domestics' level of inactivity as they complete the outside worker criteria is concomitant with an increase in debris removal by them near the nest entrance, but no significant correlation was found between the two behaviors over the 68 days of observation.

Debris removal was the one behavior that did not approach an outside-worker-like level as the domestics aged. Significantly more time was spent by domestics in within-nest debris removal than by outside workers ($t = 4.89$, $p < .01$). Debris removal increased from 1.4% of observed time to 9.1% through the outside worker criteria period. There was a strong correlation between time spent in debris removal and average age ($r = .87$, $p < .05$) on the part of the domestics. For the outside workers, no such correlation with time existed. Neither was there a significant correlation between the amount of time spent in debris removal by domestics and that spent by outside workers over the same period of time. Further, there was no significant difference between the average time spent by domestics and that spent by outside workers in debris removal. In an effort to find a behavior on the part of the outside workers that might contribute to increased debris removal by domestics and, at the same time, might take away from time available for outside workers to maintain the nest, a correlation analysis was done on the domestics' debris removal as a function of time spent by outside workers foraging. No significant correlation was found. Further, during the 4-day criteria period, at the peak of domestic debris removal, domestics were also spending more time foraging than the outside workers, 3.6% to 1.6%, respectively. None of the domestics had been observed foraging prior to these last 4 days.

The remainder of the domestics' time at any sample point shown in the figures was spent in grooming, walking, and moving slowly about the nest while feeling the substrate with the antennae or waving the antennae slowly in the air.

Outside-worker removal. Domestics of N. albisetosus developing in the absence of outside workers exhibited outside worker behavior in 19 days (range, 14-24 days) which is less than one third of the time that domestics in the baseline colony needed to develop similar patterns of behavior. Twenty-six of the thirty marked domestics in this manipulation survived. Callows marked as domestics eclosed from Day 1 through Day 5 of the experiment.

Figure 1 demonstrates sharply accelerated behavioral development for these domestics compared with baseline domestics. The finality of the behavioral change in Figure 1 was evident when no contact with the brood was observed for 2 weeks after the completion of this manipulation. The low percentage of time spent tending the queen in Figure 1 can be accounted for by the fact that only 3 of the 26 domestics were ever observed having contact with her.

Figure 2A demonstrates that less time was spent feeding within the nest than was observed for baseline domestics ($t = 4.6$, $p < .01$). Slightly more time was spent in feeding inside the nest than was seen for baseline outside workers 2.3% compared with 1%; $t = 2.58$, $p < .05$). No significant age-related trend was found, however, and this conforms with within-nest feeding behavior ot the outside workers. Figure 2B shows the same pattern for inactivity within the nest among these domestics as existed for the baseline domestics, although noticeably accelerated over 19 days. Similarly, as for baseline domestics, there was a significant negative correlation between inactivity and time spent on brood and queen ($r = -.8$, $p < .05$).

Experiment 2

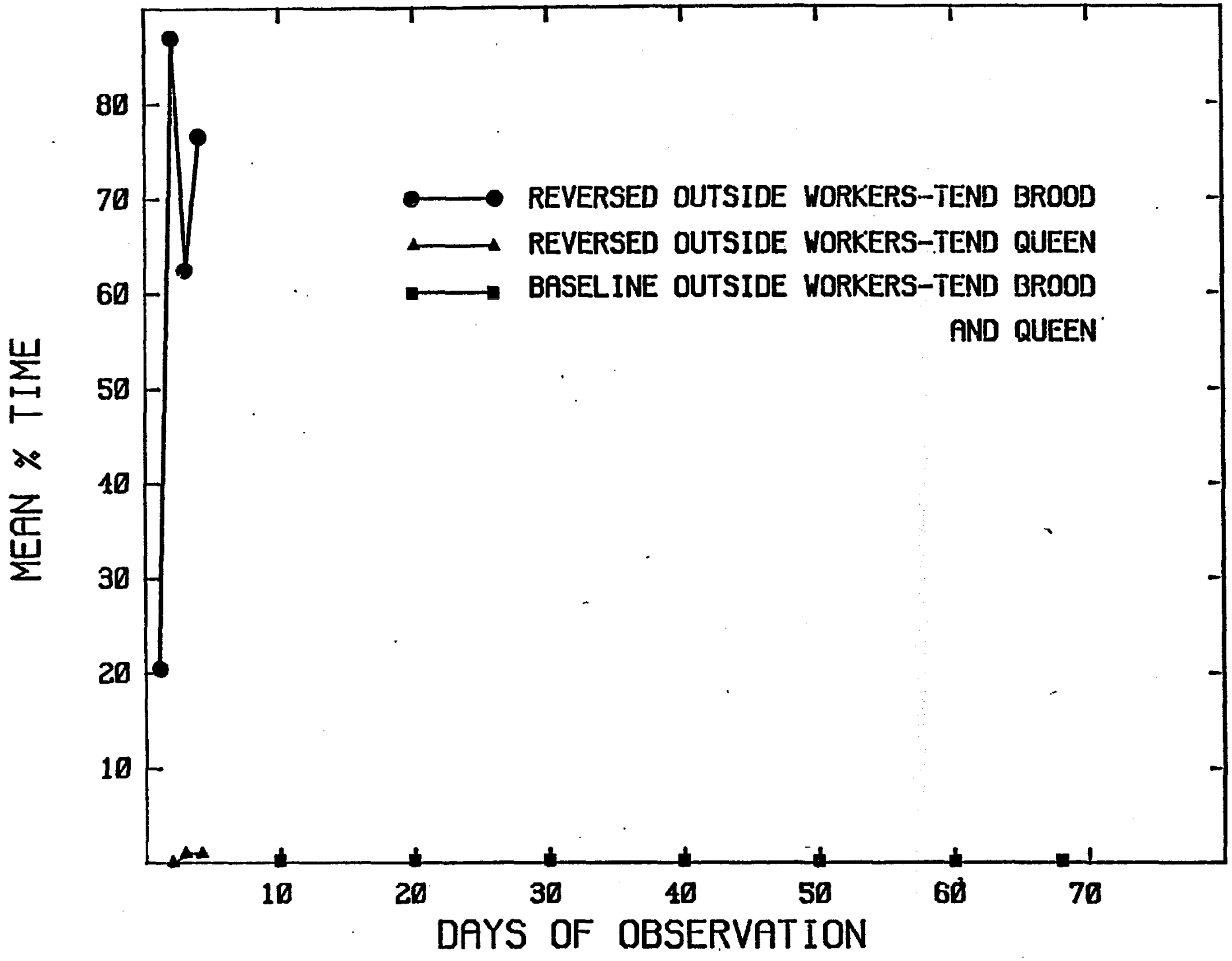
Procedure

Domestic removal. This experiment was designed to determine whether outside workers would revert to domestic-like behavior (e.g., tend brood and queen) in the absence of domestics. In a second colony the queen was left alone in the nest with 95 larvae and 36 pupae. All age-groups were removed but for 26 previously marked outside workers which were returned to the arena. The outside workers ranged in age from 185 days to an estimated 210 days. The older outside workers had not been observed tending brood for 180 days from the time they were marked, and the youngest outside workers had not been seen on the brood for 98 days. Observations were conducted until the outside workers averaged at least as much time as baseline domestics inside the nest and tending brood.

Results

Figure 3 demonstrates that reversion to domestic-like behavior was immediate for the 26 outside workers. Whereas outside workers in the baseline colony shunned brood and queen for more than 80 days of observations, outside workers in this manipulation reverted to tending the brood and queen in less than 1 day. They continued doing so for the next 4 days, spending 92.2% of the observed time inside the nest. When compared with time for baseline outside workers, this proved highly significant ($t = 42.52$, $p < .001$).

Figure 3. Effect of domestic removal on outside workers. (Outside workers revert to caring for brood and queen.)


REVERSED OUTSIDE WORKERS-TEND BROOD
REVERSED OUTSIDE WORKERS-TEND QUEEN
BASELINE OUTSIDE WORKERS-TEND BROOD AND QUEEN
MEAN % TIME
80
70
60
50
40
30
20
10
10
20
30
40
50
60
70
DAYS OF OBSERVATION

Experiment 3

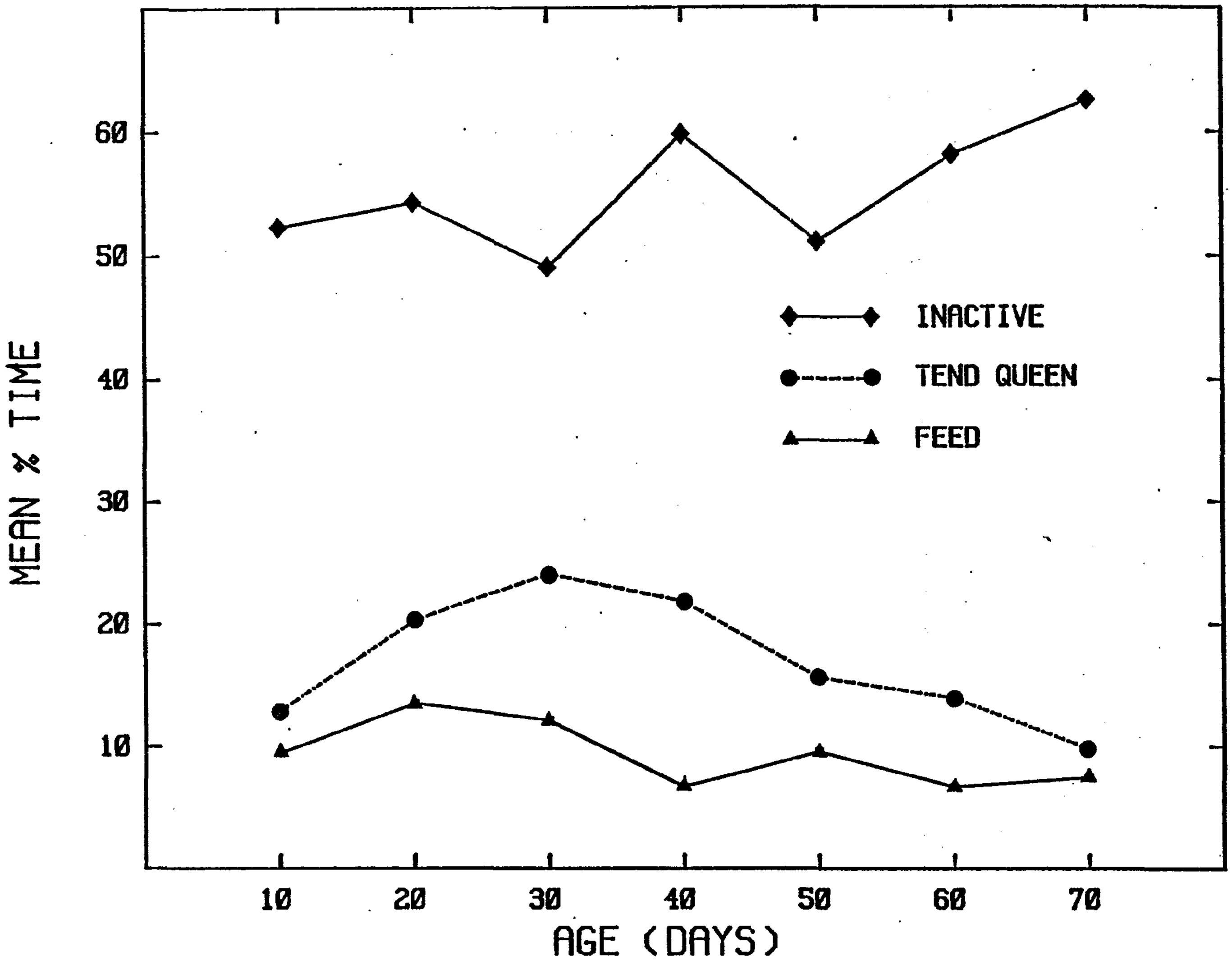
Procedure

Queen-no brood. In order to determine the relative contributions of queen and brood to the accelerated behavioral development of domestics in the outside-worker removal manipulation, 30 domestics were marked and dated as they eclosed, and they were left alone with the queen. Eggs were removed as they were laid.

Results

When the domestics were left alone with the queen, and without brood to tend, their behavioral development was retarded. Figure 4 shows that levels of feeding and inactivity within the nest are elevated compared with levels in baseline and the outside-worker removal manipulation (Figures 2A and 2B, respectively), and that they lack the noticeable age-related trends of those first two conditions. Similarly, domestics in this condition spent a larger portion of their time tending the queen than either outside workers or domestics did in the first three conditions, and all of the 28 surviving domestics tended the queen. However, there is no peak in tending the queen as there was for baseline domestics (Figure 1). Further, only 8 of the 28 domestics were ever observed outside the nest; as a

Figure 4. Retarded behavioral development of domestics in the queen-no-brood manipulation.


MEAN % TIME
60
50
40
30
20
10
10
20
30
40
50
60
70
AGE (DAYS)
INACTIVE
TEND QUEEN
FEED

group, the 28 domestics spent 99.2% of their time within the nest.

Experiment 4

Procedure

Chemico-larval stimulation. Because larvae must be fed, it is reasonable to assume that their need is communicated to the workers and that this stimulation, unlike the presence of the queen alone, may eventually elicit foraging behavior outside the nest. Ant larvae release liquids from their mouthparts and hindguts which may communicate the condition of the larvae to the workers (LeMasne, 1953), and thereby affect foraging. Free (1967) found that the smell of brood alone is partly responsible for honeybee foragers collecting pollen. In order to test for an airborne pheromone from the larvae, the center nest box of the apparatus was divided by two parallel screens approximately 7 mm apart. The queen and 50 larvae were placed on one side of the barrier, and 23 recently eclosed domestics were placed on the other side. The domestics could not touch the larvae across the dual barrier but could receive olfactory stimulation from them. Every 6 days three mealworms and a drop of honey water were provided; old worms were removed. Dead larvae were removed and replaced with live ones.

Only the domestics had access to the other nest boxes and foraging arena with ad-lib water and food.

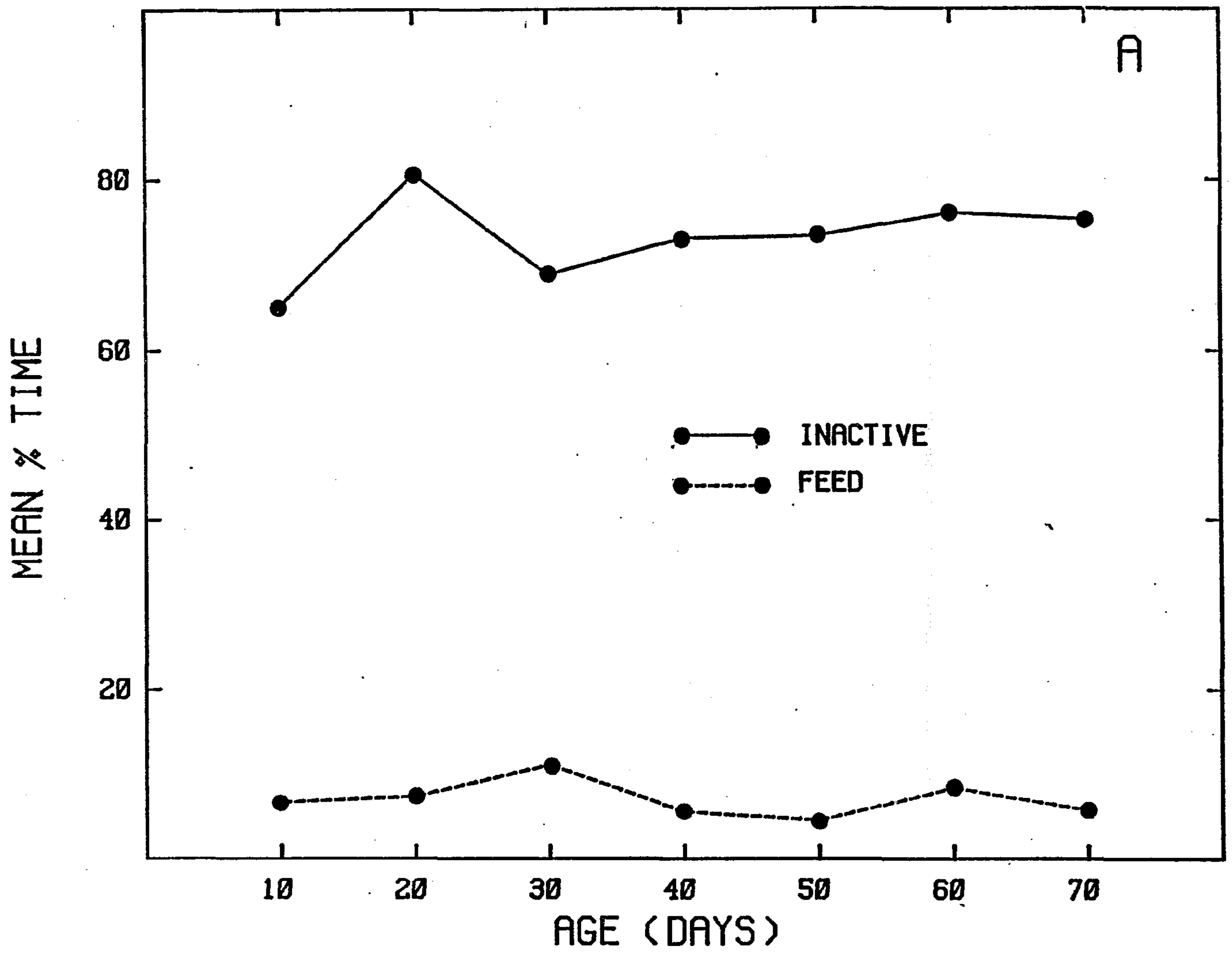
In order to test for chemotactile stimulation from the mandibular region of the larvae, the middle nest box was divided by a single fine-mesh screen which could be penetrated only be the antennae of a group of 23 recently eclosed, marked, and dated domestics. Thirty larvae were placed with their mandibular regions near one side of the screen, and the domestics were placed on the other side. So that the larvae could be groomed, they were rotated each day to a box of workers, and 30 other larvae took their place. Larvae were sparsely fed by placing two cut up mealworms in the box where they were groomed, every third day. No queen was provided because she would move the larvae from the screen. The domestics had access to the nest boxes and foraging arena.

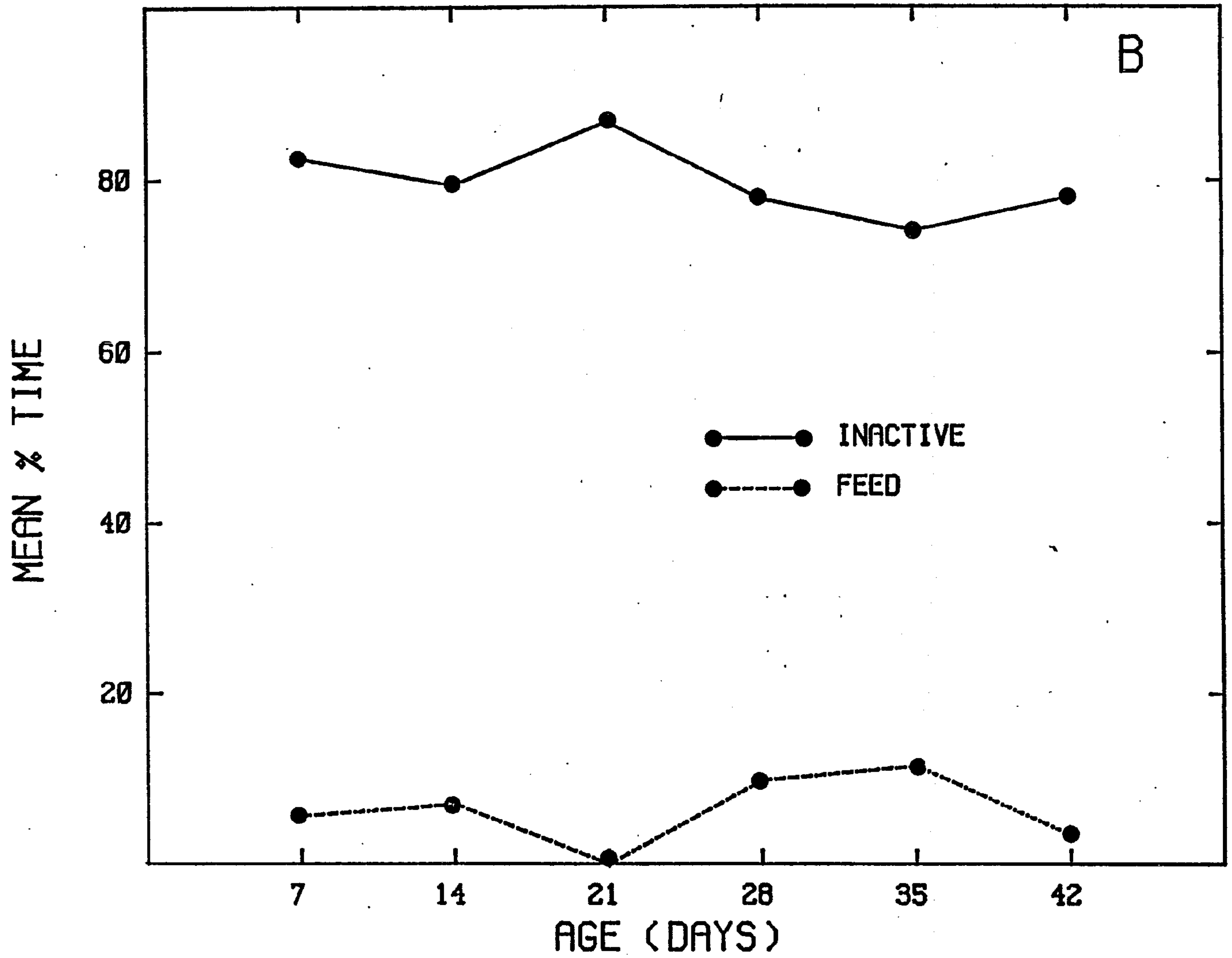
Results

In this condition, 13 of the 20 surviving domestics were observed outside the nest. As a group, domestics spent 2.1% of the observation time outside the nest, which was significantly greater than time spent outside in the queen-no-brood manipulation ($t = 2.59$, $p < .02$). Thus olfactory contact with the larvae had a greater effect on time spent outside the nest than did physical contact with the queen alone. Figure 5A shows that for those with olfactory contact only, levels of inactivity and feeding within the nest were elevated compared with baseline and the levels in the outside-worker removal manipulation (Figure 2). When inactive, domestics often tended to cluster next to the barrier, as close to the queen and larvae as possible. Although the domestics were obviously attracted by the presence of the larvae, olfactory stimulation from the larvae is apparently not enough to affect the behavioral development of the doemstics to the point at which they will spend most of their time outside the nest, as they did in baseline and the outside-worker removal manipulation.

Because of a lack of larval supply, domestics in the chemotactile condition were observed for only 42 days. However Figure 5B shows levels of feeding and inactivity within the nest similar to those in the olfactory condition. Again, there were no significant age-related trends. Eleven of the twenty surviving domestics were observed outside the nest, which accounted for 2.4% of all observed time for the group . This was not significantly different from time spent outside in the olfactory condition but significantly greater than in the queen-no-brood manipulation (t = 3.36,

Figure 5. Elevated levels of inactivity and feeding in the nest for domestics having only olfactory contact with larvae (A) and chemotactile contact with anterior portions of larvae (B).


A
80
60
40
20
MEAN % TIME
INACTIVE
FEED
10
20
30
40
50
60
70
AGE (DAYS)


B
80
60
40
20
MEAN % TIME
7
14
21
28
35
42
AGE (DAYS)
INACTIVE
FEED

$p < .01$). Again, contact with the larvae affects time spent outside the nest to a greater extent than does contact with the queen alone. The callows were observed touching the anterior portions of the larvae through the screen, and on six occassions food was found placed against the barrier, though there was no significant difference in foraging between the two conditions.

Experiment 5

Procedure

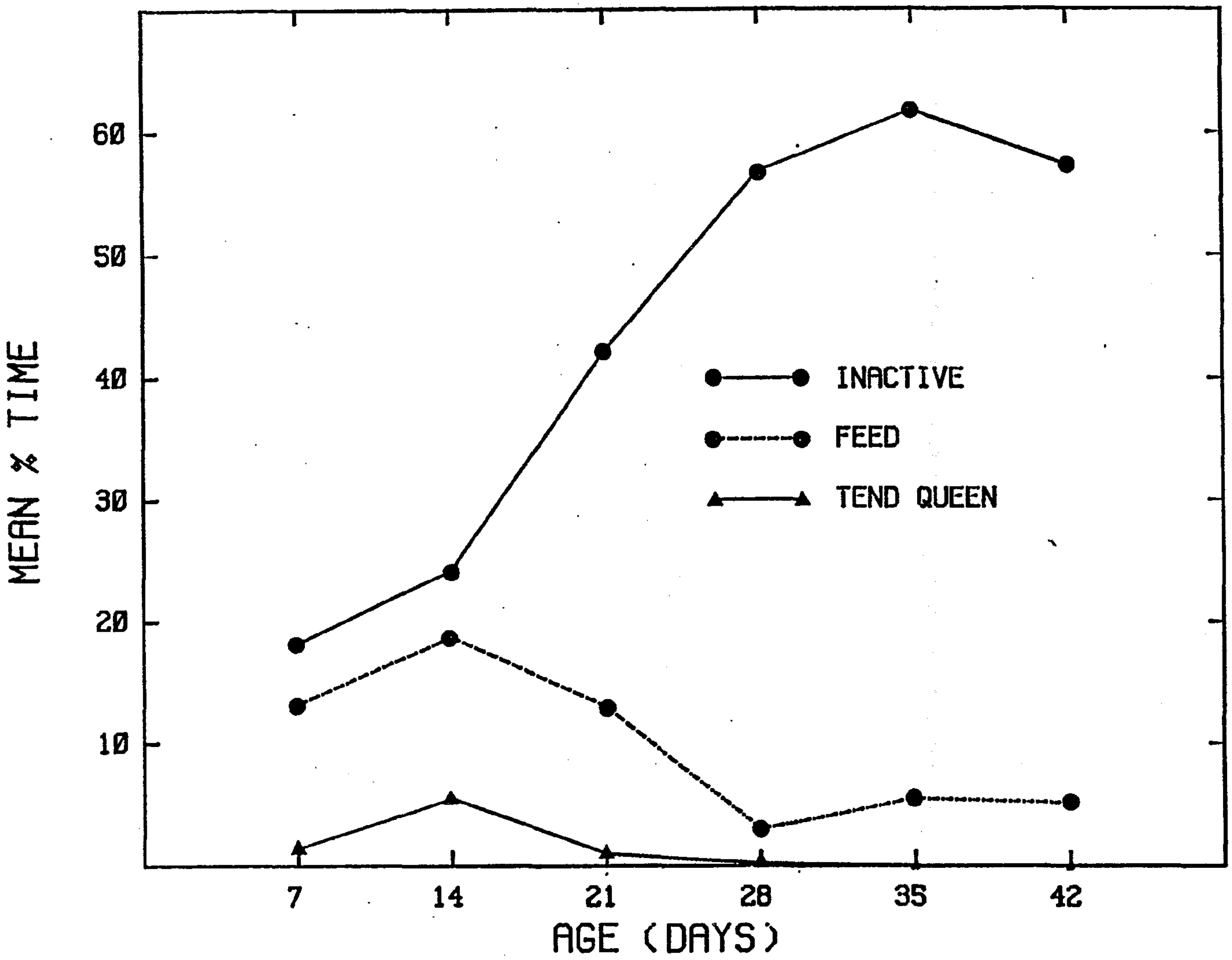
Eclosion stimulation. Because the brood had been a mixture of larvae and pupae in both baseline and the outside-worker removal manipulation and because callows were eclosing from these pupae during those conditions, the effect of eclosion on the behavioral development of domestics had to be studied. In order to test for an eclosion stimulation, 140 pigmented pupae of various shades were placed in the central nest box with the queen. The first 24 callows to eclose were dated and marked as domestics. The effect on these domestics from the eclosion of the remaining pupae was observed.

Results

Of the 24 marked domestics, 21 survived in this condition; 15 pupae died. When the domestics were exposed to the eclosion of more than 100 pupae over a 2 week period, they were stimulated to spend an average of 15.7% of their time outside the nest during the 6 weeks of observations. This is significantly greater than time spent outside the nest in the larval stimulation manipulation (olfactory, $t = 20.02$, $p < .001$; chemotactile, $t = 20.39$, $p < .001$). All of the domestics were observed outside the nest.

Once baseline domestics left the nest, they spent a significantly greater percentage of their time outside than domestics in the present condition ($t = 37.48$, $p < .001$). However, at a comparable age, baseline domestics spent an average of only 0.8% of their time outside the nest, considerably less than the 15.7% of the eclosion-stimulated domestics. After the peak of eclosion activity, during the fourth week of their lives, domestics spent 32.1% of their time outside, but this level of activity was not sustained during the remainder of the condition. Although feeding and inactivity within the nest were significantly elevated above the levels of baseline domestics ($t = 2.07$, $p < .05$ and $t = 8.74$, $p < .001$, respectively), Figure 6 shows that the trends followed by both are roughly the same (cf. Figures 2A and 2B, respectively). Tending the queen also follows a pattern roughly similar to that of baseline domestics

Figure 6. Age-related trends for domestics stimulated by recently eclosed callows.



60
50
40
30
20
10
MEAN % TIME
7
14
21
28
35
42
AGE (DAYS)
INACTIVE
FEED
TEND QUEEN

(Figure 1). Eight of the twenty-one callows were observed tending the queen.

Experiment 6

Procedure

Total larval contact. Given the results up to this point in the study, it was evident that some control of callow behavioral development resided in the brood: Eclosing callows stimulate older callows to forage; perhaps larvae stimulate the older callows to continue foraging and working outside the nest. In both baseline and outside-worker removal conditions, domestics had complete contact with the larvae. It was necessary to present a group of recently eclosed domestics with a large number of larvae, to see how much larval stimulation alone would contribute to behavioral development.

Accordingly, 200 larvae and 30 callows were placed in the central nest box without the queen. Nonfeeding larvae were removed and replaced with feeding larvae. Two clusters of behavior were observed: those involved in within-nest brood contact, and outside-the-nest activities.

Results

Twenty-seven domestics survived in this condition. Figure 7 shows how the two types of behavior display an inverse fluctuating relation to one another: When brood care percentage drops, the percentage of time spent outside rises. This behavior continued through 70 days of observation. In the eclosion stimulation manipulation there was no such fluctuating relationship between brood care and time spent outside (This was in part because of the peaking nature of eclosion and the fact that, once eclosion was complete, there was no more brood to care for).

Experiment 7

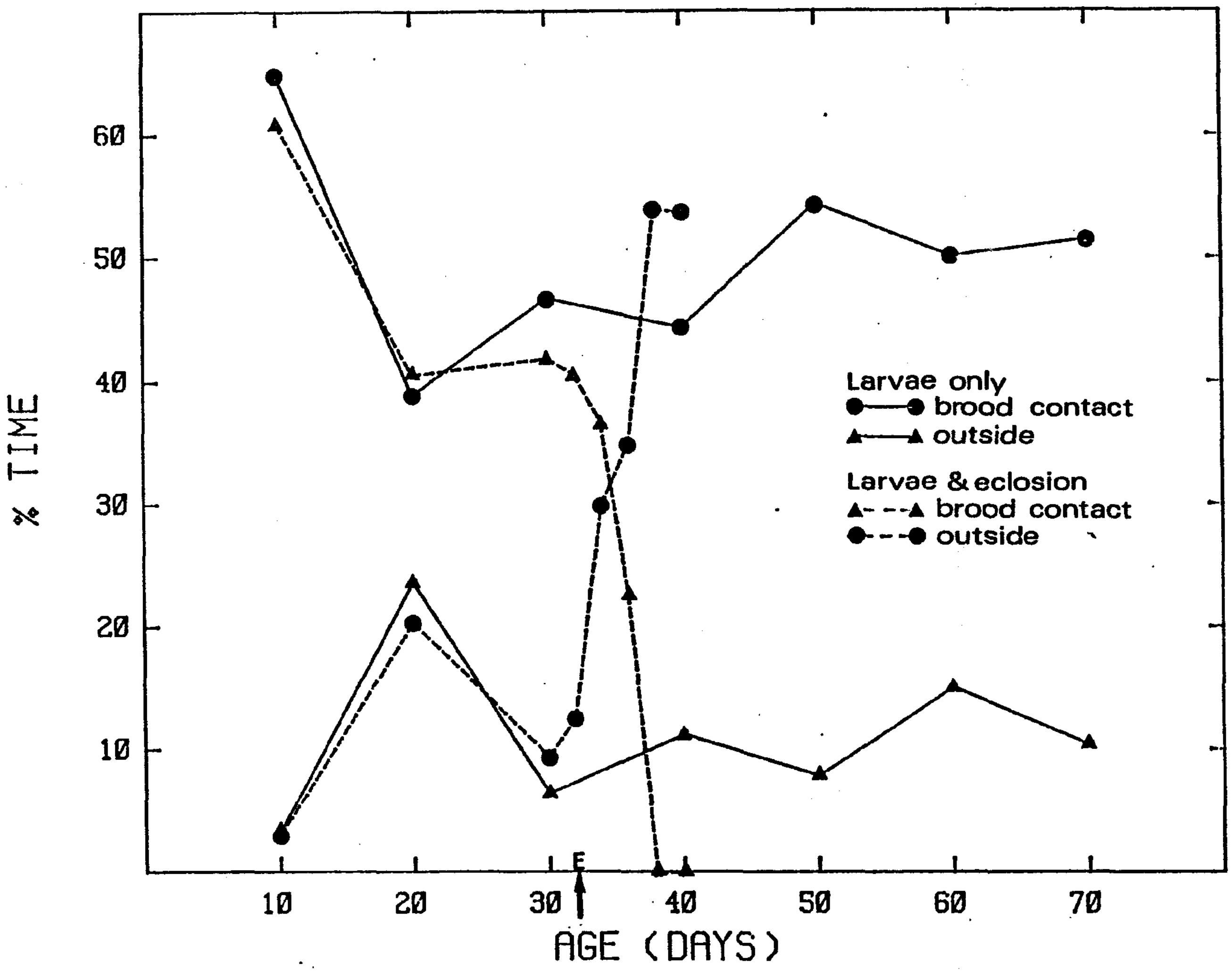
Procedure

Larval plus eclosion stimulation. The following was done to test an hypothesis that I had formed: Eclosion provides the initial stimulation for rapid behavioral development, while larval stimulation is necessary to maintain the gains made during this acceleration into outside worker behavior. The previous condition was repeated, again using 30 callows and 200 larvae, During the 4th week, this condition was combined with the eclosion stimulation conditions. Accordingly, 100 darkly pigmented pupae that were near to eclosing were placed in the nest with the larvae.

Results

Twenty-eight domestics survived in this condition. Figure 7 again shows the fluctuating relationship between

Figure 7. Domestic response to intense larval stimulation (Experiment 6), and to a combination of larval and eclosion stimulation (Experiment 7). "E" at the arrow is the point of greatest eclosion stimulation.


% TIME
60
50
40
30
20
10
10
20
30
E
40
50
60
70
AGE (DAYS)
Larvae only
●—● brood contact
▲—▲ outside
Larvae & eclosion
▲- - -▲ brood contact
●- - -● outside

time spent outside the nest and brood care. However, with eclosion taking place at about 32 days (beginning toward the end of the 30th day, with heaviest eclosion by far on the 32nd day, and ending early on the 34th day), there occurred the sudden change in behavior represented by the crossed broken lines. There was an abrupt increase in percent time spent outside to 53.9%, or baseline level; and a concomitantly abrupt decline in brood contact to 0%, again baseline level.

Experiment 8

Procedure

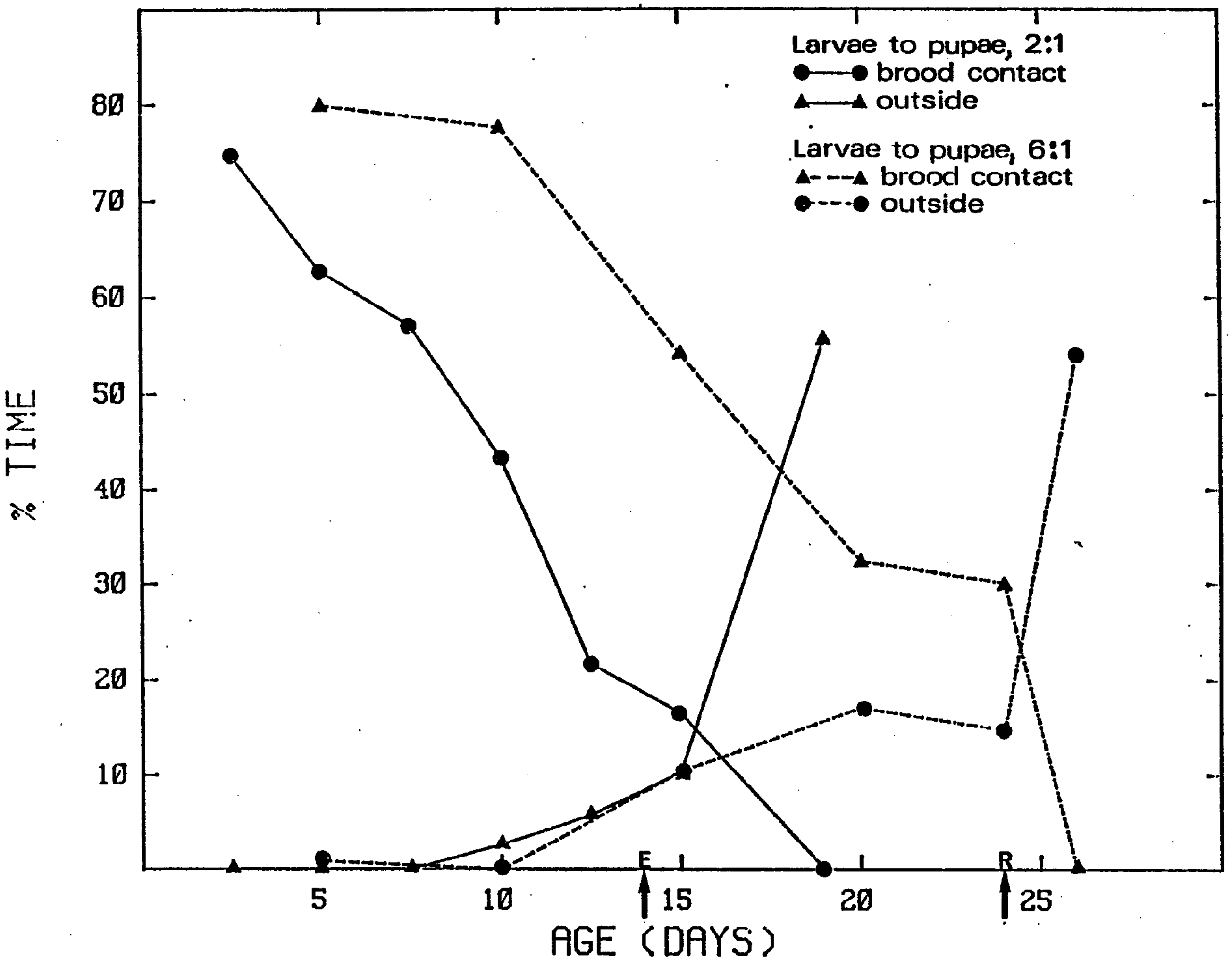
Elevated larval stimulation plus eclosion. After the eclosion stimulation and total larval stimulation manipulations it was evident that these factors by themselves were not enough to affect behavioral development to the extent that was seen in the outside-worker removal condition. By pairing the two, however, domestic behavioral development was accelerated to outside worker levels. While both stimulation from the larvae and eclosing pupae each directly affected the marked domestics, there remained the possibility of a synergistic effect. For example, the recently eclosed callows may have provided the work force necessary to properly care for the larvae, thereby diminishing larval stimulation that would otherwise have kept the marked domestics within the nest.

To test this possibility, 300 larvae and 55 pupae were placed with the queen in the center nest box. This represents three times the amount of larvae to pupae in the outside-worker removal manipulation. Eclosion stimulation was not well regulated in the outside-worker removal condition, but the greatest number eclosed on the 14th day. Accordingly, the bulk of the eclosion stimulation occurred at the end of the second week in the present manipulation. If by the 24th day (the maximum range for domestics in the outside worker removal manipulation to exhibit outside worker behavior) domestics were not conforming to the levels of outside workers for brood contact and time spent outside the nest, two-thirds of the larvae would be removed so that the proportion of larvae conformed to the outside-worker removal condition. Twenty-five callows were marked as the domestics for this condition.

Results

Of 25 callows marked for testing, 23 survived. Most of the eclosion stimulation took place on the 14th day, the same as in the outside-worker removal condition. Figure 8

Figure 8. Domestic response after eclosion (E) in the outside-worker removal manipulation (solid lines); and after eclosion followed 10 days later by removal of two thirds of the larvae (R).


Larvae to pupae, 2:1
●—● brood contact
▲—▲ outside
Larvae to pupae, 6:1
▲---▲ brood contact
●---● outside
% TIME
80
70
60
50
40
30
20
10
5
10
15
20
25
E
R
AGE (DAYS)

shows the outside-worker removal condition (Larvae to Pupae, 2:1) and the present condition (Larvae to Pupae, 6:1). There was a response to eclosion in both conditions: Whereas the behavior of domestics in the 2:1 condition reached baseline levels, behavior in the present 6:1 condition began to approach those levels more slowly, then turned away from them. Marked domestics after 24 days were still spending 30% of their time on the brood and only 14.7% outside.

On the 24th day, which represented the maximum range for domestics in the outside-worker removal condition to exhibit outside worker behavior, I removed two-thirds (200) of the larvae so that the proportion of the larvae conformed to the outside-worker removal condition (about 95 larvae, 55 pupae). Within 2 days the average level of within-nest brood care dropped abruptly to 0%; time spent outside increased to 53.6%--both outside worker levels conforming with baseline (both are not significantly different from baseline levels). These levels remained the same for the next week, at which time I had no more larvae to keep the brood replenished to the required numbers. The experiment was then terminated.

Discussion

According to Schneirla's (1957, 1971) theory of brood stimulation, the eclosion of the pupal brood provides the stimulation that initiates the nomadic phase in the army ant, Neivamyrmex nigrescens (Cresson). He assumed that larval excitation was not strong enough to start the nomadic phase, although necessary to maintain it. Topoff, Mirenda, Droual, and Herrick (1980) and Topoff, Rothstein, Pujdak, and Dahlstrom (1981) corroborated part of Schneirla's theory by demonstrating that the presence of newly eclosed callows alone is sufficient to initiate a nomadic phase and that artificially overfeeding colonies elicited statary behavior in nomadic colonies. Although N. albisetosus is not nomadic, perhaps it is the same kind of brood stimulation that influences its behavioral development, as gauged by temporal polyethism, to the point at which it is stimulated to leave the nest, become active outside, and leave tending of brood and queen to new ranks of callows.

The outside-worker removal manipulation clearly demonstrates that in the absence of outside workers and presence of brood, behavioral development is rapidly accelerated. Whereas Lenoir (1979) found similar behavioral plasticity in Tapinoma erraticum L., young N. albisetosus workers did not return to former tasks within the nest but remained outside workers. From this manipulation it is not yet clear whether the mechanism affecting accelerated behavioral development was stimulation from the brood or removal of inhibition from outside workers.

The domestic-removal manipulation shows that tasks normally associated with the domestics still remain within the repertoire of outside workers. Some of the adults that tended the brood and queen in this manipulation had not been observed touching either for 6 months. Perhaps a reciprocally inhibitory function is at work here, whereby the outside workers keep domestics from leaving the nest and domestics keep outside workers from tending the brood and queen (more on this later). Unlike Lenoir's (1979) outside workers (permanent foragers), the outside workers of N. albisetosus made good brood nurses and cared for the larvae and pupae until pupation and eclosion when new callows replaced them in these tasks.

According to the queen-no-brood manipulation, the absence of brood seems to retard behavioral development. When exposed to brood for the first time in their lives after 3 months, these domestics made poor brood nurses: Larvae, pupae, and eclosed callows were stunted. These stunted callows were significantly smaller (in both head width and head length: $F = 243$ & 200, 116 df, $p < .001$, respectively).

than workers taken randomly from other colonies. Though they did not reject brood as Jaisson's (1975) workers rejected cocoons after having been deprived of them for the first 15 days of their lives, there may be a critical period for brood tending as he suggested. Apparently, the queen alone has little effect on that part of behavioral development marked by the domestics' eventually leaving and spending much of their time outside the nest. Brood seems to be a necessary ingredient.

The larval stimulation manipulation demonstrates that olfactory stimulation both alone and in combination with chemotactile stimulation from the anterior regions of the larvae increases the amount of time spent by domestics outside the nest--significantly more so than in a condition in which no brood is present. This is interesting in light of Free's (1967) finding that the smell of brood alone is also partly responsible for the type of foraging that honeybees do, although physical contact with the brood played a much larger role in affecting foraging patterns. Brian and Abbott (1977) gave evidence that the larval head stimulates food collection in Myrmica, and it is not necessary that the head be able to swallow or to move. Though in the present study antennal contact with the heads of larvae had no more effect on time spent outside or foraging than did the smell of larvae, food was found placed against the screen on six occassions. This was not found in the olfactory condition. However, neither of these conditions by itself provides a strong enough inference of a causative factor in behavioral development.

The eclosion manipulation provides such an inference. Rather than eclosion stimulation per se, the stimulation necessary to arouse the marked domestics to leave the nest seemed to come from the accumulating numbers of recently eclosed callows. Although eclosion caused visible excitement within the nest, the marked domestics were not aroused to forage en masse until the third and fourth weeks when most of the callows had already eclosed. During these weeks, domestics spent 23% and 32.1% of their time, respectively, outside the nest. This dropped to 16.3% in the fifth week, after which it was noticed that some of the unmarked callows were also outside feeding and foraging. After the fourth week no domestic was seen tending the queen; after the third week, what brood remained were tended by the recently eclosed unmarked callows only. Clearly, stimulation from recently eclosed callows is enough to arouse young domestics to leave the nest and to keep them from returning to some tasks (tending the queen and remaining pupae) within the nest. As such, it very likely plays a role in the normal behavioral development of N. albisetosus.

The total larval contact manipulation reveals the dual nature of stimulation coming from the larvae. Perhaps there are two clusters of stimuli, one of which communicates the hunger of the larvae, the other of which communicates their general condition necessitating grooming. Hereafter I will use "hunger" and "grooming" stimulation simply as conveniences descriptive of those stimuli which arouse workers to leave the nest to forage or to maintain brood contact within the nest, respectively. It is evident from this manipulation that young domestics will spend more time outside the nest at an earlier age than they would in the presence of older outside workers, as in baseline.

Eclosion stimulation and larval stimulation were not by themselves enough to effect the behavioral development of domestics to the point where baseline levels for spending time outside the nest were reached. But together they stimulated domestic behavioral development to mature outside worker levels. While eclosion stimulation is evidently a positive and direct force, it also seems to work indirectly in the sense that eclosing callows provide the work force necessary to take care of the brood's needs, thereby decreasing the brood's grooming stimuli and dropping it below the thresholds of the older, marked domestics. That this may be the case is confirmed by the fact that it is the growing number of newly eclosed callows that is associated with domestics leaving the nest rather than an eclosion stimulation per se. Similarly, in army ants it is callow excitation rather than eclosion stimulation which initiates nomadism (Schneirla, 1957). It is to be noted that about twice the amount of larvae and pupae were used in this condition than were used in baseline; however, the ratio of larvae to pupae remained the same. Though this elevated the larval stimulation, enough callows eclosed from the pupal stage to respond to that stimulation and take care of the grooming needs of the larvae, thereby allowing the marked domestics to spend more time foraging outside the nest.

In the final manipulation, elevated larval stimulation plus eclosion, I presented marked domestics with both larvae and pupae but in a ratio disproportionate to the other conditions in which they appeared together. This ultimately demonstrated that any large fluctuation in brood composition should also have an effect similar to that found when outside workers or domestics are removed from the colony.

Evidence points away from direct stimulation from older age groups, namely outside workers, as a major factor in the behavioral development of callows. It seems less likely that outside workers or other adults directly inhibit or slow down behavioral development than it does that their presence in large enough numbers helps regulate the levels of other stimuli, such as those coming from larvae and eclosing callows, that would in the outside workers' absence (or presence in inadequate numbers) drive the callows out to forage where they would come in contact with other stimuli that may further contribute to their behavioral development.

I have shown that eclosion and larval stimulation together will speed callow (domestic) behavioral development to mature adult levels in one-third the baseline time, but that separately they do not. I have further shown that there must be a "proper" proportion of the two groups of brood stimuli: If too many larvae are present in relation to pupae, eclosion will not provide the spur to behavioral development that was seen in the outside-worker removal condition. Given that some pupae and callows did not survive, the proportion of larvae to pupae should be anywhere from 2:1 to 3:1. This proportion seems to provide enough domestics to care for the brood within the nest; they seem to be particularly sensitive to brood stimuli that evoke a brood contact response. Given enough recently eclosed callows to act as domestics caring for the brood within the nest, the older domestics are free to respond to the hunger stimuli of the brood. On returning to the nest, the stimuli for brood contact may become eventually too low for the older foraging domestics' thresholds. Thus they stay off the brood. Their thresholds may further ascend because of this lack of stimulation. No longer very responsive to larval grooming stimuli, they then become full-fledged outside workers.

I had at first thought that stimulation from recently eclosed callows starts the process of accelerated behavioral development, while stimulation from larvae stabilizes the gains made during this acceleration into mature outside worker behavior. While some kind of stimulation evidently does come from the youngest age group and evokes a foraging or other outside-the-nest response on the part of older domestics, it does not start the process of behavioral development. The last three manipulations show that larval stimulation present before eclosion, can and does influence behavioral development. The question of what stabilizes the gains made during an acceleration toward outside worker behavior can best be answered by the effect that the youngest age group has upon certain stimuli coming from the larvae. The new ranks of eclosing callows seem to have a very low threshold for the grooming stimuli of the larvae and spend much of their time in contact with them. The older domestics are stimulated by these younger ones to leave the nest to forage, thereby decreasing their time spent with the brood. This diminished contact coupled with new contact with other stimuli as a result of foraging may serve to elevate the older domestics' thresholds to the larval grooming stimuli. At the same time, the new, recently eclosed domestics are diminishing the volume of grooming stimuli by responding to it themselves. There may also be a concomitant decrease in the older domestics' thresholds to the larval hunger stimuli, which further serves to keep them foraging outside the nest and coming into contact with the brood. In the case of the domestic-removal condition where older outside workers cared for the brood, larval grooming stimuli may have increased to the point of reaching the outside workers' high thresholds; this, with renewed larval contact, may then have served to lower their thresholds of response to the grooming stimuli.

These, I think, are the dynamics of brood stimulation of behavioral development in their simplest form. That these hypothesized clusters of "hunger" and "grooming" stimuli are not the sole motivating stimuli is evident from the outside workers doing other things than sheer foraging outside the nest, such as feeding, remaining inactive, guarding (inactive or moving about close to the nest entrance), transporting and burying debris, and nest excavation.

Part II

Introduction

T. C. Schneirla (1938, 1971) became a pioneer in the field of social regulation of behavior through his analysis of behavioral development in army ants. He discovered that the cyclic pattern of army ant behavior--their nomadic and statary phases--was governed by the condition of the brood. Recently, McDonald and Topoff (1985) found evidence that Schneirla's theory of brood excitation may have broader developmental implications. They found that changing brood stimulation by removing certain age groups, controls behavioral development in an ant that is not closely related, behaviorally or taxonomically, to army ants. This ant, *Novomessor albisetosus*, is preyed upon by army ants and, like them, has evolved a high degree of social organization. Nowhere is this organization more apparent than in their elaborate and cooperative defense against army ants. The defensive effort takes place both within and outside the nest and includes all the age groups considered in Part I of this dissertation.

In Part II, I report the results of a study designed to extend the scope of developmental studies on ants, by focusing on behavioral interactions between *N. albisetosus* and their chief predator.

Development of Defensive Behavior

The nearctic army ant, *Neivamyrmex nigrescens*, of the southwestern United States preys primarily upon other ant species, termites, and their brood. These prey constitute relatively localized concentrations of food.

To deter predation ants have evolved various adaptations for defense, including venom apparatuses (comprehensively reviewed by Blum and Hermann, 1978 a & b) and physical castes often called soldiers (Wilson, 1971; Oster and Wilson, 1978). Behavioral adaptations also have evolved for colony defense (Wilson, 1971, pp 158-165), such as the protean behavior of workers fleeing from the nest while evacuating brood and queen (Topoff et al., 1980). In the case of *Camponotus* spp, protean behavior includes climbing vegetation where *N. nigrescens* rarely follows (LaMon and Topoff, 1981).

Rapid dispersal with the brood and queen helps diminish the effectiveness of army ants attacking in large numbers an isolated nest. Among species displaying rapid nest evacuation are *Camponotus festinatus* (LaMon and Topoff, 1981), *Pheidole desertorum* and *P. hyatti* (Mirenda et al., 1980), *Novomessor cockerelli* and *N. albisetosus* (author's observations).

Nest defense, like most social behaviors of ants, is also affected by age. Dobrzanska (1959) showed that in Formica sanguinea there is a gradient of aggression correlated with the location of different-aged workers within and about the nest, and Weir (1958, a & b) has shown a similar pattern for *Myrmica ruginodis*.

Cammaerts-Tricot (1975) took advantage of the fact that cuticular pigmentation is related to age in many ants (lightly colored young ants darken as they age) to show how *Myrmica rubra* reacts differently according to age toward an intruder, *Lasius flavus*. LeRoux and LeRoux (1979), while finding that nest defense varied among *Myrmica laevinodis* individuals of a year or more in age, also found that defensive behavior could be altered by experience and changing group composition.

Initial observations suggest that *N. albisetosus* exhibits a graded system of nest defense like that reported by Wilson (1976 b) for *Pheidole dentata*, where defense is followed by nest evacuation when a predatory species penetrates the nest. The protean behavior of *N. albisetosus* of evacuating the brood and queen while the entire colony flees from the nest appears to be a response used only against the army ant genus Neivamyrmex, a natural predator. Closer observations reveal that darkly-pigmented N. albisetosus workers defend the area immediately around the nest entrance by approaching and attacking the army ants. Darkly-pigmented ants also remove most of the brood, with the proportion of brood removed declining the lighter the pigmentation of the ants. The lightest pigmented ants (callows) emerge with empty mandibles from the nests. Darkly-pigmented ants also return for more brood, whereas the callows rarely do so.

The plasticity of temporal polyethism underlying defense and protean behavior in N. albisetosus is not known. For example, at what age do patterns of colony defense such as nest evacuation, approaching and attacking a predator emerge? Will these complex behaviors emerge in N. albisetosus workers without their having had prior experience with a predator such as army ants? Can the development of defensive behavior be altered by repeated experiences with army ants? The present study was designed to elucidate the development of these behaviors both in the laboratory and the field.

Care was taken that trials were performed during the same time of day or night for both the laboratory and field colonies. This was done in deference to what I call the "Gordon doctrine": In each ant colony there is a daily temporal pattern of activities performed; hence experimental manipulations at one time of day may yield quite different results at another (Gordon, 1983).

Section 1 - Isolated Age Groups

The purpose of the following was to study the development of defensive reaction to army ants by groups of *N. albisetosus* workers which had been raised in isolation. After exposing them to army ants the study was repeated using the same workers, in order to see if their defensive behavior was altered due to their single experience with the predator.

Methods

Subjects

Several intact colonies of *N. albisetosus* were flushed from their nests by releasing army ants, *Neivamyrmex nigrescens*, into the nest entrances. Darkly-pigmented pupae from the various colonies were pooled, and those eclosing on the same day were placed about 80 to a group in plastic boxes with food and water. These callows provided the age groups tested under the various conditions of the experiment.

Apparatus

The nest box was made of wood, 30 cm square x 3 cm high, fitted with a glass top. The box was situated between two foraging arenas with plywood floors: the larger arena in which army ants were released measured 48 cm x 61 cm; the smaller arena, which provided an escape route for the N. albisetosus workers, was 40 cm x 45 cm. Both arenas had sheet aluminum walls 20 cm high, coated with Fluon GP-1, a dry lubricant, to prevent ants from escaping over the walls. The floors of both arenas were covered by filter paper which was replaced after each trial. Free access to both arenas was provided from the nest box by 13-mm diameter Plexiglass tubing. Food and water were provided in the large arena; only water, to prevent possible dehydration, was provided in the escape arena.

Procedure

In order to study behavioral processes of N. albisetosus unaffected by experience with army ants, groups of about 80 callows were left isolated from eclosion until the day of testing, when 60 from each group were exposed to army ants. The 16 age groups were as follows: 3 days, and 1-15 weeks inclusive. Three days before a given age, 60 larvae and 60 pupae (conspecific) were presented to each age group so that they could become accustomed to them (workers were raised with small numbers of available brood and seemed to nurse them well). At testing, the 60 workers along with their pile of brood were exposed to 150 army ants released into the large arena. The day before the trial, the brood pile was put in the nest box and the age group to be tested was placed in the small arena (designated the escape arena). This was done so that the age group would gain some familiarity with the passage to the escape arena as they went from the escape arena to the nest. The nest box was kept moist and it was covered with cloth for a few hours, until the age group had gone inside and gathered around the brood. These workers are called the naive groups.

Survivors of each age group in these initial encounters with army ants were tested again the following day. Because of varying mortality in the first encounters these groups were culled to 40 workers, 40 larvae and 40 pupae, and tested against 100 army ants. In the few instances where mortality was greater than 20 workers, the extra workers from the same age group were tested with a proportionate amount of brood against army ants, and the necessary number of survivors added to the group to be tested the next day. Groups were tested a second time in order to reveal any changes that might have occurred in some aspects of their protean and defensive behavior as a result of their single experience with army ants. These workers are called the experienced groups.

Pilot studies suggested the following behaviors to be observed: running away (without brood to the escape arena); evacuating brood from the nest; carrying brood within the nest; recruitment (running to the large arena after having been alerted by incoming workers that army ants were present); approaching the army ants (to within antennal distance); attack.

For comparisons with defensive behaviors manifested by foragers taken from the field, foraging workers of _N. albisetosus_ were collected at least 2 m from the nests of three widely separated colonies in the field. (Two meters was chosen because at that distance and beyond, all workers observed were darkly pigmented and generally engaged in foraging, an indication that they were among the older, more experienced members of those colonies). In one trial, 60 foragers, 60 larvae and 60 pupae were tested against army ants; in two other trials, 50 and 40 foragers respectively were tested with proportionate amounts of brood. The latter two groups served as comparisons for the fewer number of workers tested in the experienced groups. Their experience with army ants, as well as the experience of any foragers collected in the field for these studies, was not known.

Following the same procedures outlined above, separate isolated groups were exposed to Formica spp and Pogonomyrmex occidentalis. These species are sympatric with N. albisetosus and served as controls for those defensive behaviors which might not develop specifically as a reaction to army ants.

Results

Table 1 shows that for both the experienced and naive groups, evacuation and relay of brood (piling brood closer to the exit to the escape arena), approaching and attacking army ants, and recruitment, improved as a function of age. Both also ran from army ants significantly less as they got older, but the naive groups, on average, ran more than the experienced groups. The naive groups also recruited significantly less. These latter two items may in part explain why the naive groups suffered fewer overall injuries than the experienced groups (though mortality as a result of army ant attacks was not significantly different for the two groups).

Figure 1 shows the steady increase in the percentage

Table 1

Isolated Age Groups: Correlation of Observed Behaviors With Age and Experience in Naive and Experienced Conditions

Behavior	Correlation	Dependent Sample t-tests
Evacuation		2.08*[a]
Naive	.94**	
Experienced	.89**	
Relay		ns
Naive	.55*	
Experienced	.52*	
Run		2.21*[b]
Naive	-.84**	
Experienced	-.84**	
Recruit		2.05*[c]
Naive	.75**	
Experienced	.82**	
Approach		ns
Naive	.83**	
Experienced	.93**	
Attack		ns
Naive	.88**	
Experienced	.89**	

[a] After 6 weeks, Experienced evacuates more than Naive.

[b] Naive runs more than Experienced.

[c] Experienced recruits more than Naive.

*$p < .05$

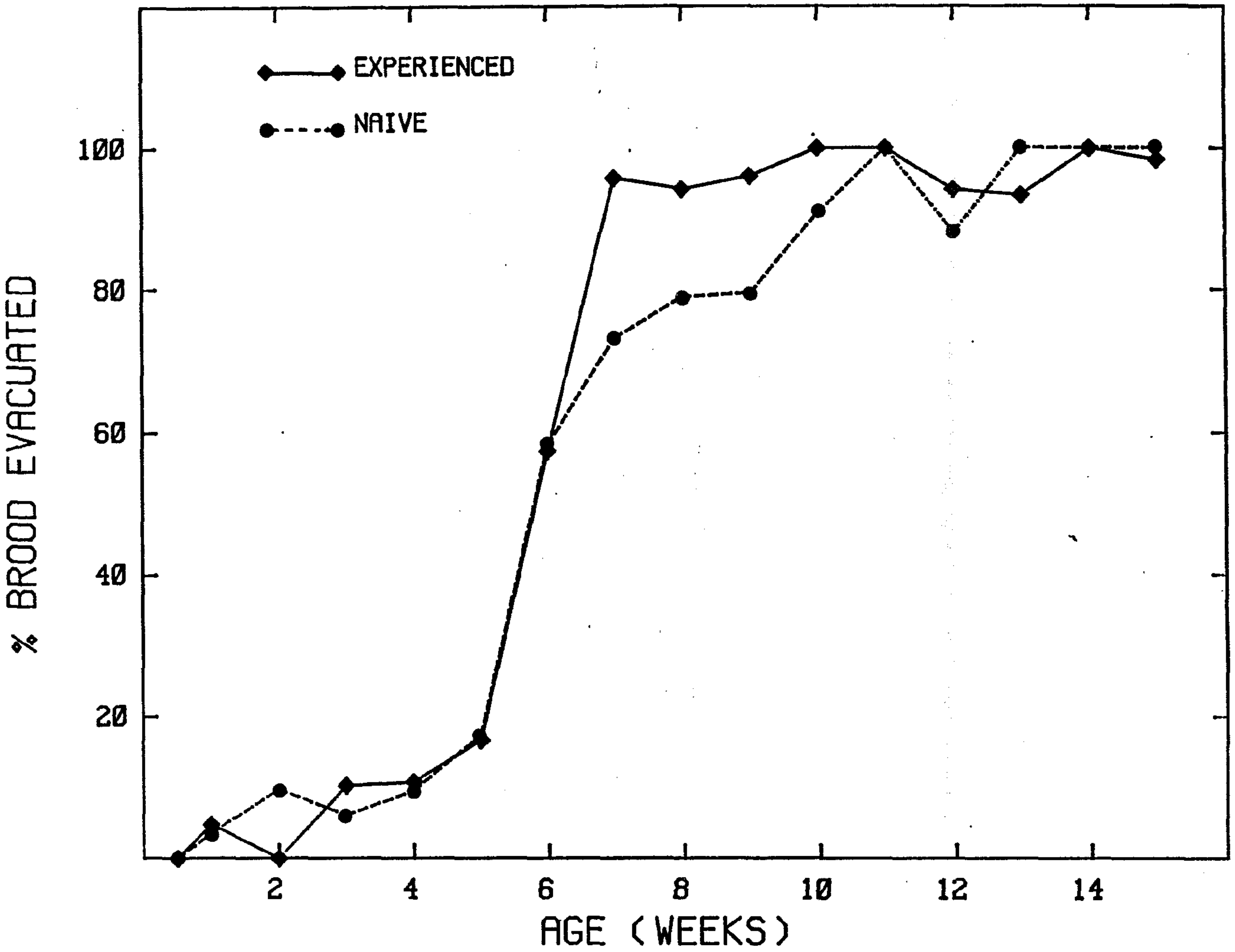
**$p < .01$

of brood removed for both groups. While both exhibit similar trends, the experienced groups removed a greater percentage overall from the 7th to the 10th week of age. As can be seen in Table 1 this was enough to make the difference significant. The three comparison groups of foragers evacuated 100% of their brood.

From the 1st week on, some of the naive groups approached their attackers and this number increased with age (experienced workers did not approach until their 2nd week). However, they did not begin to attack the army ants until their 8th week (a single incidence of attack in experienced workers was observed at 6 weeks, two at 7 weeks--too few to be of significance when compared with naives over the same period). Whether they approached or not, running empty-mandibled from the nest and away from the army ants declined as a function of age.

Recruitment to the large arena where army ants were released increased with age. Traffic was typically low in and out of the nest prior to the release of army ants in the large arena; it was also low after contact with army ants for age groups 1-5 weeks. At 6 weeks of age and after, recruitment began to be noticeable as workers ran into the nest, touching clusters of resting workers, causing both a

Figure 1. Percent of brood removed as a function of age in groups confronting army ants for the first time (naive), and for the second time (experienced).


EXPERIENCED
NAIVE
% BROOD EVACUATED
100
80
60
40
20
2
4
6
8
10
12
14
AGE (WEEKS)

general alarm marked by picking up and dropping brood or otherwise rushing about the nest, and a focused alarm characterized by running out to the large arena where the cause of the disturbance lay.

Two patterns of defense became evident from these results: 1) brood relay, brood evacuation, and flight to the escape arena; 2) recruitment, approach, and attack in the large arena. At times the same worker would help in relaying or removing brood, and attacking the army ants. This occurred most often when army ants succeeded in penetrating the nest and were attacking the brood. Evacuation-flight entailed combinations of genaral alarm within the nest, carrying of brood to the escape arena, fleeing empty-mandibled to the escape arena, and relay. Relay consisted of some workers carrying brood from the center of the nest and depositing it near the tube to the escape arena; from there it was usually taken by other workers to the escape arena. Occassionally brood was deposited yet again in the tube before being removed to the escape arena. Relayers shuttled back and forth between brood piles and escape arena.

Aggressive defense entailed recruitment to the large arena where fighting took place immediately if army ants were beginning to enter the tube to the nest. If army ants had not yet discovered the nest entrance, N. albisetosus workers would disperse into the large arena, approach the army ants, run away, or attack. Up to 8 weeks of age running was the typical response; afterwards attacks increased.

No significant correlation with age was found for the development of any of the behaviors in the control groups tested against Formica spp or P. occidentalis. In the evacuation-flight component of the protean behavior, no significant difference was found in the amount of brood relayed in the presence of the three intruder species. However, N. albisetosus ran to the escape arena and evacuated brood significantly more in the presence of army ants F = 37.71, $p < .01$ and F = 22.49, $p < .01$, respectively) than in the presence of the other two species.

In the aggressive defense complex of behaviors, differences became apparent after 8 weeks of age. Up through 8 weeks the naive groups approached and attacked the three intruder species equally. From 9-15 weeks, however, they approached and attacked army ants significantly more (F = 13.79, $p < .01$ and F = 8.78, $p < .01$, respectively) than they did the other two species. Though up through 8 weeks of age N. albisetosus recruited to P. occidentalis more than to Formica or army ants (F = 4.92, $p < .05$), from

9-15 weeks no significant difference was found. Thus after 8 weeks of age they recruited equally to all three species, but approached and attacked army ants more.

Section 2 - Laboratory Colony

In contrast with Section 1, which dealt with isolated age groups having no more than two experiences with army ants, this part of the study was designed to determine the effect of repeated exposures to army ants on the development of protean behavior in several age groups together; and to see how an intact colony might react within the nest when threatened by army ants.

Methods

Subjects

A large colony of *N. albisetosus* was collected in the field and housed in the laboratory. The first three clusters of eclosion that provided at least 50 callows each were used as experimental groups which differed in age. The colony was then constituted as outlined below.

Procedure

In order to simulate as closely as possible a small natural colony, while still being able to observe various age groups, a colony of *N. albisetosus* consisting of 50 marked 2-day, 14-day, and 28-day olds, along with 50 marked foraging workers collected at least 2 m from the nest entrance in the field, and 100 unmarked workers collected at various distances from the field nest, were tested together with the queen, 250 larvae and 250 pupae. They were exposed to army ants and to two other species on the following repeating schedule: day 1, about 500 army ants; day 2, alternated between 300 Pogonomyrmex or 300 Formica spp; day 3, rest. This cycle was repeated nine times over 27 days. Pogonomyrmex and Formica were chosen to compare with defensive behavior toward nonpredatory ants that are sympatric with N. albisetosus.

All intruder species were dropped in the end of the large arena opposite to the nest entrance. Unmarked workers (chosen as a buffer for the marked groups against decimation by repeated army ant attacks) killed during this experiment were replaced after each test; callows eclosing from the pupal brood were allowed to remain in the nest. Filter paper was replaced in the arena after each trial.

Results

The defensive part of the protean reaction (recruit, approach, attack) did not change significantly as a result of age or experience for any of the age groups. Table 2 shows that more noticeable change was taking place in the development of the evacuation-flight part of the protean reaction: as the age groups ran less, they relayed and evacuated brood from the nest more. The marked foraging workers (those taken at least 2 m from the field nest) exhibited no change in any of these behaviors as a function of repeated experience with army ants.

In all behaviors with the exception of running away, however, the foraging workers outperformed the other age groups. The marked foragers removed more brood ($F = 212.8$, $p < .01$), relayed more ($F = 46.1$, $p < .01$), and attacked more ($F = 209.6$, $p < .001$) than the other three age groups, which did not differ significantly in these behaviors. They also ran away less, recruited and approached more than the other groups, and the 28-day olds ran away less, recruited and approached more than the 2-day and 14-day olds ($F = 34.4$, 303.8, 41.5, $p < .001$ respectively), which did not differ significantly. These results are summarized in Table 2.

Since both marked and unmarked foragers did the bulk of the fighting, the other age groups got little opportunity to aggressively defend the nest. Those beginning as 2- and 14-day olds were still spending much of their time on the brood by the end of the study, 24 days after their first

Table 2

Laboratory Colony: Development of Observed Behaviors as a Result of Experience With Army Ants.

Behavior	2-day[a]	14-day[b]	28-day[c]	fw[d]
Evacuation	.72*	.79**	.88**	ns
Relay	.85**	.91**	.89**	ns
Run	-.78*	-.88**	-.93**	ns
Recruit	ns	ns	ns	ns
Approach	ns	ns	ns	ns
Attack	ns	ns	ns	ns

ANOVA[e]

Behavior	F	Comment
Evacuation	212.8**	fw remove more brood than other 3 age groups (which don't differ).
Relay	46.1**	fw relay more than other groups (which don't significantly differ).
Run	34.4**	fw run less than others; 28-day olds less than 2- & 14-day olds.
Recruit	303.8**	fw recruit more than others; 28-day olds more than 2- & 14-day old.
Approach	41.5**	fw approach more than others; 28-day olds more than 2- & 14-day old.
Attack	209.6**	fw attack more than others (which don't significantly differ).

[a,b,c] Those beginning as 2-day, 14-day, and 28-day olds, respectively.

[d] Foraging workers.

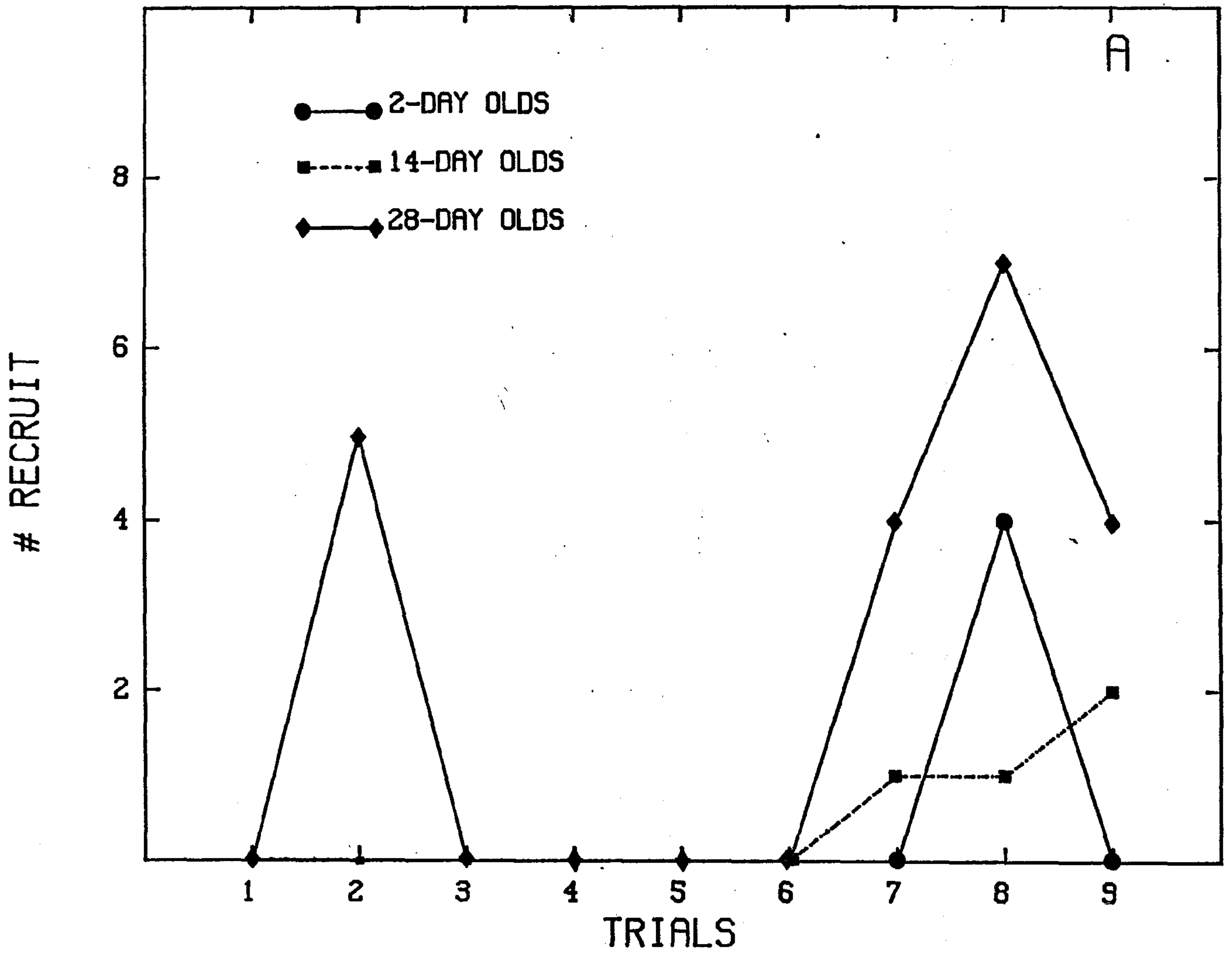
[e] Post hoc comparisons by Newman-Keuls test.

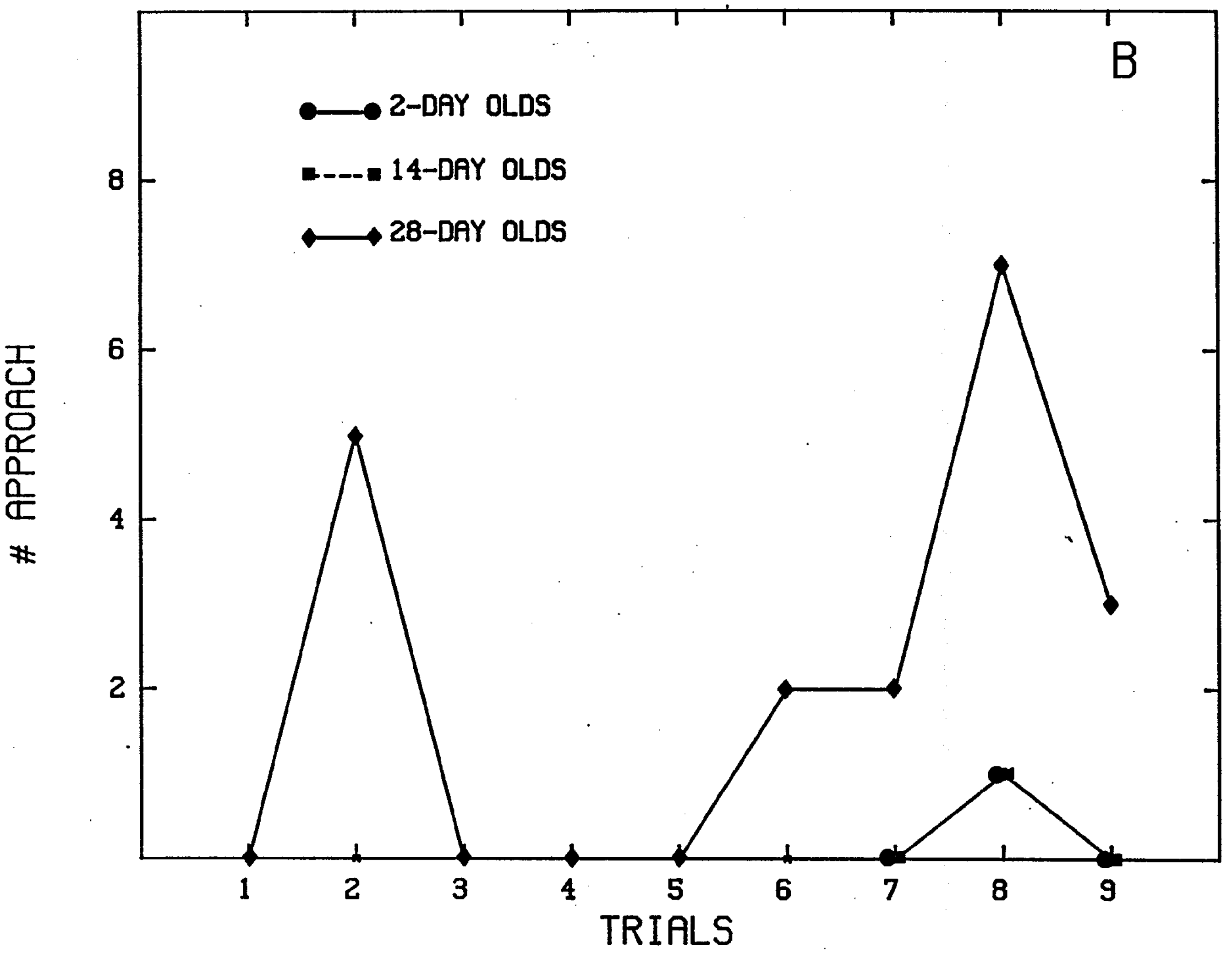
*$p < .05$ **$p < .01$

exposure to army ants. They were then 26 and 38 days old respectively. Those starting as 28-day olds, however, were 52 days old at the end, and were spending less time with the brood. This may explain in part their greater availability for recruitment to and approaching the army ants. Figure 2A-2C shows how few responses there were in any of the defensive cluster of behaviors, but that they were beginning to become more apparent as the marked groups aged and gained experience with army ants.

Though no significant difference was found among the three age groups in the amount of brood evacuated, it can be seen in Figure 3 (B and C) that those callows beginning as 2-day olds showed the most dramatic increase in performance, equalling the older two age groups by the end of the experiment. Running away (Figure 3A) decreased with age and experience. During the first few exposures to army ants young marked workers running around with brood got caught up in the focused rush and nest evacuation of the older brood-carrying workers, and had their own typically disorderly carrying tactics directed toward the exit to the escape arena. Even given their best brood evacuation performance during the later trials, however, the three age groups removed no more than 50 brood and relayed 52 more.

Figure 2. Development of aggressive defense--recruit (A), approach (B), attack (C)--in three laboratory age groups having repeated experience with army ants. (Note: Trials were performed every 3rd day. Ages given are for the first trial.)


A
2-DAY OLDS
14-DAY OLDS
28-DAY OLDS
RECRUIT
2
4
6
8
TRIALS
1
2
3
4
5
6
7
8
9


B
2-DAY OLDS
14-DAY OLDS
28-DAY OLDS
APPROACH
8
6
4
2
1
2
3
4
5
6
7
8
9
TRIALS

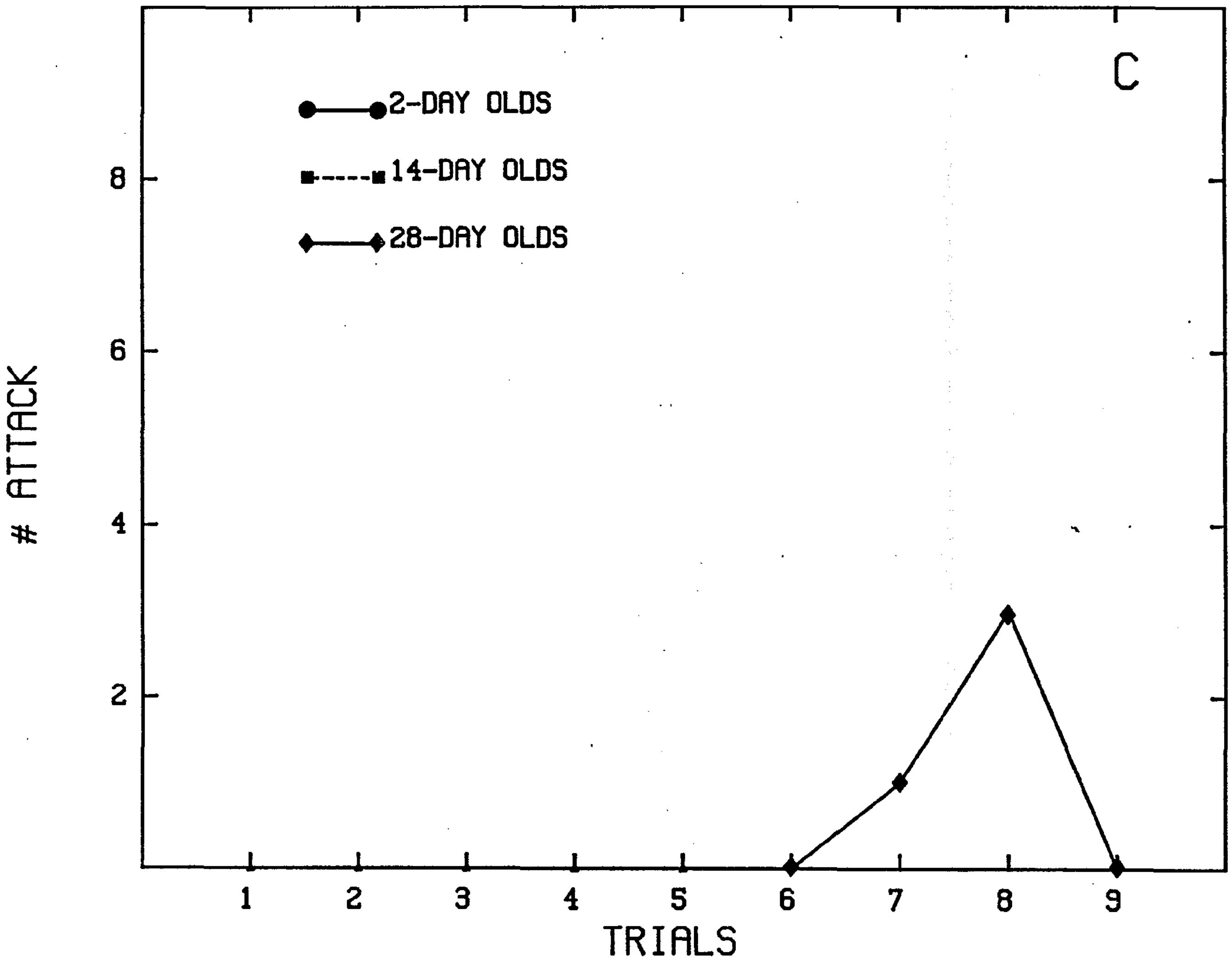
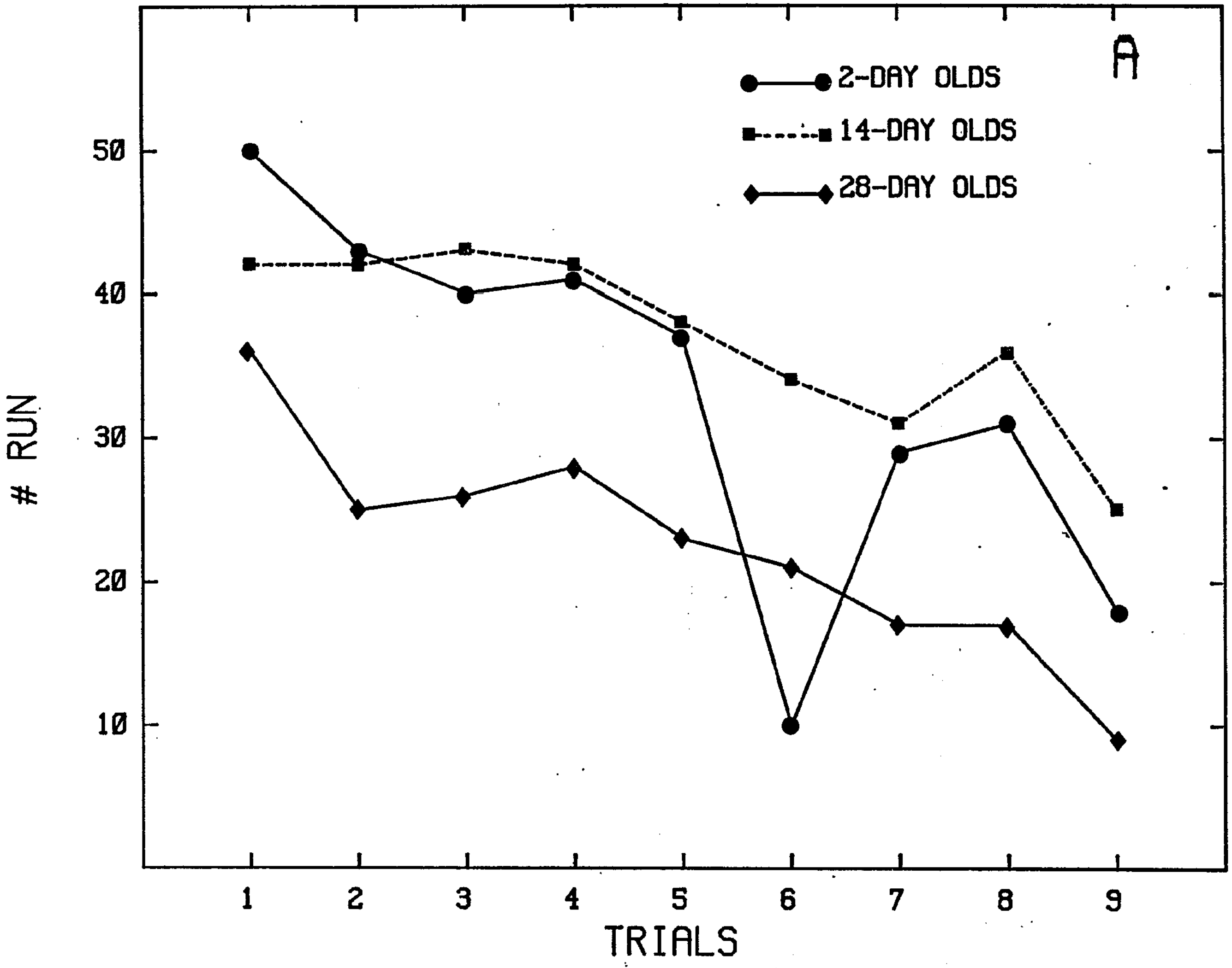
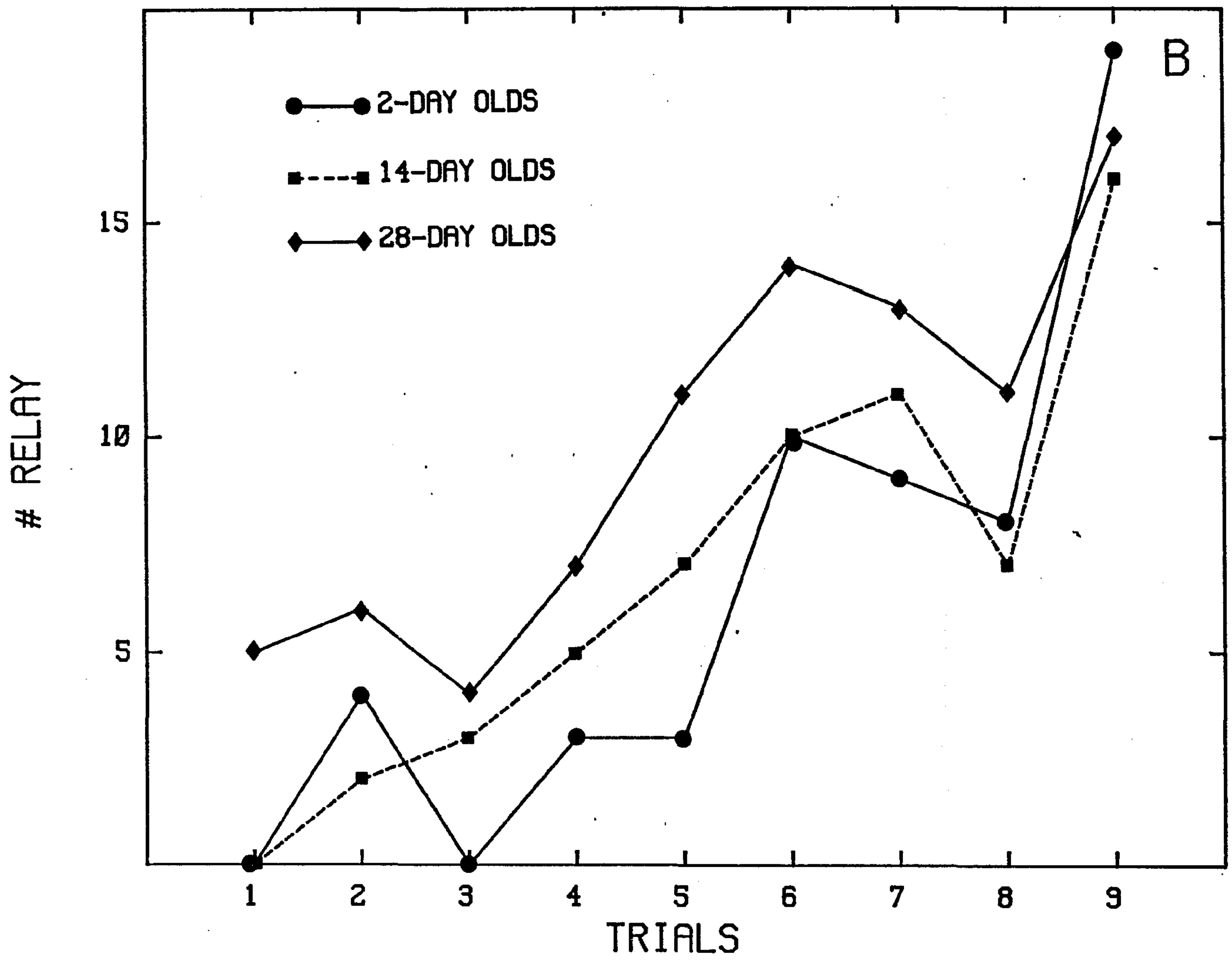
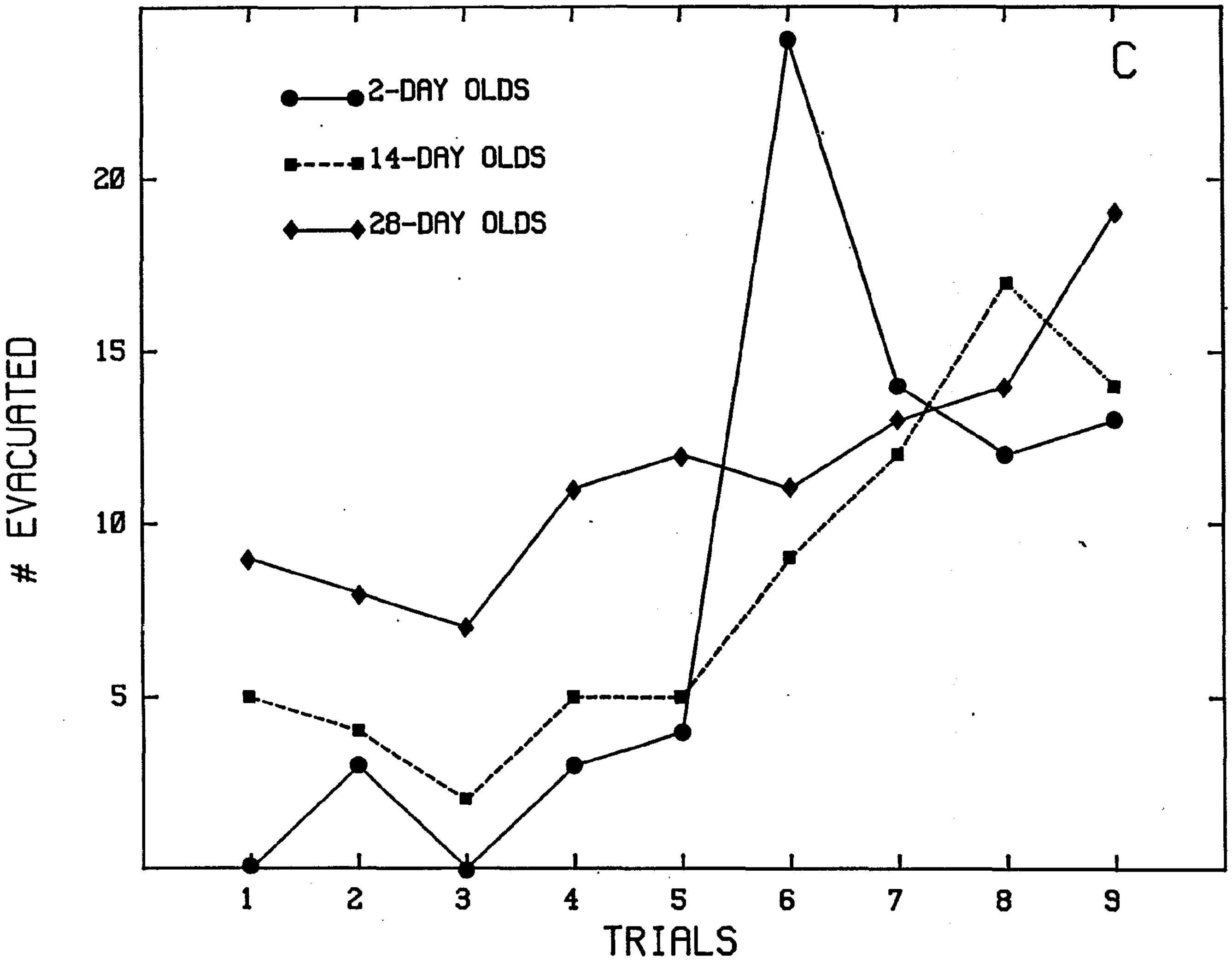

C
2-DAY OLDS
14-DAY OLDS
28-DAY OLDS
ATTACK
8
6
4
2
1
2
3
4
5
6
7
8
9
TRIALS

Figure 3. Development of the escape component of protean behavior--run(A), relay (B), evacuate (C)--in three laboratory age groups having repeated experience with army ants. (Note: Trials were performed every 3rd day. Ages given are for the first trial.)


A
2-DAY OLDS
14-DAY OLDS
28-DAY OLDS
RUN
50
40
30
20
10
1
2
3
4
5
6
7
8
9
TRIALS


B
2-DAY OLDS
14-DAY OLDS
28-DAY OLDS
RELAY
15
10
5
1
2
3
4
5
6
7
8
9
TRIALS


C
2-DAY OLDS
14-DAY OLDS
28-DAY OLDS
EVACUATED
20
15
10
5
1
2
3
4
5
6
7
8
9
TRIALS

Though the number of brood had been reduced by eclosion, this still represents less than 25% of the brood being handled by about 40% of the workers (the three age groups initially comprised 50% of the workers, but some died or were killed by army ants during the course of the experiment). The marked and unmarked foragers handled the bulk of the fighting and brood removal.

The entire colony became more efficient in removing brood from harm: There was a significant decrease in time to complete evacuation as a function of repeated exposures to army ants ($r = -.75$, $p < .05$). While initial contacts with army ants produced much fighting followed by brood removal, later trials saw workers removing brood as soon as recruitment to the large arena occurred. Evacuation then proceeded steadily until all brood was removed to the escape arena.

Over the 9 trials a fighting style emerged. Pogonomyrmex spp were contested with antennae-waving, open-mandibled displays, slow rushes and stand-offs. When attacking, N. albisetosus grasped the pedicel of a Pogonomyrmex from behind, thereby avoiding its sting. Fighting rarely occurred, however, and the two species would separate to opposite sides of the arena. No brood carrying and little if any alarm within the nest occurred during these episodes. *Formica* spp is a faster, more energetic ant than *Pogonomyrmex*: Though *N. albisetosus* confronted *Formica* with the aforementioned displays and stand-offs, it rushed this intruder more vigorously and attempted frontal assaults distinguished by grasping at the head, and neck. This tactic prevented *Formica* from squirting formic acid into a mandible-inflicted wound. Even so, combat tended to be more severe than with *Pogonomyrmex*, leading to dead on both sides. When *Formica* were introduced into the large arena, there would be some alarm along with scattered brood carrying and relaying within the nest, and recruitment to the large arena. When attacking an army ant, *N. albisetosus* leaned forward as if to keep its legs out of the way (a typical tactic of *Neivamyrmex nigrescens* is to have several of them grasp the legs and antennae of a prey ant and spread-eagle it into submission); it then lunged its head down while snapping the mandibles shut and scraping backwards, while at times taking quick steps in reverse, again as if to keep its legs free.

Section 3 - Field Colony

The following was designed to observe the effects that a natural environment would have on the development of protean behavior by N. albisetosus with repeated exposures to army ants.

Methods

Subjects

A colony of N. albisetosus was brought into the laboratory to be returned to the field with the age group proportions outlined below.

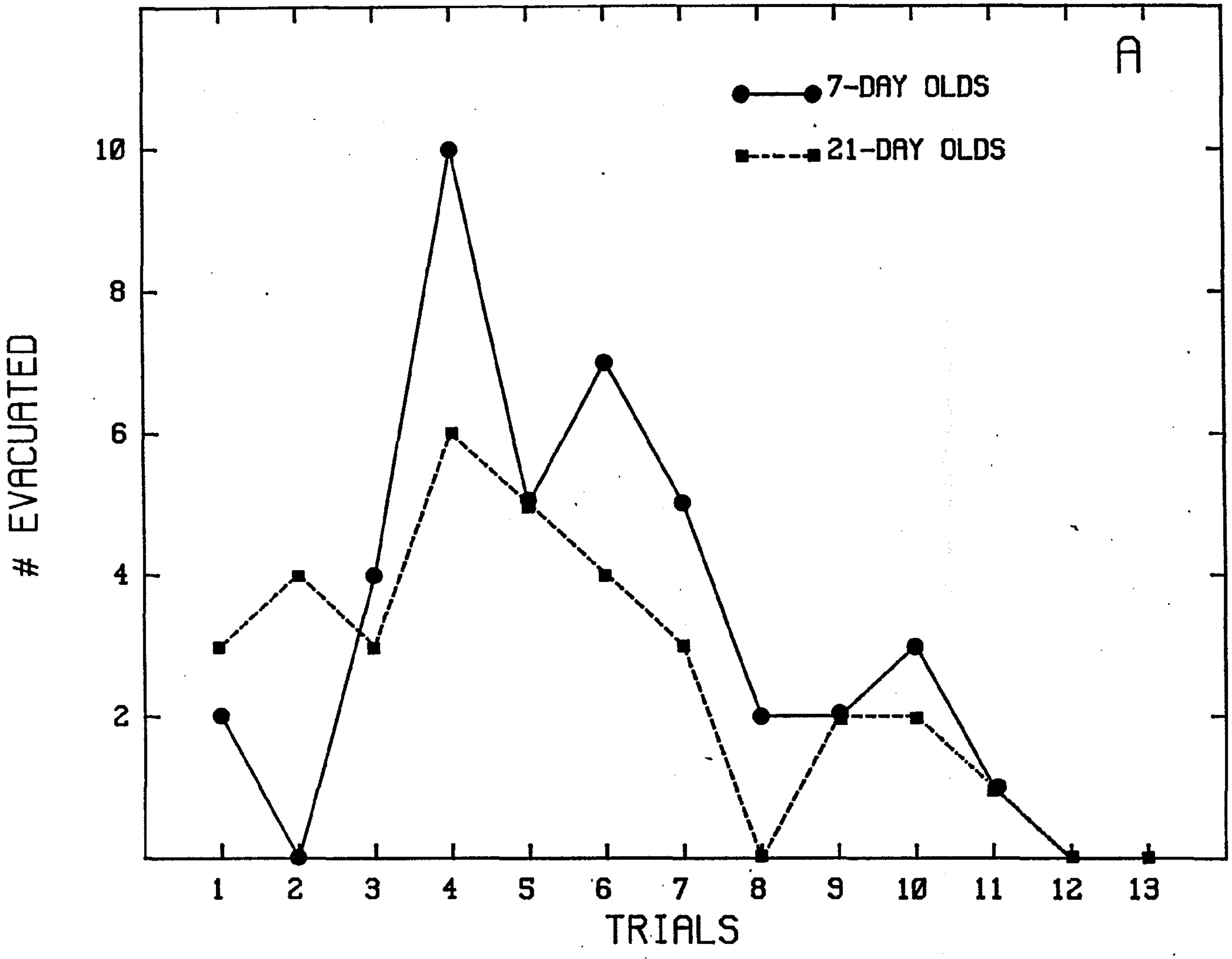
Procedures

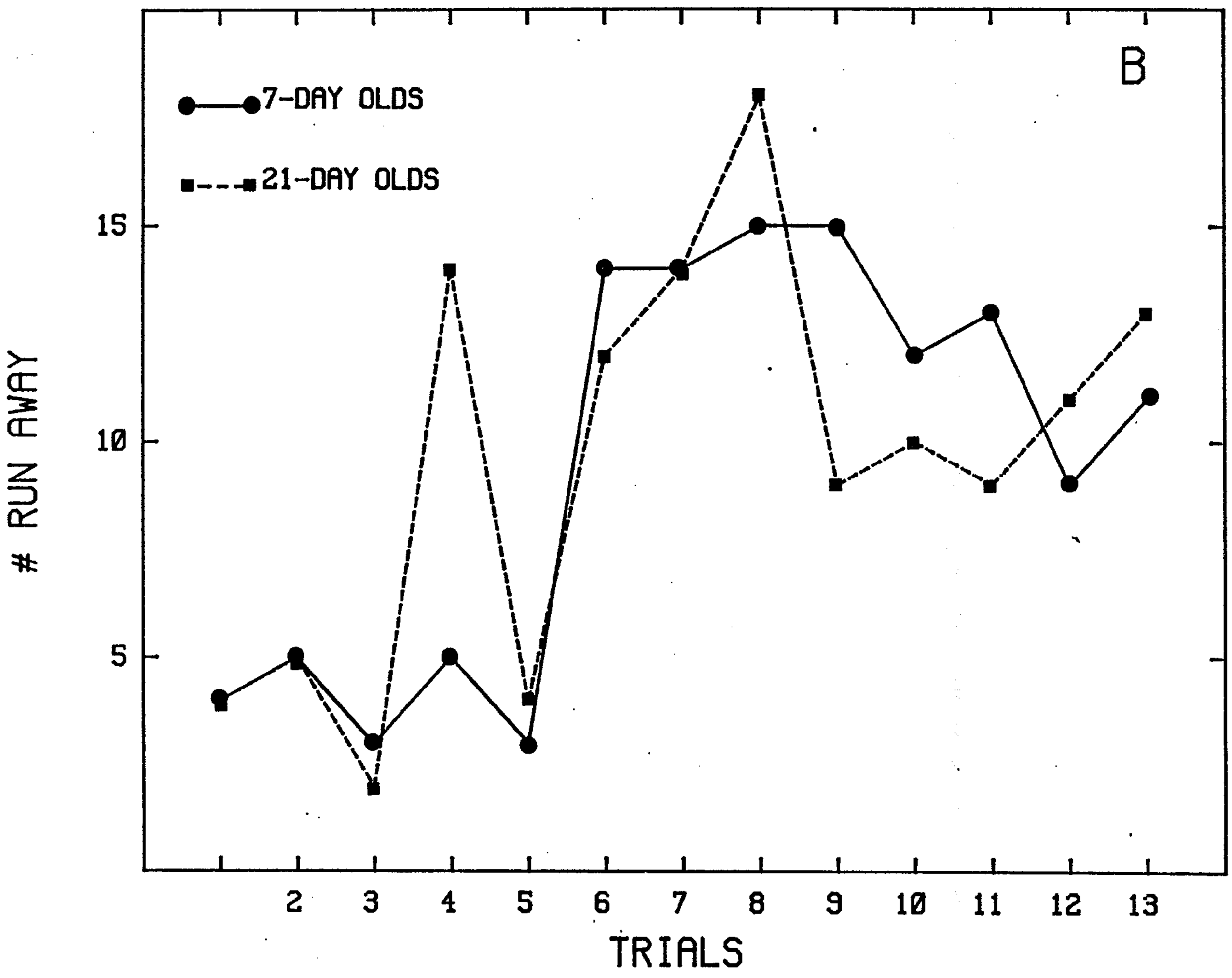
Two age groups were assembled and marked from this colony: 50 3-day olds and 50 17-day olds. Fifty foraging workers collected 2 m or more from the nest were also marked. These were returned to their field nest with the queen, 250 larvae, 250 pupae, and 150 workers taken from outside the nest. The colony was returned to the field nest by placing them in a wide-mouthed jar which was upended and left standing over the nest hole in early morning. Those not entering the hole immediatley, did so as the sun raised the temperature within the jar. Four nights later, when the age groups were then 7 days and 21 days old, the trials began. On that night and every 3rd night thereafter for 36 days, 1,000 army ants in groups of 200 were dropped on and around the nest entrance (more army ants were used per trial than in the previous section because some ran away from the nest entrance, and the rest had much greater distances to cover underground through various tunnels and chambers than they would in the laboratory). Because it was not possible to see inside the nest and because of the dim light of our headlamps, only 3 behaviors were recorded: brood evacuation, run, and attack. Since it was not possible to obtain an accurate count of the dead, foragers were not replaced after each trial; callows eclosing during the experiment were not removed.

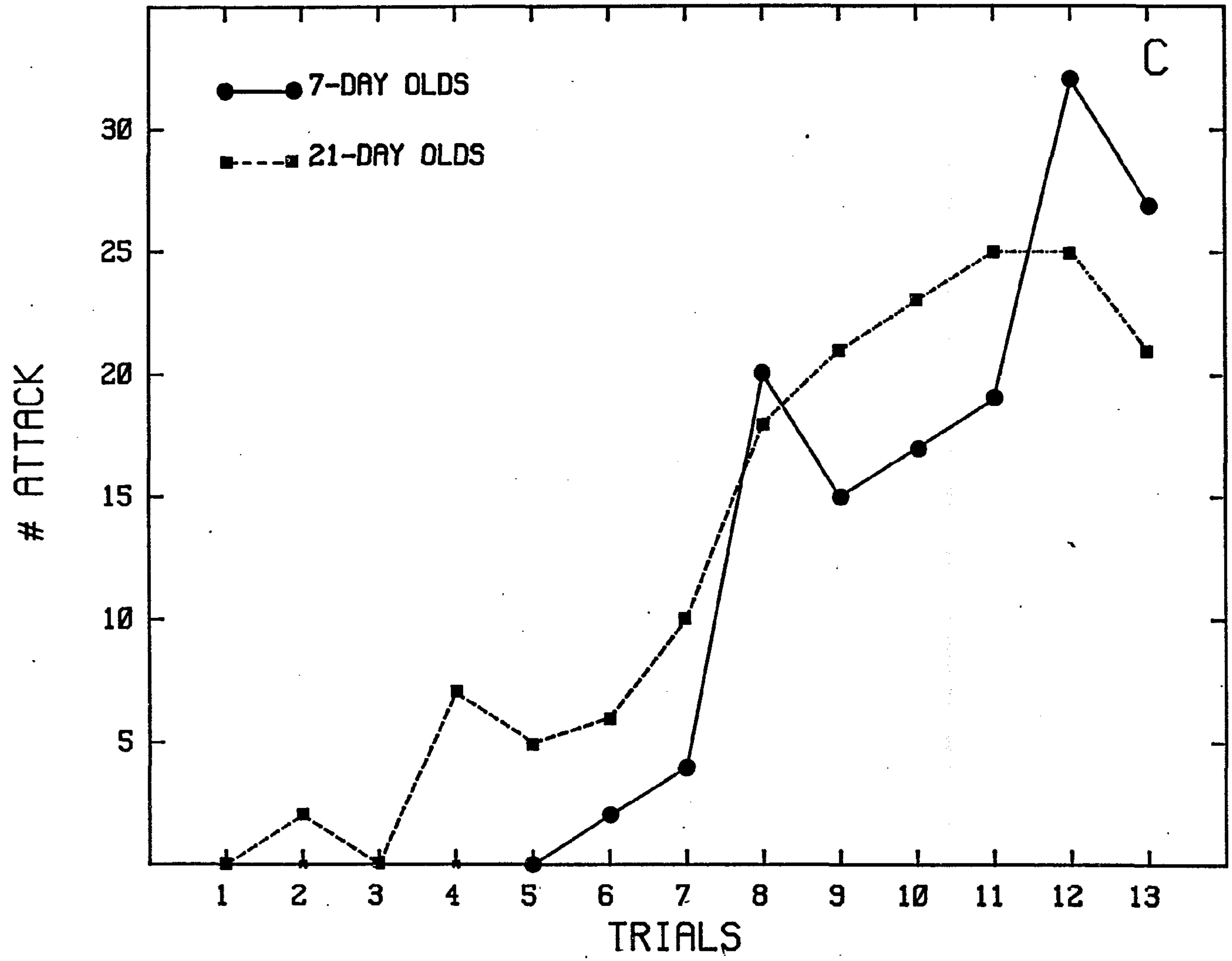
Results

Figure 4A shows that, unlike the isolates in Section 1 or the laboratory colony in Section 2, brood evacuation diminished as a function of age or experience with army ants ($r = -.7$, $p < .01$ for the 21-day olds; a negative but not significant difference for the 7-day olds). Neither removed significantly more brood than the other, but the marked foraging workers removed significantly more than both ($F = 19.44$, $p < .01$). Also unlike the experimental groups in the first two sections of this study, running away increased (Figure 4B) as a function of age or experience for the 7-day olds ($r = .7$, $p < .01$; it was not significant for the 21-day olds). The two age groups did not differ in this behavior from one another, but both ran away significantly more than the foragers ($F = 4.84$, $p < .01$). Figure 4C shows that both 7- and 21-day olds increased their attacks over the course of the trials ($r = .89$ and $.94$, $p < .01$ respectively) just as the groups did in the first two sections of the study. However, the field colony groups began attacking at a much earlier age than the laboratory groups, and they attacked much more frequently than the groups in the laboratory colony: While those beginning as 7- and 21-day olds in the field did not significantly differ in number of attacks from one another or from the foraging workers in the field and laboratory, all differed from 2-, 14-, and 28-day olds in the laboratory ($F = 6.21$, $p < .01$). Similarly, the 7- and 21-day olds did not differ in the number of those running away, but both ran significantly less than the groups in the laboratory colony ($F = 32.38$, $p < .001$). All age groups in the laboratory and field ran significantly more than the foraging workers from either colony. The 7- and 21-day olds did not significantly differ in amount of brood evacuated, but they evacuated significantly less than the laboratory colony age groups ($F = 7.1$, $p < .01$). Laboratory foragers evacuated more than foragers from the field colony, and both evacuated more than all the other age groups.

Figure 4. Development of three protean behaviors--evacuate (A), run (B), attack (C)--in two field-colony age groups having repeated experience with army ants. (Note: Trials were performed every 3rd day. Ages given are for the first trial.)


A
7-DAY OLDS
21-DAY OLDS
EVACUATED
2
4
6
8
10
TRIALS
1
2
3
4
5
6
7
8
9
10
11
12
13


B
7-DAY OLDS
21-DAY OLDS
RUN AWAY
15
10
5
2
3
4
5
6
7
8
9
10
11
12
13
TRIALS

C
7-DAY OLDS
21-DAY OLDS
ATTACK
30
25
20
15
10
5
1
2
3
4
5
6
7
8
9
10
11
12
13
TRIALS

Section 4 - Graded Introduction

The following was done to ascertain the preferred method of defense by colonies with which I had not tampered.

Methods

Subjects

Fifteen colonies of *N. albisetosus* were located and studied in the field.

Procedures

Beginning at 8 pm when foraging activity was high, army ants were introduced near each colony accordingly: 30 were placed 1 m from the nest entrance; after 1 hr, 30 were placed 30 cm from the entrance; an hour later 30 more were placed in the nest entrance. After each introduction of army ants, the number of brood evacuated was counted as each was carried from the nest. One hour after placing 30 army ants into the nest entrance, large numbers of army ants were repeatedly poured into the entrance until all the brood had been evacuated. The total number of brood from each colony was counted. All counts were taken by hand counter.

Results

N. albisetosus evacuated significantly more brood when 30 army ants were placed in their nest entrances than when placed at 1 m or 30 cm from the entrances ($F = 16.79$, $p < .01$). There was no significant differences in amount evacuated for the latter two distances. No brood was evacuated from any of the colonies when the army ants were placed 1 m from the nest entrances. The amount of brood removed when 30 army ants were placed directly into the entrances represented only about 10% of the total brood over all 15 colonies.

N. albisetosus recruited quickly to army ants at 1 m and 30 cm from the nests, and many workers surged out of the nests to fight when 30 army ants were dropped into the entrances. Whatever brood was evacuated during these skirmishes was usually returned within 10 minutes. After total evacuation when large numbers of army ants were dropped into the nest entrances, the bulk of the brood generally was not returned for two or more hours.

The preferred method of defense was to fight off the army ants even when some had gained entrance to the nest. Only when defenses had been heavily breached did _N. albisetosus_ remove all brood.

Discussion

The species typical complex of behavior in _N. albisetosus_ suitable for protecting its nest against the encroachments of its natural predator, _Neivamyrmex nigrescens_, emerges without _N. albisetosus_ having had prior experience with this army ant. That the responses were of a specific nature and of greater intensity toward army ants than toward the other species, renders doubtful possible order or stress effects that might be inherent in the type of experiments performed.

Escape and brood evacuation evidently are responses specifically used against army ants. These behaviors are exhibited when the workers are still callows. Clearly they can distinguish between the predator species and other species at an early age. However, they do not approach and attack the predators in any significant numbers until they reach the age when they would normally develop into foraging workers, that is, at about 7-9 weeks of age (McDonald and Topoff, 1985).

Across all groups in the first three sections of this study, the escape response cluster--run, relay, evacuate--appeared and developed earlier than the aggressive-defense response cluster--recruit, approach, attack. In both laboratory studies, attacks against army ants occurred at about the same age: 6-8 weeks for the groups raised in isolation and 6.5 weeks for the laboratory colony. This is interesting in light of the fact that the isolates did not have older age groups acting as a first line of defense, as did the laboratory colony.

At first it seems that young workers are not prepared to fight until 6-8 weeks of age. Yet the different circumstances of the field colony, shows that young workers will fight at least as early as 3 weeks of age. Otto (1958) and Weir (1958) have found temporal differences in the readiness of _Formica_ and _Myrmica_, respectively, to perform certain behaviors rather than in their ability. Similarly Topoff et al. (1972 a and b) have found that army ant callows are able to follow scent trails but do not do so readily. Evidently the same holds true for the attack response in _N. albisetosus_, even though the callows are softer and more susceptible to injury at 3 weeks of age. The difference in attacks and evacuation between the groups in Sections 2 and 3 may be an artifact of the laboratory nest apparatus with its two exits, one to the escape arena and one to the large arena. Though it had been thought that _N. albisetosus_ had nests with multiple entrances (Whitford, et al., 1980), I was not able to find such a nest in the field. Where there were entrance clusters, each entrance proved to be a single nest occupied by or available to workers from the same colony. I found no evidence of underground connections among these nests. Consequently, the two age groups in the field colony had to evacuate brood or otherwise escape through the incoming army ants and out of the single entrance. This may have increased the number of attacks and diminished the amount of brood evacuated. The three age groups in the laboratory colony were buffered from contact with army ants not only by the older workers' defense, but also by the escape arena exit where they could flee the nest without touching an army ant.

As the 28-day olds in the laboratory colony aged, they spent less time with the brood and remained off by themselves. It has been noted that as some ants age, they spend less time with the brood and go through a transition phase where they do little work, but are available for tasks both inside and outside the nest (Lenoir, 1979). When the 28-day olds had reached this stage, they may have become available to fight army ants. Certainly they attacked significantly more than the younger two age groups.

The peculiar fighting styles toward the intruder species was limited to older foragers of unknown age. Since systematic styles were not observed among the workers raised in isolation in Section 1, experience with other species may be an important factor in the development of fighting styles. Since I was not studying this behavior, I report these observations only to suggest a possible fruitful line of study.

Given that the appearance of attacks began at approximately the same age for the laboratory (7 weeks) and field (3-4 weeks) groups within their respective colonies, it would seem that age rather than experience is the more important factor governing attack. However, Figure 3C (attack) along with the lack of significance in the number of attacks between the 7- and 21-day olds in the field colony, shows that the 7- day olds had caught up with the 21-day olds in exhibiting the attack response by the end of the experiment.

A similar phenomenon occurs with the evacuation response in the laboratory colony. There the 2-day olds, which evacuated little or no brood over the first three trials, eventually equalled the 14- and 28-day olds in the number of brood removed. They removed so much eventually that, by the end of the study, there was no significant difference among the three groups. The same holds true for the relay component of brood evacuation.

The results of Section 4 indicate that fighting is the preferred method of defense, with brood evacuation increasing as a result of defensive breakdown marked by greater numbers of army ants gaining access to the nest. Results in Section 2, however, demonstrate that this process can be altered by repeated experience with army ants. Brood removal began as soon as army ants were detected in the large arena, before fighting began. No such alteration in their behavior toward <u>Formica</u> or <u>P. occidentalis</u> was observed.

Experience, then, may play a part in the development of relay, evacuation, and attack. If it does, its effects may be limited to a certain age range. It would be difficult to explain, otherwise, the fact that the older age groups did not progress at an equal rate in these three behaviors and thereby stay ahead of the younger age groups, so that a significant difference would be found among them (It must be remembered that all groups improved in the three behaviors over the course of the trials). Of course, it may be argued that rather than experience having its greatest effect within a certain age range, some behaviors may simply develop faster within a certain age range because of maturational processes intrinsic to the young workers. Figure 1 seems to suggest this for the naive groups' evacuation response between the 4th and 10th week of age. That the experienced group developed significantly more in this behavior between the 6th and 10th weeks, however, would argue instead for an inextricable union of the age and experience factors.

Finally, it seems that after a while in the life of a worker neither age not experience will have much effect on protean behavior: For all the behaviors studied, the older foraging workers did not change significantly, negatively or positively, though they typically outperformed the younger age groups. The single exception was the attack response in the field colony--there was no significance among those groups. In that case, it may have been that the older foragers were away foraging during the trials. Nevertheless, their number of attacks did not change significantly over the course of the trials.

Part III

Introduction

I have outlined the development of N. albisetosus from eclosion, through domestic tasks within the nest, to foraging outside the nest in Part I of the dissertation. A study of the development of defense in the same species makes up Part II. The present study focuses on the development of the ovaries, poison gland, and exoskeleton and their relationship with one another. These structures are easy to get at and have already been implicated in certain stages of behavioral development.

Otto (1958) found that the ovaries of young Formica polyctena workers contain eggs, but toward the end of their domestic period (Innendienst, that early part of their lives when they spend most of their time within the nest) resorption of the eggs begin. By the onset of their outside worker period (Aussendienst) the resorption of eggs is complete.

In a review of Otto's work, Wilson (1971) noted that, apart from the state of the ovaries, glandular changes in Formica polyctena are not closely correlated with tasks performed or with the change from domestic to outside worker, as they are for example in honey bees. However, there are some interesting findings which indicate that glandular production in some ants may be correlated with temporal polyethism. Cammaerts-Tricot (1974) and Cammaerts-Tricot and Verhaege (1974) have shown with Myrmica rubra that the poison gland, Dufour's gland, and mandibular gland reservoirs fill with exocrine secretions as they age. The callow's poison gland reservoir is devoid of trail pheromone. Further, they found that young workers are less sensitive than older workers to secretions from the poison and Dufour's glands. This may be why callows of many species do not engage much in foraging. However, Topoff et al. (1972 a and b) have shown that callow army ant workers are able to follow scent trails but do not do so readily. In this case, motivation as well as sensitivity may affect the behavior.

In N. albisetosus, the poison gland is the source of the alarm and trail pheromone (Hölldobler, Stanton, and Markel, 1978). Not surprisingly, callows seem to be less sensitive to it than are older workers, but it is not known how readily older workers will follow scent trails made from the poison glands of younger workers, or if they are able to do so. Further, it is not known at what age a worker has to be before older, foraging workers will readily follow its trail in the same numbers as if it were one of their own trails.

Concomitant with developing ovaries and poison glands are increasing pigmentation and hardening of the exoskeleta. Weir (1958) showed that, as an ant aged and became more darkly pigmented, it would more readily attack and intruder. Within a given species, the exoskeleta of darkly pigmented ants are harder than the lighter colored callows. It is not known if there is a correlation between the ultimate hardness of an ant's exoskeleton and a particular stage of behavioral development, such as aggressive defense,

Exoskeleton Pressure Resistance

Methods

Apparatus

A Semmes-Weinstein Pressure Aesthesiometer was used. The set consists of a series of nylon bristles of various diameters, each fastened in a plastic handle. It is based on a system developed by Von Frey (the Von Frey hairs) and designed for the same purpose: psychophysical tests of touch sensitivity. Each hair bends at a known force, measured in grams. In the present study, however, they were used to test the relative hardness of the exoskeleton. Also used were a stereotaxic device and a dissecting microscope.

Procedure

Ants were marked as they eclosed and dates were recorded so that ten each from the following age groups could be studied: 1 day, 4 days, and each week for 1-15 weeks. Ten workers of at least 6 months were also tested. At the appropriate ages the ants were pinned in a dish of hardened wax. The nylon bristles were clamped to the stereotaxic device and inserted gently under the microscope onto the ant's thorax. Three thoracic locations were selected: directly on top of the pronotum and avoiding the suture with the mesonotum; the side of the pronotum; the side of the metanotum and avoiding the suture with the epinotum. If the nylon bristle bent, then the next stiffest bristle was used, and so on until the exoskeleton directly under the hair was observed to give slightly. The number of that bristle was then recorded and used as an indicator of the exoskeleton's relative hardness. The dissecting microscope was set at 20 power.

Results

The difference in the thorax's resistance to pressure as the workers aged proved significant for the three selected areas (pronotum top: $F = 235.27$; pronotum side: $F = 145.86$; metanotum: $F = 164.74$, all at $p < .0001$, Newman-

Keuls post hoc test used). Figure 1 shows that the hardening of the exoskeleton progresses steadily up to the 12th week of age, after which it reaches an asymptote. However, for the side of the pronotum and the metanotum there was no significant difference in hardening from about the 9th week on; and for the top of the pronotum, from the 12th week on.

The general trend for the overall hardness of the exoskeleton is that rapid significant increases in hardness are taking place from the 1st day through the 4th or 5th week. From the 5th through the 8th week changes are slower and not significant; but the exoskeleton hardness of this group is significantly different from the group represented by the 9th through the 15th week and beyond.

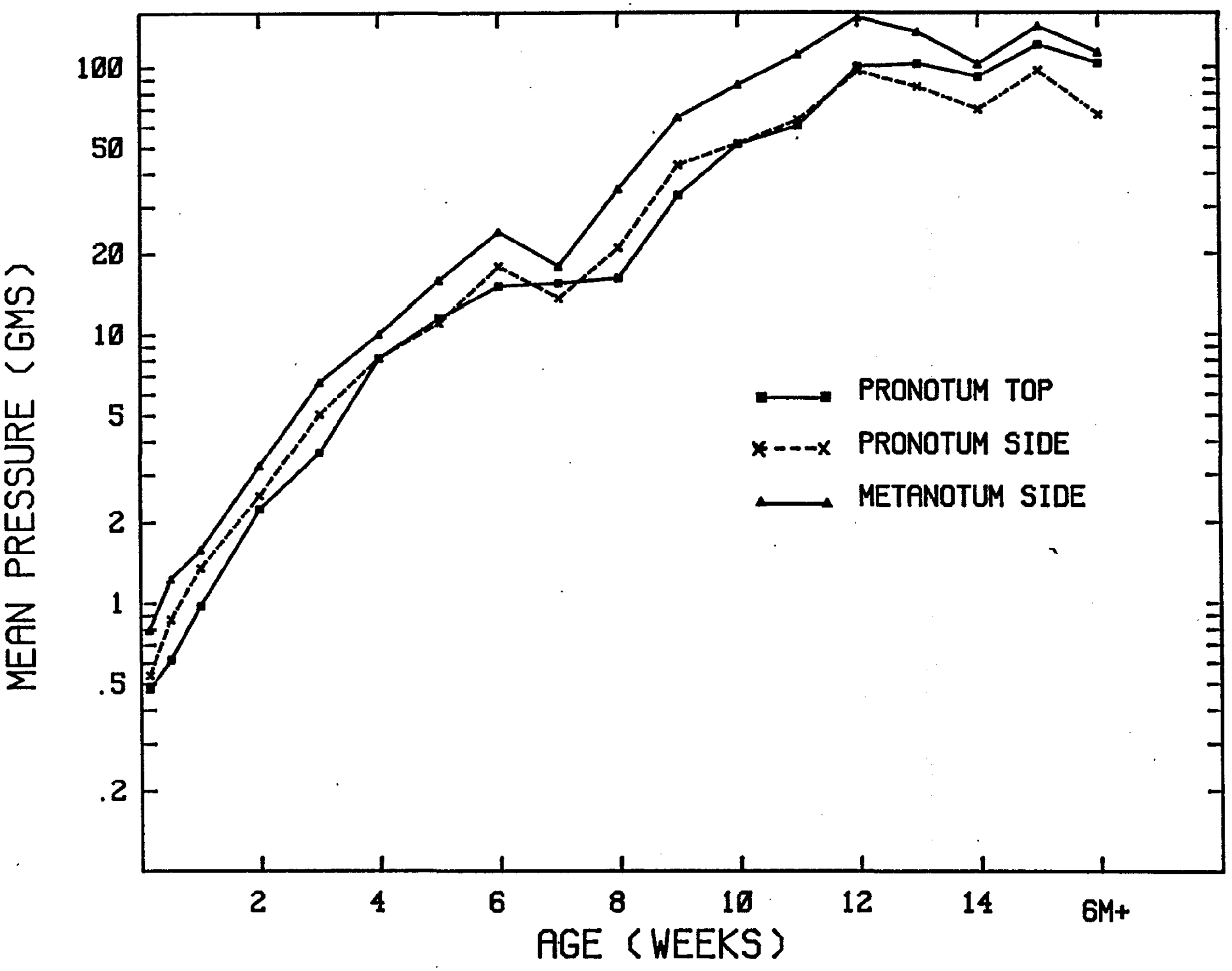
Development of Ovaries

Method

Procedure

Ten ants from each of the following groups were dissected: 1 day old, 4 days old, each week from 1-12 weeks, and outside workers of greater than 5 months. Ovaries were excised while the gaster was under a drop of water so that they would float free and not collapse upon touching the hard surface of the tweezers or the small wax filled dish in which the worker was dissected. The number of ovarioles

Figure 1. Exoskeleton resistance to pressure (measure of relative hardness) as a function of age. The ordinate is logarithmic.


MEAN PRESSURE (GMS)
100
50
20
10
5
2
1
.5
.2
AGE (WEEKS)
2
4
6
8
10
12
14
6M+
PRONOTUM TOP
PRONOTUM SIDE
METANOTUM SIDE

and eggs were counted and the largest egg was measured for length and width.

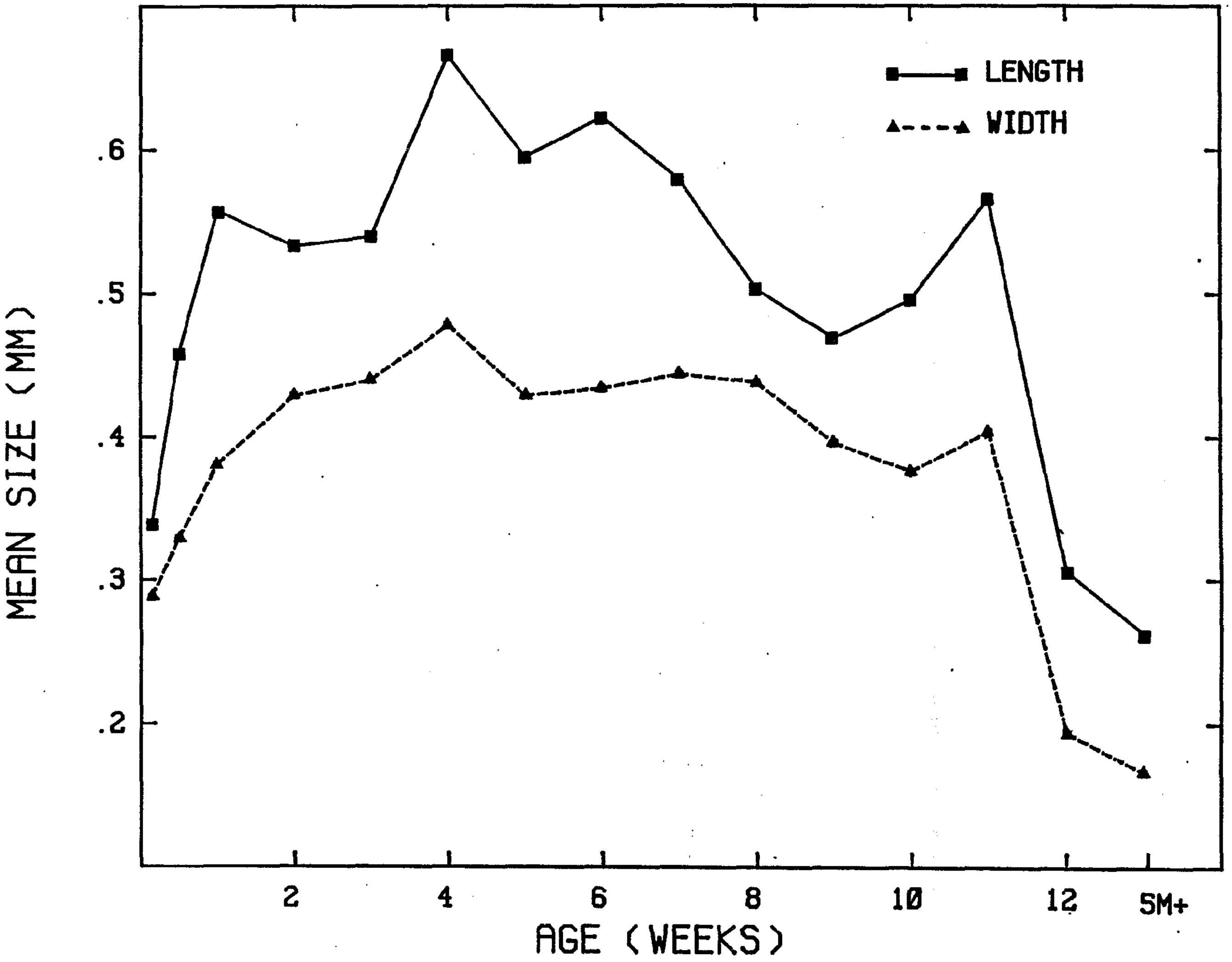
Results

Differences among the age groups for length and width of the largest eggs were significant (F = 9.11 and F = 11.56, $p < .0001$, respectively). These differences occurred mostly in the comparison of the midrange of ages with 1-day olds, and 12-week olds and beyond. This is depicted in Figure 2 where it can be seen that there is an increase in egg length until the 4th week, followed by an overall decline to the 11th week, after which there occurs a precipitous drop in egg length to below that of newly eclosed callows.

This trend is repeated for egg width. There are no significant differences from the 1st through the 11th weeks of age, but they are all significantly different from new callows at the one extreme and 12 weeks old and greater at the other. The quality of the eggs also begins to decline from the 10th week on; that is, they lose their whiteness and become more transparent. Some are almost completely transparent. This may be from yolk resorption.

The number of ovarioles declines with age ($r = -.56$, $p < .05$). Though there is a significant difference in the number of ovarioles among the groups (F = 8.7, $p < .0001$,

Figure 2. Measurement of largest egg: an indicator of egg growth and resorption as a function of worker age.


LENGTH
WIDTH
.6
.5
.4
.3
.2
MEAN SIZE (MM)
2
4
6
8
10
12
5M+
AGE (WEEKS)

Newman-Keuls test), much of it can be accounted for by those greater than 5 months old. Differences among the other age groups do not follow any particular trend: For example, 2- and 6- week olds do not differ significantly from any of those from 1 day through 12 weeks old; yet 5-week olds have significantly more ovarioles than do 7- and 8-week olds, and 4-week olds have fewer than do 4-day olds. This pattern can be seen in Figure 3.

For the number of eggs, however, there is a decided decline with age ($r = -.7$, $p < .01$, including the 1-day olds). Figure 3 shows that the decline is precipitous after 8 weeks of age. Overall there was a significant difference in the number of eggs among the age groups ($F = 15.4$, $p < .0001$, N-K test). Those over 5 months had fewer eggs than those of 4 days to 8 weeks old, which were not significantly different from one another. This latter group, for which the number of eggs remains fairly constant, was significantly different from the 1-day olds.

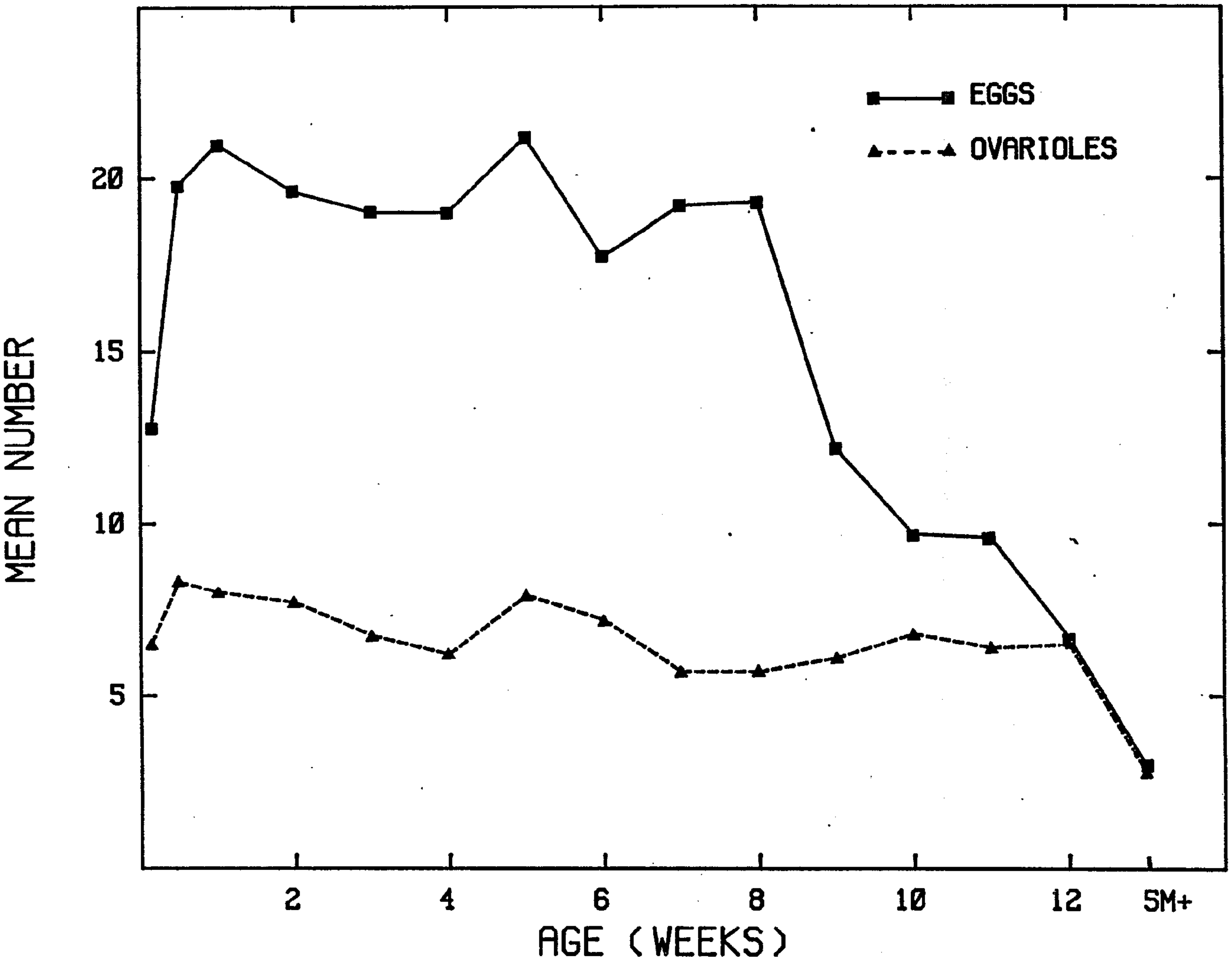
Development of Poison Gland

Methods

Procedure

Ants whose ovaries were measured in the previous section also had their poison gland reservoirs measured for

Figure 3. Number of eggs and ovarioles: an indicator of growth and resorption of ovaries as a function of worker age.



EGGS
OVARIOLES
MEAN NUMBER
20
15
10
5
2
4
6
8
10
12
5M+
AGE (WEEKS)

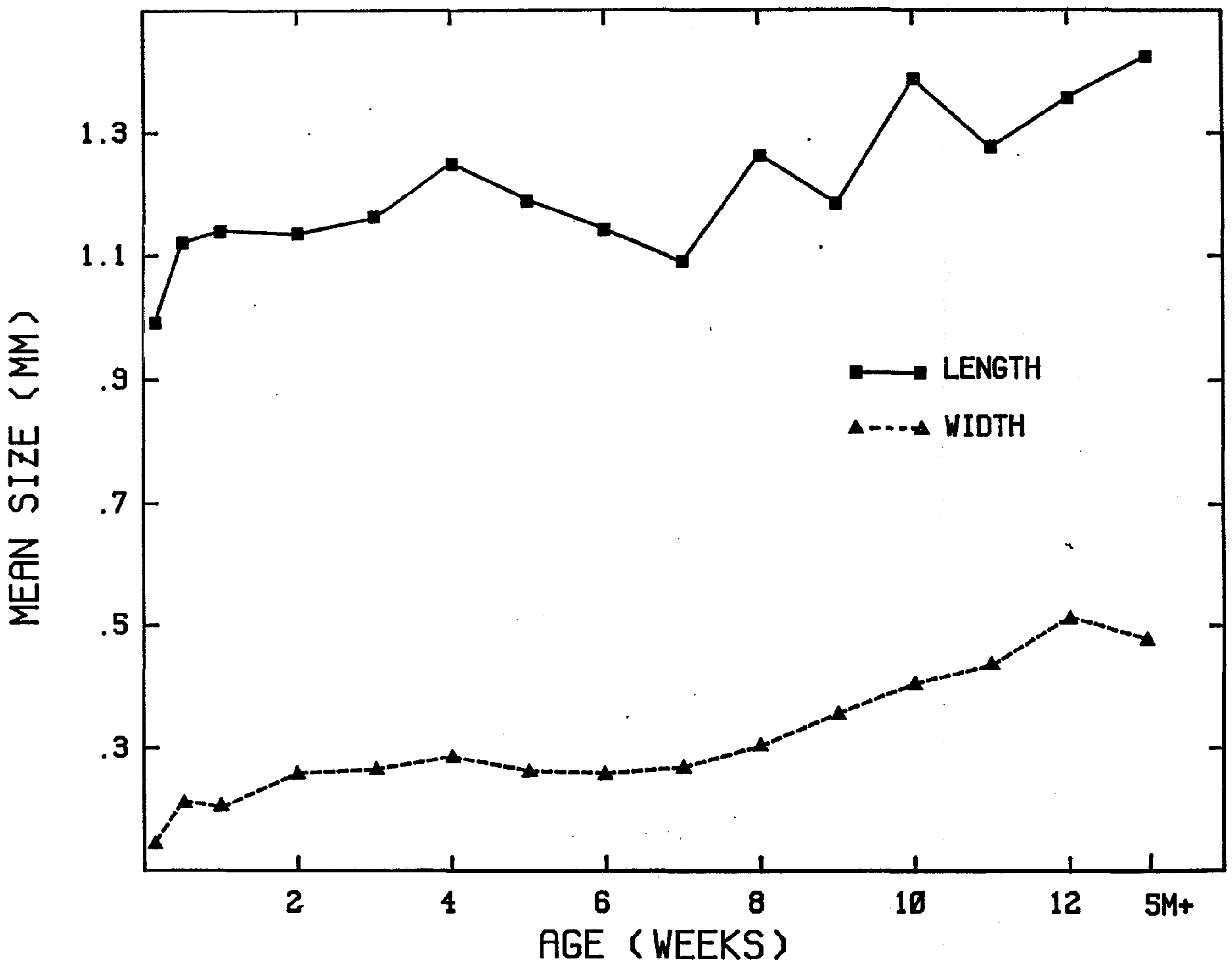
the present study. Further, 50 poison glands from each of those groups were excised and used to test the response of foraging outside workers to the trail pheromone. This was done so that the strength of the exocrine fluid as a stimulus could be assessed. The method of Hölldobler et al. (1978) was modified for this study (more glands per trial, and a shorter trail on a rougher surface were used in the present study).

Each age group contributed 50 poison glands for 10 trials (the gland is housed within the reservoir; it is actually the reservoir and its contents that were studied). For each trial 5 poison glands were placed on the tip of a hardwood stick and smeared in a 40-cm curved line across a piece of construction paper that was placed in the foraging arena. The number of ants following the trail from approximately the beginning to the end in either direction over a 5 minute observation period were recorded. Before a trial began, it was required that from 20 to 30 ants be in the arena. New paper was used for each trial.

Results

Figure 4 shows the overall increase in mean length of the poison gland reservoir. The correlation with age is significant ($r = .72$, $p < .01$). There were also signifi-

Figure 4. Measurements of poison gland reservoir: an indicator of increasing volume with age.


MEAN SIZE (MM)
1.3
1.1
.9
.7
.5
.3
AGE (WEEKS)
2
4
6
8
10
12
5M+
LENGTH
WIDTH

cant differences among the groups ($F = 6.46$, $p < .0001$, N-K test); but because of considerable variation, there were some odd results. For example, 5-month olds had significantly longer poison glands than 3- and 5-week olds but not longer than 4-week olds, and significantly longer than glands of 7- and 9-week olds but not longer than 8-week olds. This may reflect differences in volume because of pheromone use just prior to experimentation.

It can be seen from Figure 4, however, that this variability is not repeated for width. There is a steady increase in mean width with age ($r = .9$, $p < .01$). This trend is reflected in the significant differences among age groups ($F = 31.64$, $p < .0001$, N-K test). For example: 1-day olds have smaller poison gland reservoirs than all those 2 weeks and older; those of 4-day and 1-week olds are smaller than those of 8 weeks and older; those of 2- to 7-week olds are smaller than those of 9 weeks and older, and so on. The reservoir fluid in ants beyond 8 weeks, and particularly in those of 12 weeks and 5 months old, had a slightly yellow hue. This was not noticed in younger ants, whose reservoirs had clear liquid in them. Up through the 1st week the reservoirs were not well filled, a noticeable difference from the turgid poison gland reservoirs of those

9 weeks and older.

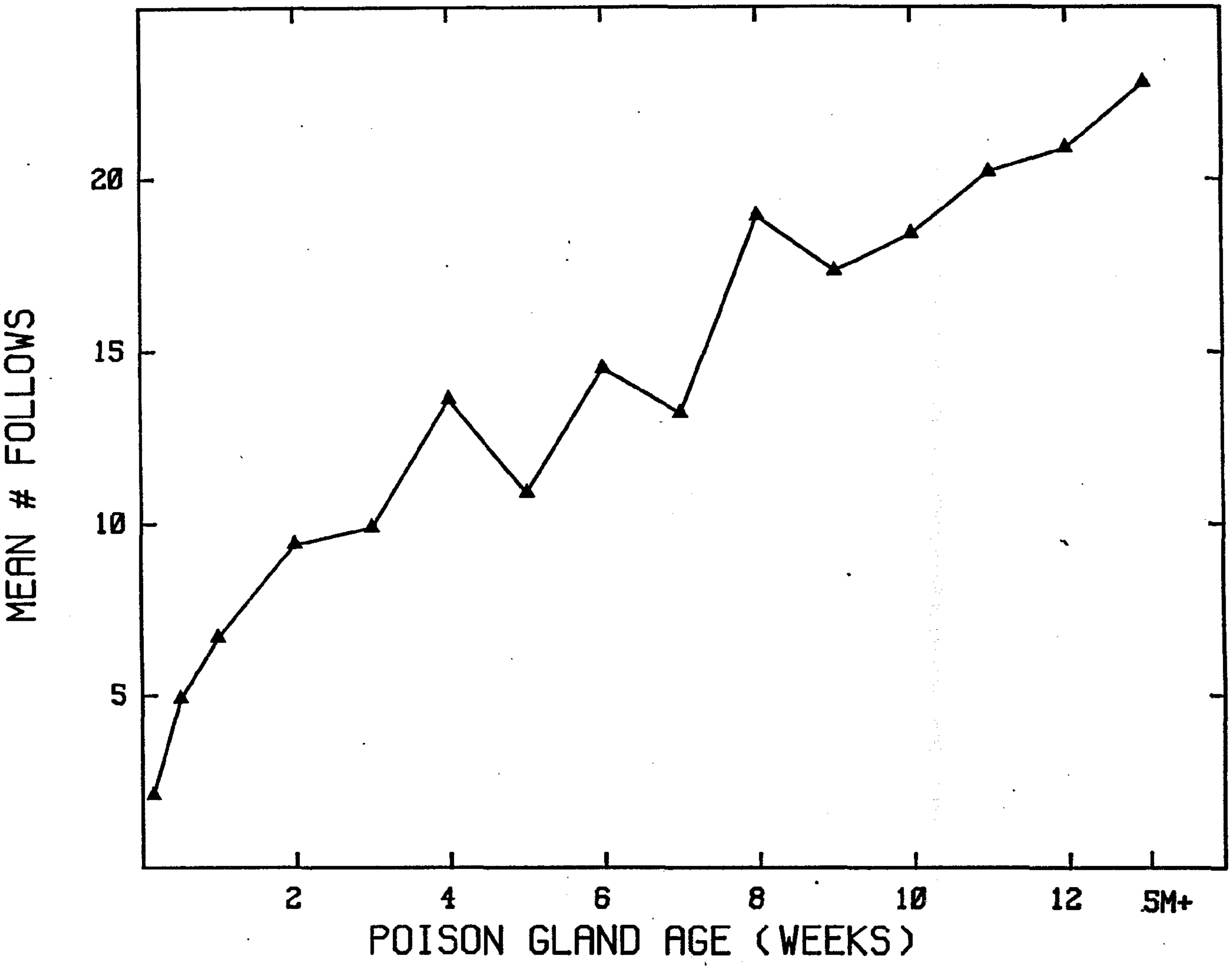
Among the foraging workers in the arena the "follow" response was significantly correlated with the age of the workers from which the glands were excised ($r = .95$, $p < .01$). The trend in Figure 5 is unmistakeable. Responses toward 8- to 12-week olds did not differ significantly. With the exception of 9-week olds, they also did not differ significantly from the 5-month olds. In general, the poison gland trails from this older group elicited significantly more follow responses than trails from those younger than 7 weeks ($F = 27.7$, $p < .0001$, N-K test). Glands from newly eclosed callows, the 1-day olds, elicited significantly less response than all the other age groups. Follow responses to poison gland trails from the 3- to 7-week olds did not differ significantly.

The initial reaction from the outside workers in the arena was one of general alarm. They swarmed on the construction paper and ran about excitedly for about 30 seconds before settling down to following the trail.

Discussion

As hardness of the exoskeleton, volume of the poison gland reservoir, and concomitant effect of the trail pheromone on outside workers increases, the general state of

Figure 5. Response of foragers to poison gland alarm/trail pheromone taken from selected age groups.


MEAN # FOLLOWS
20
15
10
5
2
4
6
8
10
12
5M+
POISON GLAND AGE (WEEKS)

the ovaries goes into eventual decline. In general, the sizes and number of eggs increase up through the 4th week, remain relatively the same for another 4 weeks, and then degenerate. The volume of the poison gland reservoir plateaus at about the same time as do the number and sizes of eggs, and remains at approximately that volume for a similar period of time, that is, from about the 4th through the 8th week. It then grows noticeably in size as the ovaries are degenerating.

The decline in the ovaries begins around the 6th week, during a time when the domestics are in a transitional behavioral stage similar to that which Lenoir (1979) found for Tapinoma erraticum. At this stage the young workers spend most of their time in the nest seemingly at rest and having little contact with the brood. Lenoir writes that they are ready to respond to any colony need, whether brood tending or foraging, during this transition to permanent forager (outside worker).

In N. albisetosus, the periods of developing, ripe, and degenerating ovaries correspond with those of Formica polyctena (Otto, 1958). Further, I have done pilot studies which corroborate other interesting findings by Otto: when kept without protein or older workers, ovaries will also fail to develop. At 3 weeks of age, for example, a group of 15 domestics kept without protein had an average of 10 eggs each, approximately half the amount in the present study. In another study, where the number of subjects was small, the number of eggs from age groups kept without older workers seem considerably less than for the same age groups in the present study.

As the poison gland reservoir increases in size, its contents affect a greater number of foragers in the arena; that the follow response also increases during that time when the reservoir remains relatively the same size indicates that the quality of the pheromone it contains may be changing, too. As Hōlldobler et al. (1978) found, the workers first responded to the pheromone with alarm before settling down to a few minutes of trail following. As in Holldobler's study, I also found the pheromone to be volatile and short-lived: after 5 minutes, trail following virtually ceased. To the pheromone taken from the youngest callows, however, there was little or no response either of an alarm or a trail following nature.

The hardness of the exoskeleton increases steadily up through 5 weeks, by which time pigmentation is close enough to that of mature adults to make it difficult to tell them apart on the basis of color. For the next 3 weeks, the rate of hardening slows dramatically. After the 8th week there is a spurt of hardening corresponding with a developmental stage found by McDonald and Topoff (1985) to be that time when the domestics become outside workers, spending half of their time outside the nest. This final hardening may be prompted by exposure to drier air outside the nest. In the field, ultra-violet radiation from the intense desert sunlight may also hasten darkening pigmentation and hardening of the exoskeleton.

General Discussion

That temporal polyethism can be altered according to the needs of the colony is not new to this dissertation (Sudd, 1967). But "colony needs" has never been a helpful construct in understanding the behavioral development of workers in an ant colony. Simply citing hunger as a general colony need inducing ants to forage, for example, would not have led to the conclusion that there are at least two clusters of stimuli coming from the larvae to which workers of different ages have different thresholds.

We can now try to ascertain the nature and sources of those stimuli and the sensitivity of those thresholds. For example, I have repeated the larval chemotactile experiment from Part I of this study, using fewer domestics, with the result of increasing domestic time spent outside. It will be interesting to see if contact with the posterior ends of the larvae have a similar effect on different numbers of developing domestics. Larval-worker trophalactic exchanges and their effects on ontogenetic processes in social organization should be a promising area of research.

As a second example, I have replicated the outside-worker removal condition using different numbers and ratios of brood and varying amounts of food, including none, and have not been able to accelerate behavioral development to outside worker levels in much under 2 weeks. It is interesting to note that this is about the limit that N. albisetosus stays alive with water and little or no food. As such it may be a hedge on the urgency of an incipient colony's need to have the callows forage at an age when they are most likely to die in the process. The flexibility of temporal polyethism, then, may be rooted in the exigencies of an incipient colony: skittish, stunted, initial workers (referred to as nanitic workers by Oster and Wilson, 1978) must forage at an early age, much earlier than they would in an older, larger colony where temporal polyethism may give the appearance of being less flexible.

Brood stimulation must combine with a host of other stimuli in the process of behavioral development. When domestics begin to leave the nest they eventually experience daylight. It is known that some ants, as they age, become slightly phototaxic, thereby reversing their preference when younger. Once outside, they forage, defend, learn local topography, patrol, excavate, and rest--all activities which keep them away from the brood. Further, the queen and brood ofter feed om trophic eggs from the domestics. Workers whose eggs have undergone resorption may have less to do with queen and brood because of this. Certainly, the Novomessor queen, which feeds largely on workers' eggs, would be less well taken care of by a worker which could not provide trophic eggs. When the behavioral development of callows is accelerated in the absence of outside workers, it may be in part because of the earlier degeneration and resorption of their eggs. Without their eggs they would have less to offer the brood and queen by way of direct contact; they would thus behave more like outside workers with their absence of brood and queen contact.

Evidence from this study indicates that there is less flexibility in the development of defensive behavior than in those behaviors associated with the development of domestics into outside workers. Oster and Wilson (1978) make a good case for the timidity of nanitic workers and why they avoid aggressive encounters: The loss of five workers from a colony of thousands may be well worth the price if it keeps enemy scouts from their territory; the loss of five from an incipient colony of ten could very likely be fatal to the colony. Oster and Wilson argue ergonomics--gain and loss, cost and efficiency.

Callows which are not nanitic, however, and are part of a larger colony in which there are many other age groups are much less timid and will attack when they are 4 weeks old, but given a choice they will flee. Even in the instances when younger workers attacked army ants, it was always the older workers which first poured from the nest entrance to set upon their adversaries.

The usual wisdom in cases of defensive behavior in the face of predators has been that these behaviors are less modifiable than others because so often they must be performed properly the first time they are needed. Death may otherwise be the result. Certainly older outside workers are more efficient killers than the young domestics: army ant injury and mortality was higher in confrontations with the older age groups.

That the early appearance of attack in significant numbers didn't happen in the field colony until the 4th to 5th weeks of age may have something to do with the hardness of the domestics' exoskeleta. Recall that between about 5 and 8 weeks there is no statistically significant hardening of the exoskeleton. It may be assumed, then, that some 4-week olds are as able as 8-week olds to resist an army ant's stings and bites. (Though army ants seem to go for joints when stinging, they will immobilize young callows by stinging through their thoracic exoskeleta). If a 4-week old's mandibular muscles are as developed as those of 8-week olds, both may be equally capable of defending the nest. It will be remembered that some time during these weeks, domestics of N. albisetosus go through a transitional behavioral period when thay begin to spend less time on the brood and become more available for outside work. One such outside task might be defense, but it took an exceptional effort to make this behavior surface at such an early age: eight confrontations with thousands of army ants over 3 weeks. These forced, prolonged exposures to the dry Arizona air may have hastened the hardening of those domestics' exoskeleta, which may have made them even more available for aggressive defense.

The effect of the trail pheromone on foragers in the arena increased with the age of the group of poison gland donors. This evidence indicates that callows would make inefficient foragers if their trails were too weak or short-lived to follow. However, it is clear that they will forage at an early age in the absence of older workers. An interesting observation regarding their early efforts is that the domestics used tactile cues more than do outside workers. They hugged the periphery of the arena and touched the Lucite walls with their antennae. Initially, they raided the midden pile against a wall of the arena and used this for food. Fresh food lay out in the sand within a few centimeters of the nest entrance. When going out into open sandy areas they explored cautiously, and there was no recruitment to the food when one of them found it. Each worker had to come across the food on her own. They could forage, but it wasn't efficient. Evidently, however, the work gets done: Out in nature all Novomessor colonies begin this way.

The changes in the hardness of the exoskeleton, size of the poison gland reservoir, effect of the trail pheromone, and state of the ovaries all occur during concomitant behavioral changes. These phenomena do not occur separately, but as a dynamic complex of developmental processes. Certainly in the case of the ovaries, development can be affected by social changes in the colony. And in one interesting development, where I replicated part of the larval chemotactile manipulation, the exoskeleta failed to harden and darken on a group of six domestics after 3 months. They were as soft to the touch as week old callows, and in every way appeared as such; yet, upon checking, their ovaries had been almost totally resorbed. In behavioral development they were somewhat like 6- or 7-week olds.

But given all the complexity in the behavioral development of N. albisetosus, this study has shown that brood stimulation is a major proximal causation underlying the social bonds of the colony. As such, it is an extension of T. C. Schneirla's work. Schneirla (1957) formulated a brood stimulation theory to account for the nomadic-statary cycle in army ants. The cycle is strongly correlated with a synchronized pattern of brood development. But brood stimulation apparently also underlies behavioral development in N. albisetosus, an ant species not closely related to the Dorylines, and definitely not nomadic. It seems likely, however that a stimulation so powerful as to cause such a noticeable change in the social behavior of an army ant colony, would be available in some form to other species of ants as well, since all are subject to stimulation from larvae and eclosion. Such stimulation, of course, would be manifest in a species typical manner; but I think that brood stimulation may prove to underlie the social bonds, by way of behavioral development, of most ant species and other social hymenopterans as well.

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