Soldier Determination in Ants: New Role for Juvenile Hormone

Abstract. *Topical application of juvenile hormone analog induces soldier development in the ant* Pheidole bicarinata. *Soldier induction takes place if the juvenile hormone analog is present during a period of sensitivity that occurs during the last larval instar. Control of worker dimorphism seems to be accomplished through control of timing of metamorphosis. The smallest size at which metamorphosis can be initiated is shifted upward from 1.2 to 1.7 millimeters by exposure to the juvenile hormone analog during the sensitive period.*

Female ants are separated into a fertile queen caste and a facultatively or obligately sterile worker caste. In some species, the worker caste itself is divided into a variable number of morphological subcastes. Large workers, called majors or soldiers, are usually behaviorally specialized and often serve a defensive function. Minor and media workers are generally responsible for the more routine tasks of colony life, including brood care, foraging, and nest construction (*1*). Although juvenile hormone (JH) plays a role in queen determination in ants (*2*), the physiological basis of worker subcaste determination is unknown. We now report that JH mediates soldier determination in the ant, *Pheidole bicarinata,* and we present a model for hormonal control of complete worker dimorphism in ants.

Like most other species of this genus, *P. bicarinata* (*3*) is characterized by a completely dimorphic worker caste. Complete dimorphism, which entails the total absence of media workers, provides a system in which the effects of JH on worker morphology can be evaluated without ambiguity. Larvae used in our study were taken from colonies maintained in the laboratory (*4*). Since queen/worker determination takes place during embryogenesis (*2*), larval queens and workers are easily distinguished. Worker larvae are bipotential until the last larval instar, when soldier/minor worker determination takes place. Larval development of workers consists of three instars, with the last larval-larval molt occurring when larvae reach a length of about 0.6 mm. Larvae of minor workers pupate soon after reaching a length of about 1.3 mm. Soldier larvae, however, continue to grow and reach a length of about 1.8 mm before initiating metamorphosis. At 27°C, the third instar lasts about 9 days in minor workers and about 15 days in soldiers.

To examine the role of JH in soldier/minor worker determination, we separated third-instar larvae, 0.6 to 1.3 mm in length, into six size classes (*5*). The JH analog (JHA) ZR-515 (methoprene) (*6*) was diluted in acetone and applied topically to individual larvae. Experimental larvae received one of three dosages of JHA. The calculated doses of JHA applied per larva were 4 to 10, 20 to 50, or 100 to 250 μg/g. Control larvae were treated with acetone. Each experimental or control group of 20 larvae from a single size class was provided with 40 minor workers as nurses to groom and feed the larvae. All groups were supplied with dead *Drosophila*, and were maintained at 27°C. After 10 days, most control individuals had terminated larval development, and the future caste of individuals in both experimental and control groups could be assessed easily. After larvae had pupated, the total body length and maximum head width of pupae were measured with an ocular micrometer.

Topical application of JHA dramatically influenced the developmental choice of worker caste (Fig. 1A), and the intensity of the effect was dose-dependent. The highest dose (100 to 250 μg/g) was effective in provoking more than 50 percent soldier production in all size classes. The lowest dose (4 to 10 μg/g) had no effect on the percentage of soldiers produced. The response to the intermediate dose (20 to 50 μg/g) demonstrated that larvae were sensitive to the effects of JHA only if treated when they were 0.9 to 1.2 mm in length. This size range represents a JH-sensitive period for soldier induction. Larvae were less sensitive in the smallest (0.6 to 0.8 and 0.7 to 0.9 mm) and largest (1.1 to 1.3 mm) size classes. The response of the smallest size classes to the highest dose was probably due to persistence of JHA until the JH-sensitive period. In the 1.1- to 1.3-mm size class, 50 percent of the larvae showed no soldier development even after treatment with the highest dose of JHA. The experiment was designed so that half the larvae in this class were larger than 1.2 mm; presumably it is this half that was unresponsive to JHA since larvae 1.1 to 1.2 mm in length were highly responsive when treated in the 1.0- to 1.2-mm size class. The JH-sensitive period, therefore, seems to end when larvae reach a size of about 1.2 mm.

Soldier development provoked by treatment with JHA was indistinguishable from normal soldier development. As larvae, the artificially induced soldiers developed imaginal fore-wing discs, which are a diagnostic feature of soldier differentiation (*4*). Furthermore,
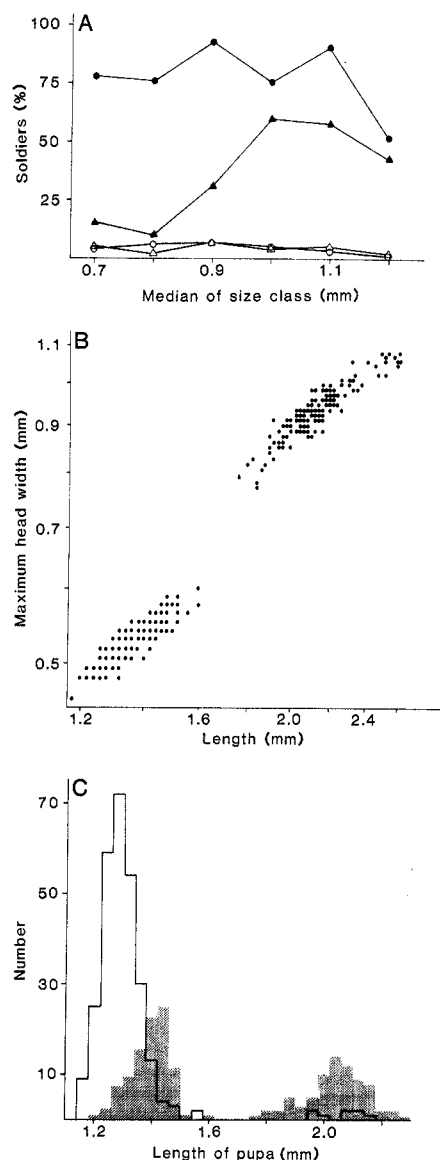

Fig. 1. (A) Response of larvae to three doses of ZR-515: (△) 4 to 10, (▲) 20 to 50, and (●) 100 to 250 μg/g. (○) Acetone-treated controls. Each size class consisted of equal numbers of larvae of sizes ranging 0.1 mm on either side of the median. Each point is based on 30 to 60 individuals; data from at least three experiments were summed. (B) Relation between pupal body and head measurements (log scales). Points represent only treated individuals that pupated. (C) Response of pupal size to an intermediate dose of ZR-515 (20 to 50 μg/g). The open histogram represents controls for this dose only; the stippled histogram represents all larvae receiving this dose, regardless of size class. Treatment resulted in bimodal distribution of pupae; soldiers are produced, and the size of minor workers is shifted upward.
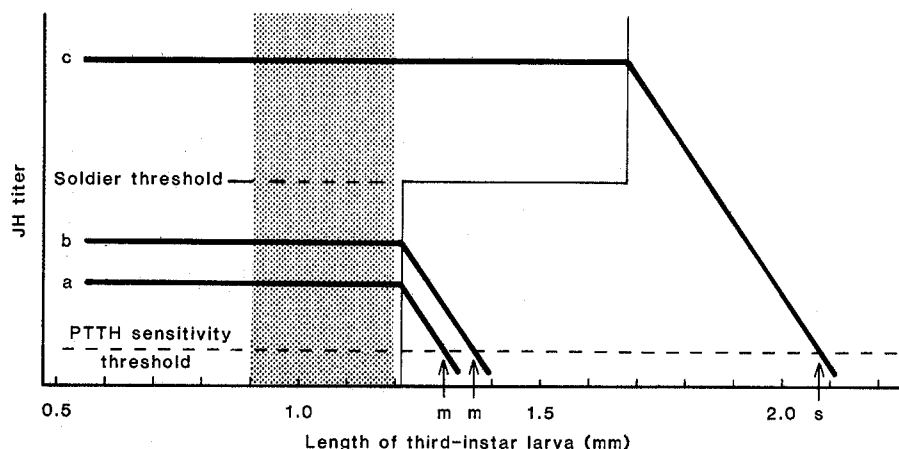
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Fig. 2. Proposed role of juvenile hormone in worker caste determination. The stippled region indicates the JH-sensitive period that controls induction of soldier development. The thin solid line represents critical larval sizes at which metamorphosis can be initiated; the upper dashed line represents the threshold titer of JH necessary to induce soldier development, and the lower dashed line represents the threshold titer of JH that inhibits PTTH secretion. Arrows represent sizes at which minor worker (m) and soldier (s) larvae stop growing and secrete PTTH to initiate metamorphosis. Thick solid lines represent hypothetical JH titers for three individuals. The rate of JH degradation is assumed to be independent of larval size. A typical minor worker is represented by line a. If JH titers are slightly higher, as in some individuals treated with JHA (line b), threshold titers are not reached but metamorphosis is delayed while superfluous JHA is degraded. If an individual has a JH titer above the soldier threshold (line c, critical size will be reset from about 1.2 mm to about 1.7 mm. JH secretion does not terminate at 1.2 mm but continues until the larva grows to the larger critical size.

the size ranges of treated individuals (Fig. 1B) duplicated the size ranges in untreated colonies of this species, and the artificially induced soldiers emerged as perfectly normal adults. We are therefore confident that JHA treatment mimics the normal mechanism for soldier determination.

The discovery of a JH-sensitive period that controls soldier determination makes this system appear analogous to queen determination in bees. In honey bees, queen determination is controlled by the presence of JH during a critical period that spans portions of the last two larval instars. In these insects, however, intermediate queen-worker forms are frequently produced by treatment with JH or JHA (7). In our study of *Pheidole*, no soldier–minor worker intermediates were ever observed (Fig. 1, B and C).

Treatment of third-instar larvae with JHA produced a second effect on caste. In addition to the induction of soldier development, the mean size of minor workers increased. Treatment with the intermediate dose (20 to 50 µg/g) caused an increase in the size of minor worker pupae from a mean of 1.29 to a mean of 1.39 mm, as well as a delay in pupation of 1 to 2 days (Fig. 1C).

We propose a model that accounts for the size frequency distributions of worker castes seen both in nature and in our experiments. Our model is based on two mechanisms through which JH participates in morphogenetic changes in other insects. These mechanisms are (i) a JH-

sensitive period that controls inductions of alternate character states and (ii) a critical size at which metamorphosis is triggered.

The JH-sensitive periods are temporal windows during which a gene or group of genes is susceptible to repression or derepression, the choice of state depending on whether JH is above or below a relevant threshold (8). In *P. bicarinata*, the JH-sensitive period that controls soldier induction occurs while the larva is 0.9 to 1.2 mm in length.

The concept of critical sizes for metamorphosis has been best studied in Lepidoptera, particularly the tobacco hornworm, *Manduca sexta*. At a critical size during the last larval instar, *Manduca* larvae initiate a sequence of events leading to pupation: secretion of JH by the corpora allata is terminated, residual JH is cleared from the hemolymph, and PTTH (prothoracicotropic hormone) is secreted to provoke release of the molting hormone. Apparently JH inhibits the secretion of PTTH in final-instar larvae. Thus, the presence of exogenous JH or JHA after the critical size has been reached postpones PTTH secretion, and hence metamorphosis, until all supplementary JH is metabolized (8, 9). Such JH-dependent metamorphic delays are common in holometabolous insects (10).

In *P. bicarinata*, the JH-sensitive period, critical size, and JH may interact to induce soldier development and produce a dimorphic worker caste (Fig. 2). If endogenous JH titers are below some

critical threshold during the JH-sensitive period, larvae will become minor workers. Upon reaching the critical size, which probably falls between 1.2 and 1.3 mm, the corpora allata are inactivated, and pupation is initiated. When the JH titer is artificially elevated by JHA treatment, some individuals will have a JH titer that is below threshold, but unusually high for prospective minor workers. Since pupation must be postponed until all JHA is cleared from the blood and tissues, a metamorphic delay occurs while JHA is degraded. Larvae continue to feed and grow during this period. Thus treated larvae that metamorphose as minor workers have a larger mean size than controls (Figs. 1C and 2).

If endogenous levels of JH are high during the JH-sensitive period, larvae will become soldiers. During this period, JH mediates changes in growth patterns in designated tissues. For example, imaginal wing discs begin to grow and become visible for the first time as soldier larvae pass the first size threshold for pupation (4). Growth patterns of other organs (for example, Dufour's gland) are presumably reprogrammed at this time. Allometric patterns seen in completely dimorphic worker castes (1) could be explained by reprogramming of growth constants during the JH-sensitive period. The most important change mediated by JH during the sensitive period, however, is resetting the critical size for metamorphosis from a body length of about 1.2 mm to a body length of about 1.7 mm. Thus after experiencing high levels of JH during the JH-sensitive period, larvae will not terminate secretion of endogenous JH until the higher critical size has been attained (Fig. 2).

The presence of two discrete critical sizes would account for the lack of worker-soldier intermediates when larval JH levels are artificially manipulated. A larva is evidently programmed to continue secreting JH beyond the lower critical size if JH titers are above a certain threshold during the JH-sensitive period. Analogous situations, in which the presence of JH at certain times can alter subsequent patterns of JH secretion, are known in other insect systems (8). In *Pyrrhocoris apterus*, embryonic exposure to JHA interferes with the normal program of corpora allata inactivation. In affected individuals, JH continues to be secreted in the last larval instar and an extra larval stage ensues (11). Also, in *Locusta migratoria*, different maternal levels of JH have been implicated in setting the very different patterns of JH secretion that are characteristic of solitary and gregarious offspring (9, 12). No

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evidence exists from any insect other than *P. bicarinata* for resetting of critical sizes by JH during larval development. However, the known role of JH during embryogenesis in forming the program of JH secretion, which includes the original setting of critical size, makes JH a likely physiological tool for revising critical size during larval development.

Finally, in order to integrate this model of caste determination with the bionomics of an ant colony, we propose that nutritional state directly controls the endogenous titer of JH. The fact that *Pheidole* soldiers are produced only when larvae are fed a highly proteinaceous diet is well documented (*13*). The link between the larval endocrine system and the nutritional environment is likely to be a rate factor associated with diet. In honey bees, artificial diets with high sugar content enhance the feeding rate of larvae and induce queen development. A high feeding rate appears to produce abdominal stretch, which triggers an increased rate of JH synthesis and secretion (*14*).

The discovery of a JH-sensitive period for soldier determination provides a physiological basis for the decision points (*15*) along larval developmental pathways. A decision point is a time in development at which one or the other of two sets of growth patterns is acquired by the larva, after which the individual proceeds in its development toward one caste (or subcaste) or the other (*15*). In ants, previous examples of decision points have correlated a period of winter dormancy with a change in the developmental pathway (*16*). The mechanism of soldier determination in *P. bicarinata* is independent of season and thus may have wide applicability to the control of polymorphism in ants.

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6. The volume of JHA applied per gram varied somewhat from individual to individual because of the size range of the larvae and the difficulty of controlling drop sizes, which were as small as 1 nl for the smallest larvae.
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Vibration Signal Transmission in Spider Orb Webs

Abstract. *Vibration transmission from the prey-catching region to the hub of the unloaded orb web of* Nuctenea sclopetaria *was measured by laser vibrometry. Compared to transverse or lateral vibrations, longitudinal vibration shows less attenuation and contains more directional information. It is transmitted well throughout the entire frequency range measured (1 to 10,000 hertz).*

A century ago, Boys (*1*) observed that a spider will attack a vibrating tuning fork touching its web. Since that time interest in vibration signals in spiders' webs has continued (*2–8*), and it has become clear that the web is not only a device for ensnaring prey but also a medium for transmitting information on the nature and position of the source of mechanical disturbances. Therefore, an understanding of vibration transmission through the web is central to understanding the use of vibration signals by web
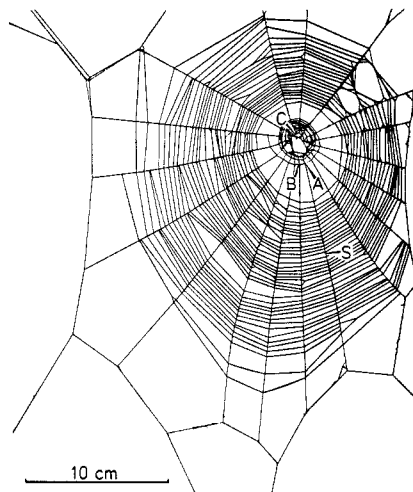

Fig. 1. A reversed-image photograph of the orb web from which the vibration measurements shown in Fig. 2a were made. The hub is the central area of dense meshwork surrounded by a spiral-free zone. Radial strands attach at their distal ends to the frame threads forming the web margin; these in turn are fixed directly, or else via supporting stays, to the wooden frame (not shown) in which the web was built. The web was stimulated at point S (or, in other webs, on any radius in the web's lower half) and the vibration measured at points A to C.

spinners. Despite a number of investigations (*4–8*), there has been no satisfactory means of measuring web vibrations that does not load the web at the point of measurement. The web's light construction—one spanning 500 cm² may weigh only 1 to 2 mg—requires a noncontact, and therefore optical, measurement technique, while the signal from entangled prey—expected to extend over a frequency range of about 1 to 10,000 Hz at amplitudes ranging from nanometers to millimeters—demands an instrument with extended frequency and amplitude response.

We now report on the differential transmission of three types of vibration signals through the unloaded web of *Nuctenea* (= *Araneus*) *sclopetaria* determined by laser Doppler vibrometry. Our results represent the first step in a program intended to describe the complete system composed of the spider, its web, and a vibration source (prey or mate, for example). It has now become possible to measure transmission over a very broad range of frequency (probably the whole range of interest to the spider), and the results show that there are several types of web vibration, not previously distinguished, each having different transmission properties, with perhaps different functions.

Normally, a spider sits either at the hub of its web (Fig. 1), the convergence point of all web radii, or hides nearby and monitors web vibration via a signal strand that runs to the hub. The radii appear to be the most important vibration-conducting elements of the web, since it is along them that the spider orients when trying to locate a vibration source (*3, 4*). We can distinguish several