Research article

Caste allometries in the soldier-producing aphid *Pseudoregma alexanderi* (Hormaphididae: Aphidoidea)

D. L. Stern[1,*], A. Moon[2] and C. Martinez del Rio[2]

Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA

Summary

Colonies of the aphid *Pseudoregma alexanderi* produce morphologically-specialized first-instar larvae, termed soldiers, that defend the colony from predators. The environmental cues and physiological mechanisms governing soldier production are currently unknown. Here we present a morphometric study of soldiers and normal first-instar larvae of *P. alexanderi*. Several morphological features (fore-leg length and width, hind-leg length, and horn length) plotted against body length display relationship that are similar to a sigmoidal curve. We found further support for an earlier finding that soldiers fall into two size categories, majors and minors, although both types of soldiers appear to follow the same allometry. The patterns of allometry in the soldier-producing aphids are very different from those found in other social insects and do not easily fit into the traditional categorization of allometries. We present two simple alternative models of soldier development as a framework for guiding future studies of the mechanisms of soldier production.

Introduction

Many social insect species have evolved behavioral, physiological and morphological castes. In most cases the different castes perform either reproductive or "worker" functions. Ants and termites have reached the highest complexity in caste systems, exhibiting considerable morphological polymorphism among workers (Wilson, 1971). Specialized castes have probably evolved to allow more efficient performance of specific tasks, and this specialization entails reduced individual functional flexibility (Oster and Wilson, 1978).

   Aoki (1977) demonstrated that some aphids produce a specialized caste of larvae, termed soldiers, that defend the colony from predators. Although aphids

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[1] Current address: Wellcome/CRC Institute and Department of Genetics, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QR, UK
[2] Current address: Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071-3166, USA
* Author for correspondence: David L. Stern, Churchill College, Cambridge CB3 0DS, UK

produce extensive morphological variation across generations (Dixon, 1985), soldiers are peculiar not only because they represent a reproductively-altruistic caste (Stern and Foster, 1996 a, b) but also because they are a case of morphological dimorphism within a single generation. For example, the soldiers of *Pseudoregma alexanderi* are first-instar larvae, but they are easily discriminated from normal larvae by their relatively large size and enlarged weaponry (Aoki and Miyazaki, 1987; Aoki et al., 1981).

Some aphid polymorphisms, for example the production of sexuals and of winged migrants versus unwinged adults, have been studied in great detail (reviewed in Lees, 1966). For wing polymorphism, the timing of the developmental switch from the unwinged to the winged morph in different species can occur from just before birth, as a maternal effect, up through early postnatal development in the first of the four larval instars. In contrast, sexuals are determined early in embryogenesis. The developmental switch for soldiers of *Pseudoregma alexanderi* clearly occurs during embryogenesis, since soldiers are first-instar larvae. [Many species produce second-instar soldiers (Stern and Foster, 1996b) and for these the developmental switch may be later.] However, the stage in embryogenesis when the switch is made is unknown.

A case similar to soldier production may be found in the aphid *Periphyllus testudinaceus* that produces both normal first-instar larvae and morphologically-specialized aestivating first-instar larvae (reviewed in Dixon, 1985). In this species poor nutrition triggers the production of the aestivating morph. Adults transferred to poor food begin producing the aestivating morph in approximately four days. Therefore, assuming the adult can quickly detect food quality, the developmental switch to production of the aestivating morph probably occurs several days before birth.

The environmental cues triggering sexual, winged, and aestivating polymorphisms are fairly well understood and include crowding, nutrition, temperature and photoperiod (Lees, 1966; Dixon, 1985). In contrast, the cues triggering soldier production are unknown. Potential cues include photoperiod, temperature, crowding, food quality, predation, adult size, and absolute time.

Here we present a morphometric study of soldiers and normal larvae of *Pseudoregma alexanderi* to begin exploring soldier-aphid allometries and the possible developmental mechanisms and selective pressures underlying the evolution of soldier morphology.

Materials and methods

The colony of *Pseudoregma alexanderi* examined was collected on 1 March 1988 directly into 70% ethanol from the bamboo *Dendrocalamus latiflorus* at Sun Moon Lake, Taiwan and kindly provided to us by Dr. Shigeyuki Aoki (collection number SA-8821). First-instar aphids were sorted into normal and soldier larvae; soldiers have much larger fore-legs relative to normal larvae for the same body size and have been characterized as "pseudoscorpion-like" (Aoki and Miyazaki, 1978). Aphids were stained in 1.5 ml eppendorf tubes by heating 1 ml 10% KOH at 70°C for approximately 45 minutes which was then replaced by distilled water and heated at 70°C for 45 minutes. The water was removed and 1 ml of a 1:1 solution of lactic
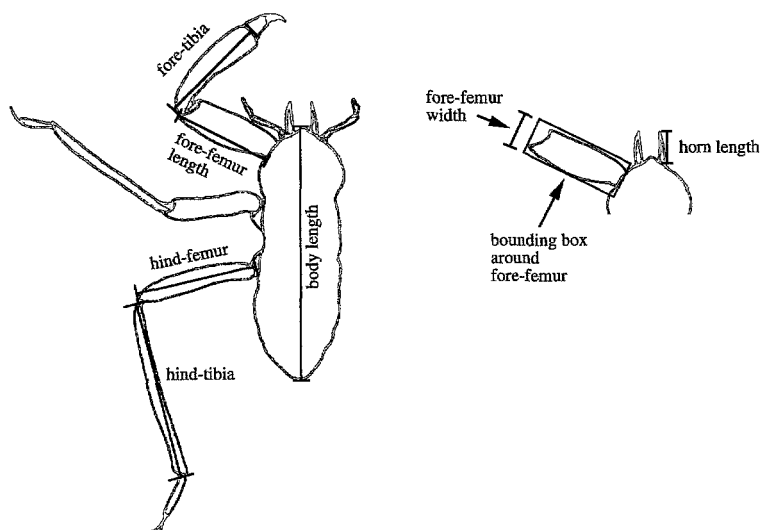
Figure 1. Diagrammatic illustration of *Pseudoregma* soldier with the measurements made on each individual

acid: water and one drop of 0.2% Acid Fuchsin were added. This solution was heated at 70 °C for 45 minutes to 2 hours. The lactic acid was removed and aphids were washed in glacial acetic acid for at least one hour. The acetic acid was removed and clove oil was added. The aphids were mounted temporarily on glass slides in clove oil. Aphids were arranged on the slide until all appendages were clearly visible. A cover slip was dropped onto the specimens which flattened the aphids.

Slides were lit from below with a light box and viewed with a Zeiss dissecting microscope with a video camera attached. Images were digitized and landmarks were recorded using OPTIMAS 3.01 software (BioScan Inc., Aldus Corp). These landmarks were converted to lengths for the characters shown in Fig. 1. To calculate the width of the fore-femur, we first traced the outline of the entire femur and then used the OPTIMAS software to calculate a bounding box around the femur. The length of the shorter side was used as the width of the fore-femur (Fig. 1).

The average of the left and right sides of traits for each individual were used for all analyses. We first examined the size distributions for body length and horn length. We then examined the relationships of each trait with respect to body length. We also examined the relationships between the following traits to search for possible functional relationships between traits: hind-femur length versus fore-femur length, hind-femur length versus hind-tibia length, fore-femur length versus fore-femur width, fore-femur length versus fore-tibia length, and fore-femur length versus horn length.

Analysis of covariance (ANCOVA), implemented with the computer program SuperANOVA (Abacus Concepts), was used to test for a difference in allometries between normal and soldier larvae. For pairs of variables we fit the following linear model:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2 + \varepsilon \tag{1}$$

where y is the dependent variable, x_1 is the covariate, x_2 is the treatment or caste (normal or soldier larvae) that can take a value of 0 or 1, β_0 is the intercept, β_1 is the effect of the covariate on the slope, β_2 is the effect of caste on the intercept, β_3 is the effect of caste on the slope, and ε is the error term. We tested for a difference in intercepts ($H_O: \beta_2 = 0$) and a difference in slopes ($H_O: \beta_3 = 0$) due to caste. If neither was significant then the two castes were co-linear and followed the same allometry. If either or both were significantly different from 0 then the allometries were different. If both were different from 0, then the allometric equations were constructed as:

1) $y = \beta_0 + \beta_1 x_1$ for soldiers, and $\qquad\qquad\qquad\qquad\qquad\qquad$ (2)

2) $y = (\beta_0 + \beta_2) + (\beta_1 + \beta_3)x_1$ for normal larvae. $\qquad\qquad\qquad$ (3)

If only one coefficient (the intercept or the slope) was significantly different from 0, then the non-significant coefficient was removed from the model and the model was recalculated. For example, if there was no significant difference in intercepts but there was a significant difference in slopes, then we fit the model

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_1 x_2 + \varepsilon \qquad\qquad\qquad\qquad\qquad (4)$$

and the allometric equations were reconstructed as

1) $y = \beta_0 + \beta_1 x_1$ for soldiers, and $\qquad\qquad\qquad\qquad\qquad\qquad$ (5)

2) $y = \beta_0 + (\beta_1 + \beta_2)x_1$ for normal larvae. $\qquad\qquad\qquad\qquad$ (6)

Results

Size distributions

The histograms of both body length (Fig. 2a) and horn length (Fig. 2b) show overlap between normal larvae and soldier larvae. The normal larvae tend to display a single modal value and an approximately normal distribution. In contrast the soldier larvae appear to show at least two modal sizes for both characters (Fig. 2) and all other characters measured (results not shown). For body size, the first mode of soldier sizes is approximately equal to the modal value for normal larvae (Fig. 2a), whereas for horn length the first modal value is greater than that for normal larvae (Fig. 2b).

Allometric relationships

Effect of body size

The forms of the relationships between body length and all leg lengths (Fig. 3 for fore-femur, others not shown) and horn length (Fig. 4) are qualitatively similar. Normal larvae fall on a shallower slope than soldier larvae for all five characters although the slopes are significantly different only for fore legs and horns (Table 1).
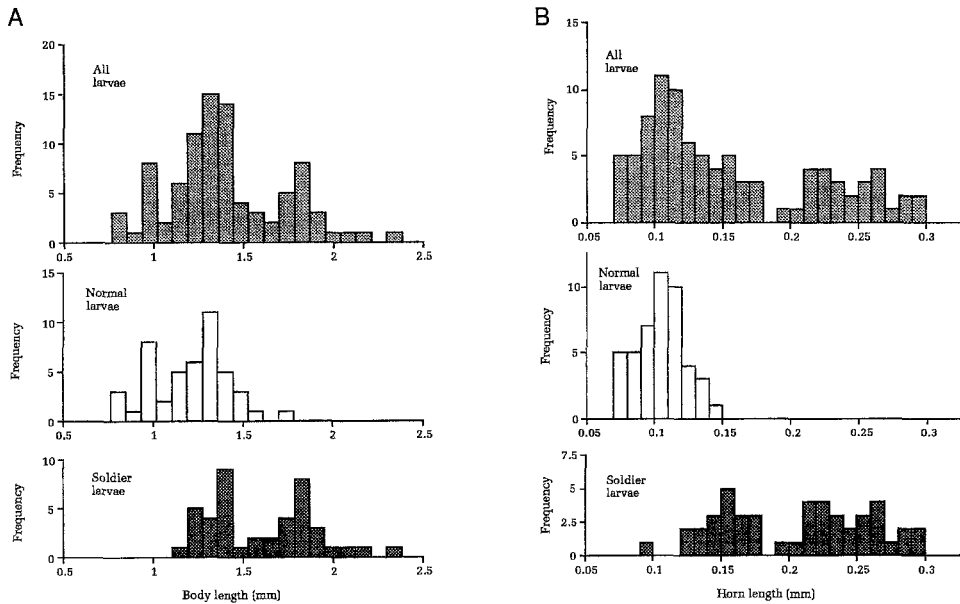
Figure 2. Histograms for (A) body length (N = 46 normal larvae and 43 soldiers) and (B) horn length (N = 46 normal larvae and 46 soldiers). The top histogram for each measure is for all larvae combined, the middle histogram is for normal larvae, and the bottom histogram for soldier larvae. Frequency on these and all following histograms are raw numbers of individuals
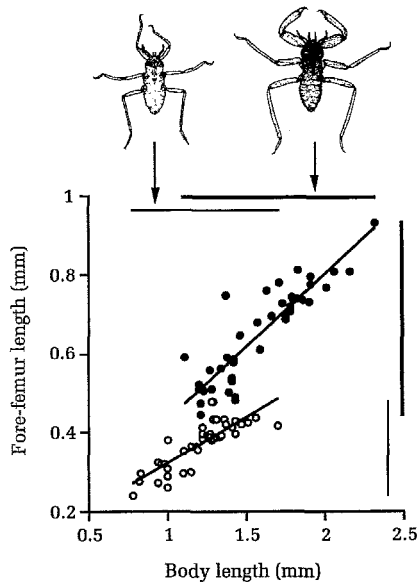

Figure 3. Scatterplot of fore-femur against body length. For this and all remaining figures the open circles (○) are normal larvae and the filled circles (●) are soldier larvae. The lines on the right and top borders indicate the ranges of values for normal (thin line) and soldier (thick line) larvae. The slopes differed significantly between normal larvae and soldiers, but the intercepts did not. The allometric relationships between body length (X) and fore-femur length (Y) are Y = 0.244 X + 0.075 for normal larvae and Y = 0.361 X + 0.075 for soldiers

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Table 1. Results of analysis of covariance (ANCOVA)

Covariate	Dependent Variable	Slope			Intercept			d.f.
		Normal	Soldier	F	Normal	Soldier	F	
body length	fore-femur length	0.234	0.368	10.637**	0.084	0.064	0.181	(1,81)
reduced model		0.244	0.361	174.807***				(1,82)
body length	fore-tibia length	0.280	0.369	4.422*	0.143	0.094	0.696	(1,81)
reduced model		0.301	0.356	35.618				(1,82)
body length	hind-femur length	0.317	0.421	3.472†	0.095	0.117	0.080	(1,82)
reduced model		0.308	0.427	94.698***				(1,83)
body length	hind-tibia length	0.394	0.551	3.531†	0.260	0.260	0.000	(1,80)
reduced model		0.393	0.551	73.433***				(1,81)
body length	horn length	0.057	0.147	19.423***	0.036	-0.030	5.379*	(1,85)
fore-femur length	fore-tibia length	1.081	0.980	2.025	0.080	0.044	1.318	(1,85)
reduced model[1]		0.808		1512.34***	0.169			(1,87)
fore-femur length	log fore-femur width	0.941	0.995	0.103	-1.300	-1.306	0.006	(1,84)
reduced model[1]		1.027		817.79***	-1.330			(1,86)
fore-femur length	horn length	0.232	0.386	8.080**	0.018	-0.047	7.465**	(1,84)
hind-femur length	hind-tibia length	1.212	1.263	0.149	0.133	0.185	0.483	(1,82)
reduced model[1]		1.273		1583.928***	0.133			(1,84)
hind-femur length	fore-femur length	0.662	0.870	13.169***	0.055	-0.031	6.633**	(1,82)

† $p < 0.07$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
[1] These reduced models are simple linear regressions ($y = \beta_0 + \beta_1 x_1 + \varepsilon$) and the F ratio therefore refers to the effect of β_1 (i.e. whether the covariate explains a significant amount of the variation in the dependent variable)
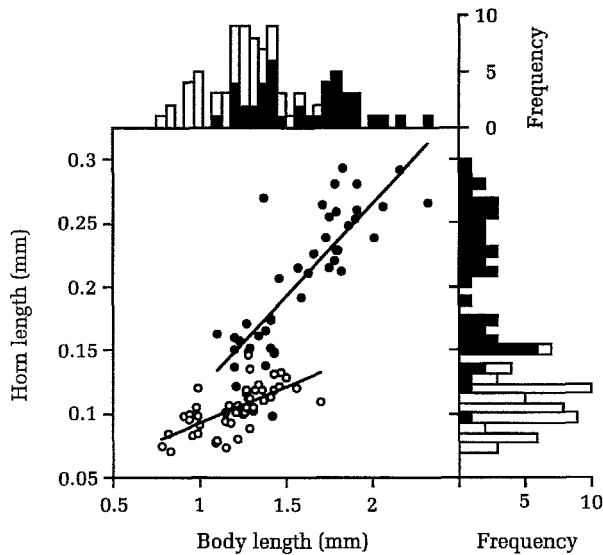
Figure 4. Scatterplot of horn length against body length. The histograms from Fig. 2 for horn length and body length are plotted on the scattergram. The height of the bars represents all larvae; the black bars represent soldiers and the white bars are normal larvae. Therefore the black and white within each category are the relative proportions of the two types of larvae. Both the slopes and intercepts differed significantly between normal larvae and soldiers. The allometric relationships between body length (X) and horn length (Y) are $Y = 0.056X + 0.036$ for normal larvae and $Y = 0.147X - 0.030$ for soldiers

For hind legs the slopes are almost significantly different (Table 1). In addition, the regression lines for soldiers are displaced to the right relative to the normal larvae. This results in an approximately sigmoidal shape for the final distribution of all larvae combined. The distribution appears to differ from a clear sigmoidal distribution because there is extensive overlap in the ranges of normal and soldier larvae for body length.

Relationships among characters

Despite the qualitative similarities among all characters relative to body length, the slopes for different characters vary over an order of magnitude (Table 1). For example, the slope for normal-larvae horn lengths is 0.056 (compared to 0.147 for soldiers) whereas the slope for normal larvae for hind-tibia lengths is 0.394 (compared to 0.551 for soldiers).

The fore-femur width plotted against fore-femur length shows an exponential relationship (not shown). Accordingly, a semi-log plot yielded a linear relationship between the variables (Fig. 5a). This was the only comparison that displayed a clear non-linear relationship between the variables.

Normal and soldier larvae appear to fall approximately along a single allometry for fore-femur length versus width (Fig. 5a), and hind-femur length versus hind-tibia (Fig. 6b) lengths. There are no significant differences between the slopes or intercepts for these characters although there is some evidence that the regressions
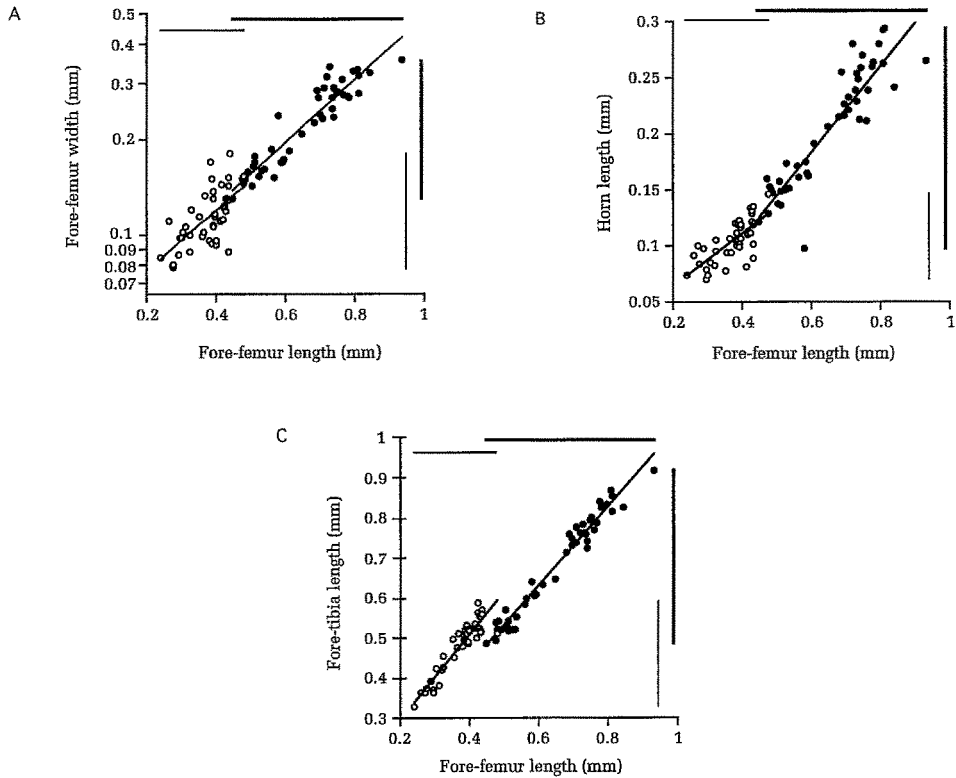
Figure 5. (A) Scatterplot of fore-femur width against fore-femur length. Note that this is a semi-log plot. With arithmetic axes the scatterplot showed a distinct exponential relationship. Neither the slopes nor the intercepts were significantly different between normal larvae and soldiers. The allometric relationship between fore-femur length (X) and fore-femur width (Y) is $Y = 0.047 * 10^{1.027X}$. (B) Scatterplot of horn length against fore-femur length showing diphasic allometry. Both the slopes and intercepts are significantly different between normal larvae and soldiers. The allometric relationships between fore-femur length (X) and horn length (Y) are $Y = 0.232X - 0.018$ for normal larvae and $Y = 0.386X - 0.047$ for soldiers. (C) Scatterplot of fore-tibia length against fore-femur length. Neither the slopes nor intercepts were significantly different by ANCOVA. However, the regression for soldiers is displaced to the right relative to normal larvae, and the residuals of a simple linear regression show clear heteroscedasticity (not shown). Therefore the results from the ANCOVA are used to define the allometric relationships. The allometric relationships between fore-femur length (X) and fore-tibia length (Y) are $Y = 1.081X - 0.08$ for normal larvae and $Y = 0.98X - 0.044$ for soldiers

are slightly shifted between soldiers and normal larvae. For example, for fore-tibia length plotted against fore-femur length soldiers are displaced to the right relative to normal larvae (Fig. 5c), even though the slopes and intercepts are not significantly different by ANCOVA (Table 1). Because of this clear shift in values the results from the ANCOVA are used to define the allometric relationships.

The distribution of horn lengths relative to fore-femur lengths shows a diphasic allometry (Fig. 5b). This is a reflection of the greatly increased size of horns in soldiers. Of all measured variable the horns show the greatest increase in regression slope, almost three times greater, in soldiers relative to normal larvae (Fig. 4 and Table 1).

Discussion

The allometric patterns found for these soldier-producing aphids suggest that the aphids use a very different set of growth rules for producing the soldier caste than is used in the production of worker castes in ants. Wheeler (1991) showed that all ant worker castes can be generated through the modification of three developmental parameters: (1) the critical size, or the size at which metamorphosis is initiated, (2) the growth parameters that regulate the relative growth rates of organs, and (3) the reprogramming of growth parameters at the critical size. In addition, Wheeler has proposed that new castes have evolved by the addition of modified growth programs onto the end of ancestral developmental pathways. This results in ant castes being relatively well defined by size. For any given body size, an individual worker ant has a very high probability of being of a certain caste. In *Pseudoregma alexanderi*, in contrast, there is considerable overlap in body sizes between normal and soldier larvae. A similar pattern of overlap in sizes between normal and soldier larvae is found in the distantly related species *Colophina clematis* (Aoki, 1976). Therefore, it is unlikely that aphids use body size, or any critical size parameter, for switching to the soldier caste. This raises two questions: (1) how is the soldier caste produced and (2) why do the aphids not use a system more similar to the mechanisms used by ants?

Three factors combine to suggest that caste in these soldier aphids is controlled hormonally or physiologically by the mother. First, the patterns of allometry presented above suggest that the aphids do not use a size-based developmental switch. Second, the offspring should be genetically identical, since aphids reproduce via thelytokous parthogenesis during the generations when soldiers are produced, so genetic differences cannot account for the morphological differences. Finally, soldiers of *Pseudoregma alexanderi* are born viviparously, indicating that all developmental decisions and growth are completed within the mothers body. The precise mechanisms controlling the difference between normal and soldier larvae await further research.

The patterns of allometry suggest two alternative models for the timing of the switch between normal larvae and the soldier caste (Fig. 6). The first involves a developmental switch early during embryogenesis that leads to two distinct developmental trajectories (Fig. 6a). The second involves the facultative adoption of a sigmoidal growth trajectory late in embryogenesis (Fig. 6b). We suggest this second model as a possibility because many of the pattern found appear sigmoidal. However, because the soldier and normal larvae shown considerable overlap in sizes, both of the models require a switch prior to the conclusion of embryonic growth.

There is probably an upper limit of larval size defined by the mother's size. This upper limit may have forced the aphids to adopt a strategy of caste production that is not strictly size dependent. In contrast, the size of ant larvae is more constrained by nutritional limits than by physical limitations. This may have led to a system that utilizes larger larval sizes as a cue for new relative growth rates in ants (Wheeler, 1991).

We have found further evidence for Aoki and Miyazaki's (1978) initial finding of two sizes of soldiers, minors and majors, in *Pseudoregma alexanderi*. Aoki et al. (1981), however, did not find a clear bimodal distribution of soldier body sizes. It seems likely, in light of our findings (Fig. 2), that two types of soldiers are at least
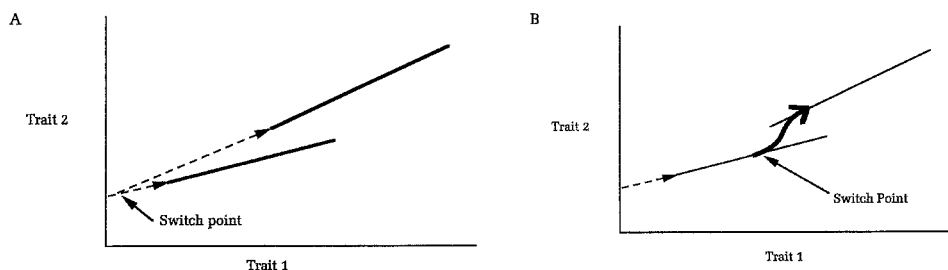
Figure 6. Two models of possible developmental pathways (dotted lines) that would produce many of the observed allometries (solid lines). During embryonic development the sizes of the two traits are assumed to follow the lines with and without arrows and stop somewhere along the solid lines. (**A**) A switch early in embryogenesis leads to divergent pathways. (**B**) A switch late in embryogenesis leads to adoption of a facultative sigmoidal developmental pathway

occasionally produced although there are no allometric differences between the two types. The behavior and defensive abilities of these two types require further investigation.

Many soldier-producing aphids do not produce larvae with an obvious morphological dimorphism (for review see Stern and Foster, 1996b). In these species, all larvae of a particular stage, for example the first instar, attack predators. However, no detailed morphometric studies coupled with behavioral observations have been performed for these species, so it is possible that within a single instar there is an undiscovered morphologically or behaviorally-specialized caste. Currently, it is believed that in aphids behaviorally-specialized defensive castes always evolve morphological specialization for this task. It will be interesting to examine the wide range of soldier-producing species (Stern and Foster, 1996a, b) to test whether morphological specialization has always co-evolved with behavioral specialization within an instar.

Acknowledgements

References

Aoki, S., 1976. Occurrence of dimorphism in the first instar larvae of *Colophina clematis* (Homoptera, Aphidoidea). *Kontyû 44*:130–137.

Aoki, S., 1977. *Colophina clematis* (Homoptera, Pemphigidae), an aphid species with "soldiers". *Kontyû 45*:276–282.

Aoki, S. and M. Miyazaki, 1978. Notes on the pseudoscorpion-like larvae of *Pseudoregma alexanderi* (Homoptera, Aphidoidea). *Kontyû 46*:433–438.

Aoki, S., S. Akimoto and S. Yamane, 1981. Observations on *Pseudoregma alexanderi* (Homoptera, Pemphigidae), an aphid species producing pseudoscorpion-like soldier on bamboos. *Kontyû 49*:355–366.

Dixon, A. F. G., 1985. *Aphid Ecology.* Blackie & Son Limited, Glasgow.

Lees, A. D., 1966. The control of polymorphism in aphids. *Adv. Insect Physiol. 3*:207–277.

Oster, G. F. and E. O. Wilson, 1978. *Caste and Ecology in the Social Insects.* Princeton University Press, Princeton, N. J.

Stern, D. L. and W. A. Foster, 1996a. The evolution of sociality in aphids: a clone's-eye-view. In: *Social Competiton and Cooperation in Insects and Arachnids: II Evolution of sociality.* (J. Choe and B. Crespi, Eds.), Princeton University Press, Princeton, N.J. in press.

Stern, D. L. and W. A. Foster, 1996b. The evolution of soldiers in aphids. *Biological Reviews of the Cambridge Philosophical Society.* in press.

Wheeler, D. E., 1991. The developmental basis of worker caste polymorphism in ants. *Amer. Nat. 138*:1218–1238.

Wilson, E. O., 1971. *The Insect Societies.* Belknap Press of Harvard University Press, Cambridge, MA.