Soldiers' Armature Changes Seasonally and Locally in an Eusocial Aphid (Homoptera: Aphididae)

by

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ABSTRACT

Size-frequency distribution of social insect castes has been reported to change temporally and spatially, and be influenced by external factors such as colony size. Here, we show for the first time that the sterile soldiers of eusocial aphids change the size and shape of their armatures, seasonally and locally. Evidence is presented that, in non-soldier individuals, the sizes of armatures were seasonally constant. Two potential mechanisms causing these morphological changes in the soldiers' armatures are discussed in relation to predation pressure on the aphid.

Keywords: caste morphology, *Ceratovacuna japonica*, defense, phenotypic plasticity, predation pressure.

INTRODUCTION

Caste lies at the heart of colonial organization in the social insects. Caste differences among colony members are principally or exclusively phenotypic rather than genetic. Environmental cues affecting caste decision include larval nutrition, egg size, inhibitory pheromones, and winter chilling (Wilson 1985). Furthermore, the worker caste is sometimes divided into subcastes, e.g., the minors and the majors, by size and body proportions (Wilson 1984), and the size-frequency distribution of workers is affected again by external factors (e.g., by colony size: Wilson 1983), and changes seasonally, locally, or ontogenetically (Wilson 1983; Johnston & Wilson 1985; Bourke 1999; Yang 2006).

In eusocial aphids, the soldier caste does not reproduce but is dedicated to defending the colony against enemies (Stern & Foster 1996). As aphid

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soldiers are principally 1st instar larvae (Aoki 1977a, b; Aoki *et al*. 1981), the caste determination (soldier or non-soldier) is supposed to be made in the egg- or embryo stages. Shibao (1999) reported that larger colonies have a higher proportion of soldiers, suggesting that colony size affects caste determination. As the soldier caste is indispensable in defending the colony against predators (Stern & Foster 1996), the caste ratio, and further, the soldiers' size-frequency distribution, are predicted to change spatially and temporally depending on predation pressure. This prediction, however, has not been tested, and the soldiers' size-frequency distribution has not been assessed (Aoki & Imai 2005).

Here, we provide the first documentation of seasonal and geographical changes in size-frequency distribution of soldier aphids. We focus on the sizes of the soldiers' armatures because they are effective to deter enemies (Stern & Foster 1996), and are supposed to change depending on the enemies' intensity. Data are presented to show that in all three investigated populations, the armature size changed seasonally and the mean size varied significantly among the populations.

MATERIALS AND METHODS

Study organism

Ceratovacuna japonica is a cyclical parthenogenetic aphid which produces galls on the leaves of its primary host *Styrax japonica* (Ebenales, Styracaceae) in the fall, and moves to its secondary host *Sasa senanensis* (Poales, Poaseae) and constitutes an open colony from spring to summer on the leaves (Aoki & Kurosu 1991). In at least central to eastern Japan, however, the gall generation is usually skipped and the aphids reproduce mostly asexually all year round in open colonies (Carlin *et al*. 1994). There are two distinct castes in the first instar larvae: non-soldiers and "pseudoscorpion-like" soldiers. The soldiers never molt to the second instar larvae and are obligatorily sterile.

Field sites and sampling

Three populations (A, B, and C) of *C. japonica* were studied in Nagano, central Japan. The host plant *S. senanensis* predominated in the forest floor at sites A and C (the distribution range extended over several kilometers). At site B, *S. senanensis* was distributed in small patches (100-1000 m^2 each)

which dispersed at intervals of hundreds of meters. At each site, *C. japonica* is abundant in open forest edges, especially along roads, and sometimes host plants wither due to the infestation.

The environmental settings of the sites are as follows. Site A: Ichino-sawa valley, N36°18'5" E137°47'50", alt. c. 1100m, a deciduous forest dominated by *Quercus serrata* and *Castanea crenata*. Site B: Norikura-kogen highland, N36°7'19' E137°37'42" alt. c. 1400m, a deciduous forest dominated by *Betula platyphylla var. japonica* and *Sorbus commixta*. Site C: Sakai-toge pass, N36°21'11' E137°42'31', alt. c. 1400m, a broad- and thin-leaved mixed forest dominated by *Larix kaempferi* and *Quercus serrata*. Here at site C, *Formica yessensis* (Formicidae, Formicinae) routinely visited the aphid colonies to collect honeydew.

Aphid samples were collected monthly from the three populations from June to August 2006. On each sampling occasion, 20-40 individuals from 15 randomly-selected colonies of *C. japonica* were sampled. "Colony" is defined here as a group of aphids living on a leaf of *S. senanensis* (c. 10-1000 individuals). The sample colonies were selected randomly from a roadside plot of 10m x 30m at each site (10m x 10m at site C) on each sampling occasion. The collected samples were preserved in 70% ethanol.

Caste morphology

We measured shape and size of first instar soldier larvae. The sample sizes are: population A, Jun: n=26; Jul: n=21; Aug: n=27, population B, Jun: n=20; Jul: n=44; Aug: n=33, population C, Jun: n=35; Jul: n=37; Aug: n=31). We also measured those of non-soldier first instar larvae of population A (Jun: n=23; Jul: n=26; Aug: n=29). These samples were cleared, stained and mounted as described in Kozarhevskaya (1968). We measured (1) body length, (2) Horn length, (3) Foretibia length, (4) Forefemur length and (5) Forefemur width (Fig. 1), using PHOTO MEASURE (Kenis limited) and a binocular microscope. The anatomical features which were the subjects of measurements (2) - (5) are considered defensive armatures because soldiers use their forelegs and frontal horns to fight against predators (Stern & Foster 1996, Aoki *et al*. 1981). We measured each character five times for each individual, and used the mean value as the definitive measurement.

Table 1. F-statistics of ANOVA for body-size measurements. For the soldiers (first instar larvae), three seasons (June, July and August) for three populations (population A, B and C) are analyzed. For non-soldiers (first instar larvae), three seasons (Jun, July and August) for population A are analyzed. For the F-statistics, the sequential Bonferroni adjustments are applied.

| Measurements | Soldier (N= 274) | | | Non-soldier (N= 78) |
	Season	Population	Season × Population	Season
Body Length	2.43	45.40***	7.31***	2.79
Horn Length	25.46***	18.64***	0.53	2.20
Foretibia length	33.40***	47.27***	4.03**	0.09
Forefemur length	33.40***	32.92***	4.37**	0.07
Forefemur width	3.78*	25.05***	2.22	2.66

*$p<0.05$; **$p<0.01$; ***$p<0.001$

(3)

(2)

(5)

(4)

(1)Body length

(2)Horn length

(3)Foretibia length

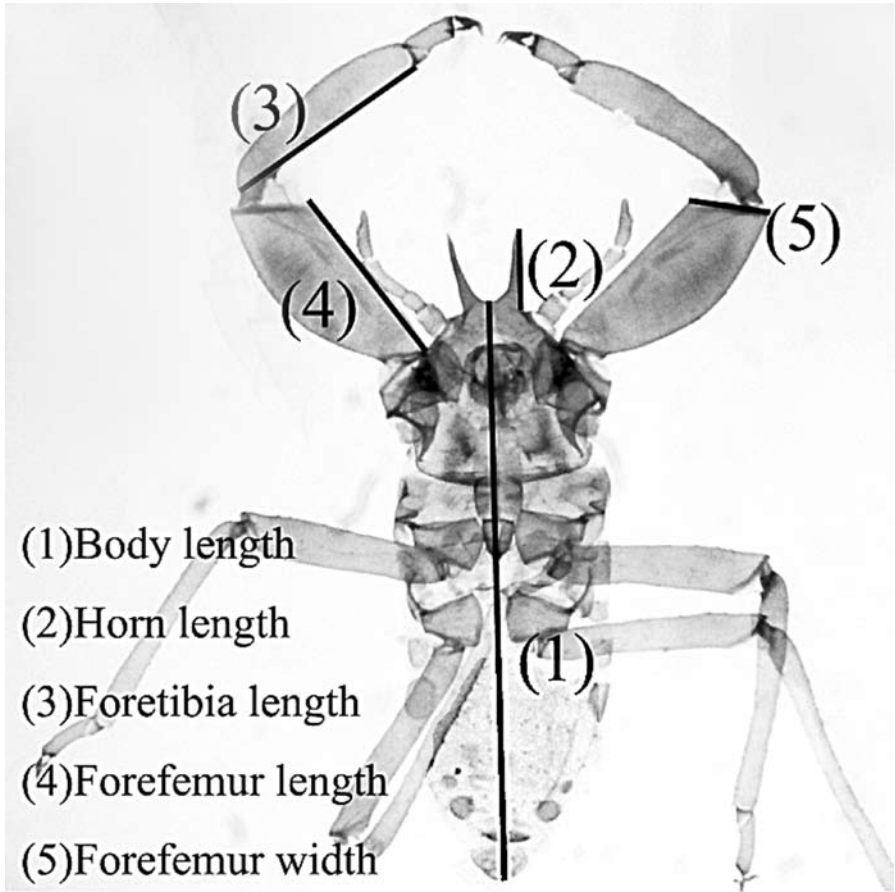(4)Forefemur length

(5)Forefemur width

(1)

Fig. 1. A soldier of *Ceratovacuna japonica*. The measured characters are indicated.

Statistical analyses

Statistical analyses were performed by using R (version 2.3.1; R Development Core Team 2005). For the morphometric analyses of soldiers and for differences between castes, we used Two-way ANOVA. For the analysis of seasonal changes in non-soldiers, we used One-way ANOVA. All statistical tests were corrected for multiplicity by the sequential Bonferroni adjustment (Rice 1989).

RESULTS

The four defensive characters of *C. japonica* soldiers changed seasonally and locally (Table 1) and were larger in the later season (Fig. 2) while body length did not change seasonally (Table 1). Contrary to this, non-soldier first instar larvae showed no seasonal morphological changes in the four defensive characters (Table 1).

In population A, we compared sizes between soldiers and non-soldiers. The sizes of non-soldiers were: (1)1.036±0.021mm, (2)0.107±0.002mm, (3)0.347±0.005mm, (4)0.284±0.005mm, and (5)0.082±0.002mm in June; (1)1.013±0.014mm, (2)0.107±0.003mm, (3)0.353±0.005mm, (4)0.288±0.003mm, and (5)0.102±0.021mm in July; (1)1.008±0.018mm, (2)0.114±0.002mm, (3)0.355±0.004mm, (4)0.289±0.003mm, and (5)0.079±0.002mm in August. The sizes of (1)-through-(5) were significantly larger in soldiers than in non-soldiers (Two-way ANOVA: F(1) between caste=571.95, $p<0.001$; F(2) between caste =1488.77, $p<0.001$; F(3) between caste =1518.07, $p<0.001$; F(4) between caste =2994.06, $p<0.001$; F(5) between caste =1658.05, $p<0.001$).

DISCUSSION

The size of armatures in soldier aphids changed seasonally and locally (Table 1). In contrast, non-soldier aphids exhibited seasonally-constant morphology (Table 1), indicating that seasonal changes in resource quality and/or quantity do not fully explain the size changes of soldiers. This proposition is supported by the fact that the host plant *S. senanensis* decreases its net primary production in summer (Sakai & Akiyama 2005) when the soldiers get larger. While morphological differences between soldiers and non-soldiers have been previously documented (Shingleton & Foster 2001; Stern *et al.*
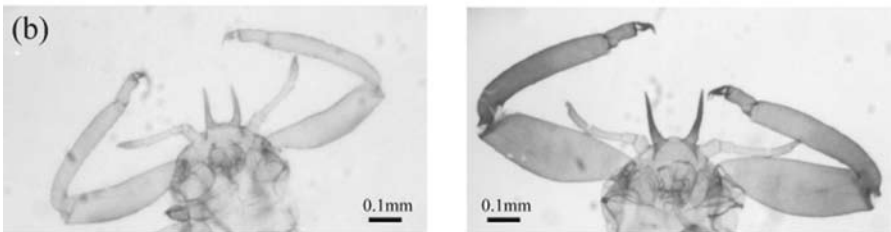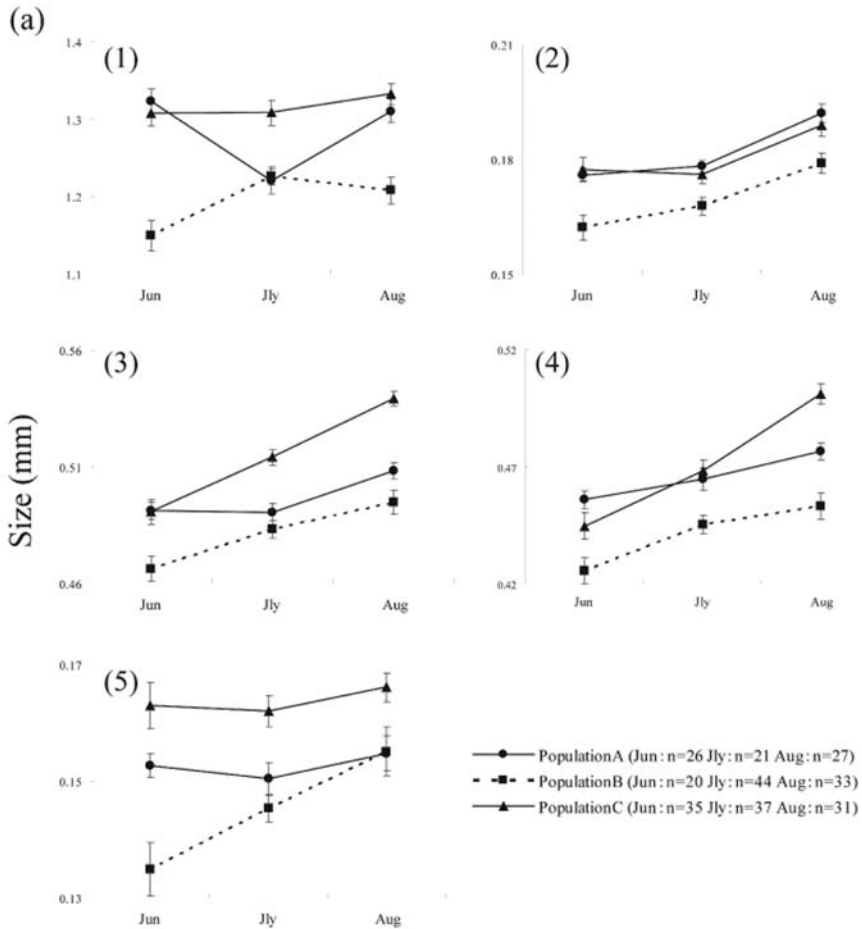
Fig. 2. (a) Seasonal changes in the mean (± s.e.) morphological measurements of *C. japonica*. (1): Body length (2): Horn length (3): Foretibia length (4): Forefemur length (5): Forefemur width. See Fig. 1 for details. (b) Left: soldier of population A sampled in June, right: soldier of population A sampled in August.

1997), we believe this is the first report of morphological variation within the soldier caste for eusocial aphids .

We propose two factors causing the change in armature size. One is phenotypic plasticity, in which armature sizes change in response to enemy pressure, especially in response to the dominant predatory moth, *Atkinsonia ignipicta* (Lepidoptera, Oecophoridae). Indeed, at all the study sites, armature size of the soldiers increased in August when predators are most abundant (M.H., personal observation), and the armature size was smaller in population C where the aphids are ant-attended and less predators were observed near aphid colonies (M.H., personal observation).

Another factor that may cause the seasonal change in soldier size is the effect of feeding on armless aphid colonies by predators. If predators prey exhaustively on clones (colonies) that (genetically) produce small soldiers, then clones (colonies) that produce large soldiers survive, resulting in a seasonal increase of average armature size of aphid population. This hypothesis assumes that clones producing small soldiers have unknown adaptive advantages over those producing large soldiers in other seasons (fall or winter). Such seasonal changes in clone composition dynamics have been reported in nonsocial aphids (Via & Shaw 1996; Vorburger 2006), but the proximate mechanisms have yet to be elucidated.

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