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Soldiers with large weapons in predator-abundant midsummer: phenotypic plasticity in a eusocial aphid

Mitsuru Hattori · Osamu Kishida · Takao Itino

Abstract Eusocial aphids produce sterile individuals ("soldiers") that specialize behaviorally and morphologically to protect their colony from predators, while production of soldiers can negatively affect colony growth because of reproductive allocation and opportunity cost. Hence, a cost-saving soldier production strategy is expected to be favored. Here, we hypothesize that, to save the cost, a eusocial aphid *Ceratovacuna japonica* produces soldiers with smaller weapon in the season when predators are not abundant. The abundance of two specialist lepidopteran predators (i.e., *Taraka hamada* and *Atkinsonia ignipicta*) of *C. japonica* dramatically increased, and aphid colony size significantly decreased, from July to August. In line with these, the soldiers in August had larger weapons (i.e., frontal horns) than those in June, indicating a correlational increase in weapon size with predation pressure. We predict that a reliable prospective signal indicating the coming of midsummer (environmental temperature) induces mother aphids to produce soldiers with larger weapons. Experiments clarified that soldiers produced at 20 °C (typical temperature of July to August) had larger weapons than those produced at 15 °C (typical temperature of May to July). Such phenotypic plasticity appears to be adaptive to maximize the fitness of *C. japonica* under a temporally variable but predictable predation environment. These results indicate that *C. japonica* aphids not merely have distinctive reproductive—and soldier castes, but also produce differentially armed soldiers in a habitat with temporally changing predation risks.

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Introduction

An important goal of evolutionary ecology is to understand how variations in organismal traits relate to ecological environments (Johnson et al. 2009). Biological interactions such as predation can lead to variations in functional traits among and within populations of prey species (Tollrian and Harvell 1999; Thompson and Cunningham 2002; Kishida et al. 2010), for example, predators bias trait distribution in prey populations by selecting for prey individuals with an effective defensive phenotype at the expense of those with a less effective phenotype. Plastic induction of trait changes in prey can also shape prey trait variation. Prey organisms exhibit defensive strategies in response to environmental signals directly or indirectly indicating predator emergence (Lively 1986a; Van Buskirk and Relyea 1998; Tollrian and Harvell 1999; Kishida et al. 2009b). Therefore, spatiotemporal variation in the strength of predator–prey interactions can generate trait variation within and among populations of prey species (Trussell and Smith 2000; Toju and Sota 2006). Understanding the causal mechanisms and consequences of trait variation can provide deep insights into how organismal phenotypes are shaped evolutionarily and how they affect population and community dynamics (Agrawal et al. 2007; Mougi and Kishida 2009; Kishida et al. 2010).

Aphids have evolved diverse anti–predation strategies (secretion of venomous protease, producing alate individuals, defensive mutualism with ants, and eusociality) through their interactions with predators (Aoki 1977; Weisser et al. 1999; Shingleton and Foster 2000; Shibao et al. 2004; Stadler and Dixon 2005). The most extreme anti-predatory strategy among aphids is eusociality: the production of obligatory sterile individuals that protect the colony from enemies (Aoki 1977). Several aphid species belonging to the Pemphigidae and Hormaphididae exhibit eusociality (Stern and Foster 1996). A colony of eusocial aphids consists of two distinct castes: (1) the reproductive caste (hereafter "reproductive individuals") and (2) the sterile caste that spends less time feeding and specializes in colony protection ("soldiers"). Soldiers maintain colony growth by lowering the likelihood of destructive attacks on their colony by a predator (Hattori et al. 2013). However, the production of soldiers itself can negatively affect colony growth, because soldiers are sterile and, moreover, the mother aphids, by producing soldiers, incur reproductive resource allocation and opportunity costs (Stern and Foster 1996). This cost-benefit perspective leads us to hypothesize that production of soldiers might be adjusted plastically depending on the spatiotemporal variation in predation risk.

In support of this hypothesis, Shingleton and Foster (2000) have experimentally demonstrated that soldier production is induced in *Pseudoregma sundanica* (Van der Goot) (Homoptera: Hormaphididae) by an environmental signal implying predation risk (i.e., presence or absence of tending ants). Some patterns observed in natural eusocial aphid populations are also consistent with our hypothesis. For example, Shibao (1998) observed a high abundance of soldiers in a *P. bambucicola* (Takahashi) (Homoptera: Hormaphididae) colony when predators were also abundant. Thus, these studies have deepened our understanding of local variation in the abundance of soldiers in eusocial aphids. However, in eusocial aphids, the efficacy of defense can also be achieved by an increase in the ability of individual soldiers to repel a predator (Pike and Foster 2008). For this reason, it is important to investigate the trait variations of individual soldiers within a single species,

although only a few studies have done so far (but see Aoki 1984; Hattori and Itino 2008). A soldier's ability to repel a predator depends on the values of the soldier's attack traits. If mother aphids produce strong soldiers in response to signals directly or indirectly indicating predation emergence, the proportion of soldiers with a high-performance phenotype should be higher in colonies exposed to high predation risk than in those exposed to less predation risk. Thus, by investigating trait variation in soldiers of a single species and its relation to predation risk, we can deepen our knowledge of the adaptive strategies of eusocial aphids and gain further insight into the ecology of social insects. In the present study, we investigated temporal variation in defensive morphological traits of soldiers in a eusocial species of cerataphidini aphids and explored its causal mechanism.

Several cerataphidini species produce both reproductive individuals (i.e., the reproductive caste) and soldiers (i.e., the soldier caste). Both castes are characterized by morphological traits which are linked to their ecological roles. Reproductive individuals have shorter horns and forelegs than soldiers. Their horns are used for competition over feeding sites on the secondary hosts (Morris and Foster 2008). To rob other conspecifics on feeding site, the reproductive individuals stab the back of other individuals with their horns (Aoki and Kurosu 1985). In contrast, soldiers have longer horns and longer and thicker forelegs than their non-soldier reproductive siblings at similar instar stages (Aoki and Miyazaki 1978; Shingleton and Foster 2001; Hattori and Itino 2008), and they protect their colony from predators by using these large morphological traits (Hattori et al. 2013). Soldiers grasp a predator that has intruded into their colony with their forelegs and then force their frontal horns into the predator to kill it. Such attack behavior is an effective colony defense and is common among many eusocial cerataphidine aphids [*P. alexanderi* (Takahashi) (Aoki et al. 1981); *P. bambucicola* (Ohara 1985); *P. koshunensis* (Takahashi) (Homoptera: Hormaphididae) (Arakaki 1992); for a review see (Stern and Foster 1996)]. Therefore, the forelegs and frontal horns of soldiers, which are larger than those of reproductive individuals, are important attack traits, and soldiers having larger forelegs and frontal horns might be expected to be more effective defenders of their colony.

Given this ecological function of the soldiers' morphological traits, we infer that soldier morphology might vary with predation risk. If mother aphids can plastically produce soldier offspring with elongated horns and forelegs in response to signals indicating predation risk, the proportion of soldiers having large horns and forelegs in a colony exposed to high predation pressure should be higher than that in a colony exposed to less predation pressure. To test this hypothesis, we investigated morphological variation in soldiers of the eusocial aphid species *Ceratovacuna japonica* (Takahashi) (Homoptera: Hormaphididae). We previously showed significant spatial and temporal variation in the morphology of soldiers of *C. japonica* (Hattori and Itino 2008). From June to August, the average size of their forelegs and horns becomes larger with time. A possible mechanism, phenotypic plasticity, could be mechanistic causes of this temporal variation in weapon size in relation to predation risk.

In the present study, we first investigated temporal patterns of variation in the number of predators and soldier morphology in three natural populations of *C. japonica* and determined the correlation between them. Our field study showed a correlative temporal relationship between soldier weapon size and predation risk. The number of predators was higher, and the soldiers in aphid colonies had larger weapons in midsummer (August) than in early summer (June to July). Since the observed temporal trend of predation pressure (i.e., high abundance of predators in midsummer) seems to be general in this species (Moriuti 1982; Banno 1997), we further hypothesized that reliable signals indicating the coming of midsummer (e.g., environmental temperature) induce mother aphids to produce soldiers with larger weapons. Therefore, we next conducted an indoor experiment to determine whether mother aphids plastically produced soldiers with larger weapons in

response to environmental temperature. The experimental results clearly showed that soldiers with large weapons were produced at a temperature consistent with coming of midsummer. Thus, temperature-induced phenotypic plasticity of mother aphids may be a mechanism shaping the trait variation dynamics of soldiers in this species.

Materials and methods

Ceratovacuna japonica and its predators

The eusocial aphid species *C. japonica* is a common woolly aphid in Japan (Kurosu and Aoki 1994). This species has a heteroecious (i.e., host alternating) and cyclical parthenogenetic (i.e., with an asexual phase and a sexual phase) life cycle and has one primary host plant species, *Styrax japonica* (Sieb. et Zucc.) (Ebenales: Styracaceae), and several secondary host plant species (Poaceae species) (Aoki and Kurosu 1991, 2011). It reproduces sexually on the primary host in autumn and parthenogenetically on a secondary host all year round except for severely cold months (Takahashi 1958). Alate individuals (sexuparae) to migrate to the primary host plant are rarely produced on the secondary hosts (Carlin et al. 1994). Here, we studied soldiers produced from June to August in the parthenogenetic reproduction phase of aphid populations on a secondary host species, *Sasa senanensis* (Rehd.) (Poales: Poaceae).

We defined an aggregation of aphid individuals on a single leaf of *S. senanensis* as a colony, because this aggregation consists of various instars of aphids including adults and nymphs and persists for a relatively long period (i.e., several months). An aphid colony is founded by a few individuals. Founders parthenogenetically produce their clone in the colony, and sometimes, the aphids propagate until they wither the leaf of *S. senanensis*. Such colonies ranging from small, founding stage to mature, overpopulated stages exist at the same season in the field (M. Hattori personal observation).

This aphid species is eusocial, producing obligately sterile, pseudoscorpion-like soldiers on secondary host plants. The only role of these soldiers, which have longer frontal horns and forelegs and a larger body size than non-soldier aphids at the same instar (Hattori and Itino 2008), is to protect the colony against predators (Hattori et al. 2013). The soldiers do not develop into second instar nymphs (Aoki et al. 1981).

Larvae of *Taraka hamada* (Druce) (Lepidoptera: Lycaenidae) and of *Atkinsonia ignipicta* (Butler) (Lepidoptera: Stathmopodidae) are the primary predators of these aphids. These predators are observed only at colonies of aphids on their secondary host plants and are thus specialist predators. Adults of these predators are abundant from June to July when they lay their eggs in aphid colonies [*T. hamada*, (Banno 1997); *A. ignipicta*, (Moriuti 1982)]. *Taraka hamada* larvae in particular sometimes exert heavy predation pressure on aphid colonies (Banno 1997). Both species of predatory larvae produce silken threads with which they make a nest on *S. senanensis* leaves, and they usually remain in their nest, where they cannot be attacked by soldier aphids, coming out only to eat aphids. Although other potential predators such as Syrphidae and Chrysopidae species are able to prey upon *C. japonica*, these predators have rarely been observed to prey upon this species in the field.

Behavior and morphology of the soldiers

The soldiers usually stay at the periphery of the colony and sometimes move around, in contrast to reproductive individuals, which are less mobile and spend most of their time

feeding by sucking phloem sap of *S. senanensis* through their proboscis. Once the soldiers find a predator, they grasp it with their forelegs and stab it using their horns (Hattori et al. 2013). Therefore, the horns and forelegs of the soldiers are inferred to be attack offensive morphological traits. In contrast, their second legs and hindlegs seem not to be important for their attack, because once a soldier has grasped the predator, it hangs on and does not use its second legs or hindlegs. Although, in many cases, soldiers which have attacked the predator are killed by the predator, they sometimes succeed in repelling the predator from their colony (M. Hattori personal observation).

Temporal variation of aphid colony size and predation risk

To estimate the temporal variation in predation risk, we measured the number of aphids in colonies belonging to each of three wild populations, and the number of predators of each colony. We used three geographically separated populations of *C. japonica* in Nagano Prefecture, central Japan (population A, 36°18′N, 137°47′E; population B, 36°21′N, 137°42′E; population C, 36°7′N, 137°37′E). All populations were located at the edges of deciduous forests, where the secondary host plant *S. senanensis* is abundant.

Our field surveys were carried out from June to August 2006, and each population was surveyed 1–3 times each month. We finished our field survey in August because aphid colonies have become very rare in September due to the predation by *A. ignipicta*. During each survey, we randomly selected 15–50 *S. senanensis* leaves with an aphid colony in each population from which to collect aphids and predators. In June, July, and August, we, respectively, collected aphids and predators from 44, 48, and 34 colonies in population A; 28, 15, and 14 in population B; and 53, 32, and 38 in population C. All aphids and predators on selected leaves were fixed in 70 % ethanol and then preserved in a plastic bag. We counted the number of preserved aphids (soldiers and reproductive individuals) and of *T. hamada* and *A. ignipicta* larvae under a binocular microscope. To examine whether colony size (number of aphids per colony) in each population varied among the months, we conducted two-way analysis of variance (ANOVA) for the number of aphids in each colony, considering month as a fixed factor, population as a random factor, and their interaction. Because there was significant temporal variation in colony size (see "Results"), we compared colony size between each pair of months to determine how aphid abundance changed from month to month. In the post hoc comparison, we also performed ANOVA, considering month as a fixed factor, population as a random factor, and their interaction. Similarly, to examine whether the number of predators associated with each population varied temporally, we conducted two-way ANOVA on the number of predators in each colony, considering month as a fixed factor, population as a random factor, and their interaction. This analysis was also followed by post hoc comparisons because we found a significant temporal effect.

Analyses of morphologies of soldiers in wild populations

Soldier morphology was analyzed using samples collected and described by Hattori and Itino (2008) ($n = 241$) along with additional samples that were collected but not measured by Hattori and Itino (2008) ($n = 82$). Although Hattori and Itino (2008) outlined the sampling methods, we describe them here in greater detail.

We randomly selected 18–63 soldiers from each population in each month (i.e., each month, 0–5 soldiers were selected per colony). In June, July, and August, we, respectively, measured a total of 28, 63, and 59 soldiers from population A; 18, 39, and 27 from population B; and 30, 34, and 25 from population C. Following the method of Kozarzhevskaya ([1986]), we cleared, stained, and mounted selected soldier specimens to make slidemount samples. We captured digitized images of the slidemount samples from their ventral side and used these digital images to quantify the length of five morphological traits: body length, horn length, foreleg length, second leg length, and hindleg length (Fig. [1]). Two of these traits (second leg and hindleg lengths) were not measured by Hattori and Itino ([2008]). We measured not only defensive traits but also other traits so that we could determine whether only defensive traits varied temporally. We used Photo Measure software (Kenis Ltd., Osaka, Japan) for the measurement.

We calculated the colony mean value of each morphological trait in each month and used these values for the following analyses. To examine whether the morphology of the soldiers varied temporally, we conducted two-way ANOVA on the morphological traits, considering month as a fixed factor, population as a random factor, and their interaction. When we found significant temporal variation in a trait (see "Results"), we compared the trait values between each pair of months to determine how the soldier morphology changed temporally. We also used ANOVA for the post hoc comparison, considering month as a fixed factor, population as a random factor, and their interaction.

Experimental test of the phenotypic plasticity hypothesis: Do mother aphids produce soldiers with different weapon sizes depending on temperature?

Objective and hypothesis

Field research provided evidence that aphid colonies are exposed to higher predation pressure in August than in June (see "Results"). This temporal trend of predation risk is probably common in this species (Moriuti [1982]; Banno [1997]). In addition, the
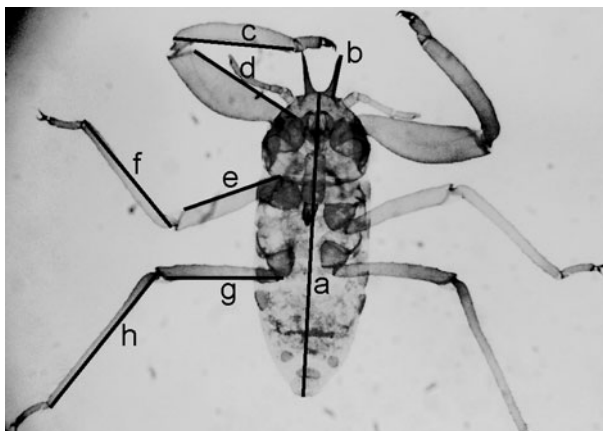

Fig. 1 Digitized image of a *C. japonica* soldier showing the measurements made on each individual. Dimensions *a–h* are as follows: *a* body length, *b* horn length, *c + d* foreleg length, *e + f* second leg length, and *g + h* hindleg length

morphological analysis results indicated that mean values of soldier weapon size were significantly larger in the high-risk month (August) (see "Results"). From an adaptationist perspective, we can predict that such temporal variation in weapon size might be caused partly by adaptive phenotypic plasticity in reproduction by mother aphids. Life history theory predicts that prey should use reliable signals, indicating the potential increase in predation risk to exhibit adaptive phenotypic plasticity (i.e., an inducible defense) (Moran 1992; Tollrian and Harvell 1999). To examine this plasticity hypothesis, we focused on temperature as a possible environmental cue. We predicted that mother aphids experiencing a summertime (July to August) temperature would produce soldiers with larger weapons than those experiencing springtime (from May to July) temperatures. To test this prediction, we conducted the following indoor experiment in which we examined the morphology of soldiers produced by mother aphids having identical genetic backgrounds (i.e., clones) on leaves of *S. senanensis* in incubators set at either 15 or 20 °C, which are typical temperatures of June 1 to June 30 (monthly mean temperature $\pm$ SE, 16.7 $\pm$ 0.38 °C), and of July 1 to July 31 (monthly mean temperature $\pm$ SE, 19.9 $\pm$ 0.37 °C), respectively, in our research fields (Fig. 2). The environmental temperature was recorded by using data loggers (Ondotori TR–57U, T&D Co, Nagano, Japan) on a 15-min basis at the aphid population where we got the aphid clone for our indoor experiment as described hereinafter.

Preparation of clonal mother aphids

To rigorously test the plasticity hypothesis, we excluded the possible effects of differences in genetic background by using a single clone lineage of this species in the experiment. To establish a clonal colony (i.e., the original colony), we arbitrarily selected one natural colony of *C. japonica* in Nagano Prefecture, central Japan (36°16′N, 137°49′E), and removed all aphid individuals other than one mother aphid (i.e., founder) from the colony in June 2009. This original colony was then covered with a fine-mesh net to prevent immigration of aphids from other colonies. After 1 month, the original colony successfully expanded. We then transplanted several reproductive aphids from the original colony to several uncolonized leaves of nearby *Sasa* vegetation. These leaves were also covered with

Fig. 2 The daily average temperature from June 1, 2010, to August 31, 2010. *Error bars* denote SE
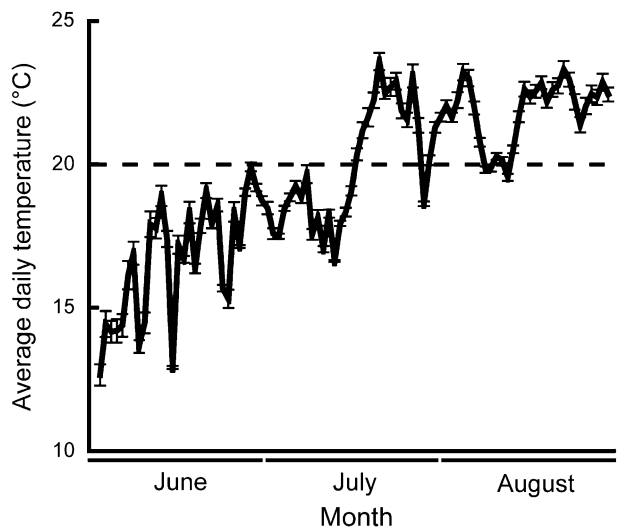
a fine-mesh net. After these descendant colonies grew, we collected several reproductive aphids from these leaves and transplanted them to *S. senanensis* seedlings cultivated in pots in environmental chambers in our laboratory. These *S. senanensis* seedlings had 20–30 leaves and had grown for at least 1 month in the chambers at 20 °C, 65 % humidity, and a 16:8 day/night cycle.

Experimental treatments and statistical analyses

To examine whether the morphology of the newborn soldiers was differentiated depending on temperature, we set the temperature of the environmental chambers at either 15 or 20 °C. Although we conducted this experiment for 90 days and collected newborn soldiers beginning 3 days after the start of the experiment, we used only data of soldiers that were collected from 30 to 90 days after the start to exclude any possible maternal effect (i.e., environmental effects of the natural habitats). Aphids produced at least three generations during our experiments. In total, we collected 41 soldiers from 4 pots and 134 soldiers from 6 pots in the 15 and 20 °C chambers, respectively. We fixed the collected soldiers in 70 % ethanol and made slidemount samples. In the chambers, humidity of over 65 % and a 16:8 day/night cycle were maintained throughout the experiment. Water (20 ml) was provided to the potted plant every second day.

We measured body length, horn length, and foreleg length. To compare these morphological traits between temperature treatments (i.e., 20 and 15 °C), we conducted nested ANOVAs on the morphological traits, considering temperature treatments as a fixed factor and the pot as a nested random factor.

Statistics

We performed the statistical analyses with the JMP v. 8.0.2.2 statistical package (SAS Institute). For the parametric tests, the numbers of aphids and predators were transformed into natural logarithms to satisfy the normality and constant variance requirements of the tests (Sokal and Rohlf 1995). Because multiple comparisons (we conducted post hoc tests in the analyses of both aphid and predator numbers and compared five traits in the morphological analyses) may increase the possibility of a type I error, we adjusted the significance level for each comparison by controlling the false discovery rate [FDR, (Verhoeven et al. 2005)] according to the method described by Benjamini and Hochberg (Benjamini and Hochberg 1995).

Results

Temporal variation in the number of aphids and predators in the wild populations

The colony size (number of aphids) varied temporally ($F_{2,4} = 13.94$, $P = 0.012$; Fig. 3). The aphid colony size increased slightly from June to July, but decreased to around half the July size from July to August (post hoc comparisons using the FDR correction: June × July, $F_{1,3} = 16.98$, $P = 0.044$; June × August, $F_{1,3} = 1.91$, $P = 0.29$; July × August, $F_{1,3} = 50.48$, $P = 0.008$).
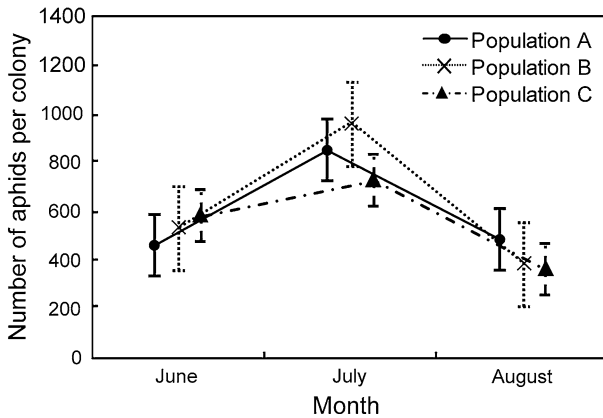
The number of predators also varied temporally ($F_{2,4} = 43.40$, $P = 0.002$; Fig. 4). Post hoc comparisons with FDR correction revealed that the number of predators increased

from July to August (June × July, $F_{1,3} = 1.71, P = 0.33$; June × August, $F_{1,3} = 137.26$, $P = 0.004$; July × August, $F_{1,3} = 111.73, P = 0.002$).

Temporal variation in morphological trait lengths of soldiers in the wild populations

Body length did not vary temporally ($F_{2,4} = 0.38, P = 0.704$). Second leg length and hindleg length varied temporally, but their statistical significance levels were marginal (second leg length, $F_{2,4} = 7.75, P = 0.042$; hindleg length, $F_{2,4} = 7.81, P = 0.041$). In contrast, horn length and foreleg length of the soldiers exhibited significant temporal variation (horn length, $F_{2,4} = 23.35, P = 0.005$; foreleg length, $F_{2,4} = 15.13, P = 0.013$; Fig. 5). Post hoc comparisons with FDR correction revealed that horn length in August was significantly longer than that in June or July (June × July, $F_{1,3} = 5.96$, $P = 0.131$; June × August, $F_{1,3} = 28.25, P = 0.031$; July × August, $F_{1,3} = 65.51, P = 0.007$; Fig. 5). We also found a significant tendency for foreleg length to be longer in July and August than in June (June × July, $F_{1,3} = 31.52, P = 0.028$; June × August, $F_{1,3} = 23.06, P = 0.040$; July × August, $F_{1,3} = 6.23, P = 0.128$), but these differences were not significant after the significance levels were adjusted by the FDR correction method (Fig. 5).

Fig. 3 Temporal variation in aphid colony size. *Error bars* denote SE. We plotted the number of aphids per colony from June to August

Fig. 4 Temporal variation in the number of predators. *Error bars* denote SE. For the number of predators (predator abundance), we summed the numbers of *T. hamada* and *A. ignipicta* larvae
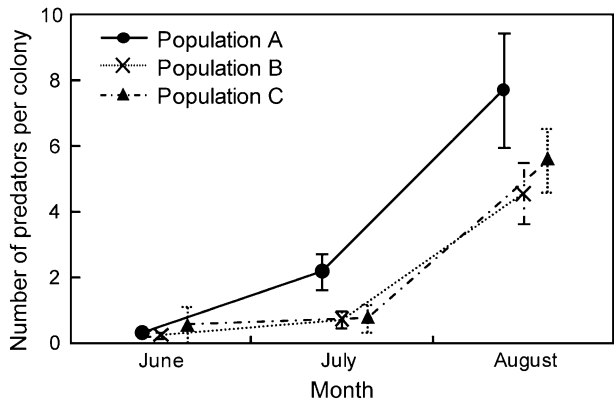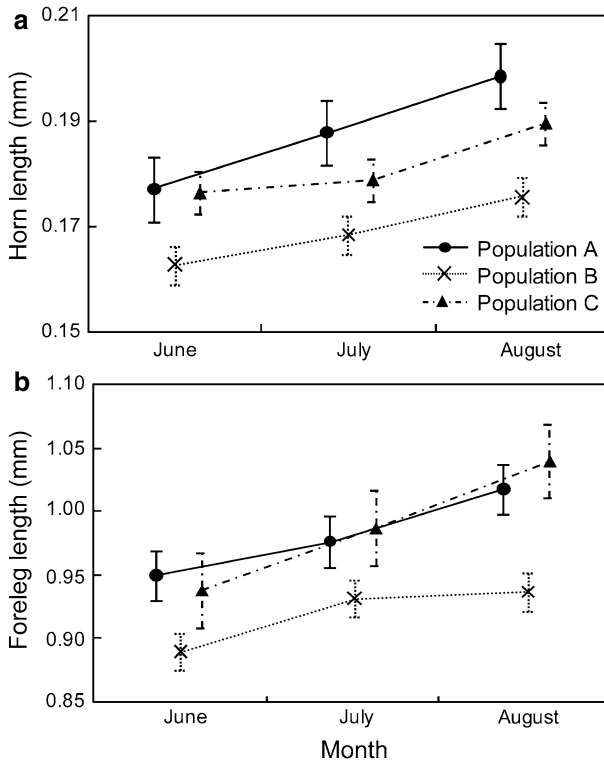
Fig. 5 Temporal variation in soldier morphology. The average lengths of horn (**a**) and foreleg (**b**) of soldiers from June to August are shown. *Error bars* denote SE

Phenotypic plasticity of mother aphids in response to temperature in the indoor experiment

Body length of soldiers did not vary between the temperature treatments ($F_{1,8} = 0.03$, $P = 0.870$; Fig. 6a). In contrast, horn length (Fig. 6b) and foreleg length (Fig. 6c) of the soldiers were significantly longer at 20 °C than at 15 °C (foreleg length, $F_{1,8} = 8.38$, $P = 0.008$; horn length, $F_{1,8} = 29.91$, $P < 0.001$).

Discussion

The number of specialist predators showed a dramatic fourfold increase from July to August, and aphid colony size decreased by about half from July to August (Figs. 3, 4). Furthermore, in September, we were not able to easily observe aphid colonies in any population. These results suggest that predation pressure to *C. japonica* by their specialist predator increased intensely from early summer to midsummer. Similar temporal patterns of predator increase, and declines in aphid abundance were described previously (Moriuti 1982; Banno 1997). By comparing population dynamics between predator-free colonies in which predators were artificially removed and natural predator-exposed colonies in which predators were not artificially removed, Banno (1997) demonstrated that dramatic decrease

in aphid populations in midsummer was caused by predation by the abundant predators at that time. Therefore, *C. japonica* populations are regarded to be exposed to intensive predation from specialist predators in midsummer. These imply that colony protection is more important in midsummer than in other seasons in *C. japonica*.

Soldier morphology also varied temporally in the wild populations. Consistent with the temporal variation in predator abundance, the soldiers' horns, which are used as weapons against predators, were significantly longer in midsummer (Fig. 5). Soldiers' forelegs, which are used as weapons against predators, second leg length, and hindleg length, which are not used as weapons, were also longer in midsummer, but their significance was marginal. On the other hand, we did not find temporal variation in soldiers' body length. These results show that when predation risk was high (August), the aphid colonies had more soldiers with large weapons than when predation risk was low (June and July). Interestingly, the horn and foreleg length of normal (reproductive caste) nymphs do not show temporal variation in this species (Hattori and Itino 2008). Temporal variation in horn length and foreleg length is thus specific to the soldier caste.

What factors are responsible for the temporal variation in the soldiers' weapons, which correlate with the temporal predation risk? We hypothesized that the temporal variation in weapon size of soldiers is caused partly by phenotypic plasticity in reproduction by mother aphids in response to predation risk. Given that emergence of predators in midsummer is a consistent phenological pattern, predation risk may be predictable for the aphids, allowing them to cope with the predation risk by responding to a reliable environmental signal indicating the coming of midsummer. So, we predicted that mother aphids would produce soldiers with large weapons in response to environmental temperature, which is a possible predictable signal, and confirmed this prediction by our indoor experiment. Soldiers' weapon sizes at 20 °C (typical temperature of July to August) were significantly larger than those at 15 °C (typical temperature of May to July) (Fig. 6). Our indoor experiment confounded environmental temperature and host plant quality because host plant may change quality in response to environmental temperature. However, the following fact suggests that the soldiers' weapon size is less likely affected by host plant quality. In the wild, even in the same season, the host plant quality has large spatial variation because density of the aphids changes dramatically between plants. Despite this, the range of soldiers' weapon size obviously differed between months (Fig. 7), indicating the limited influence of plant quality on the weapon size.
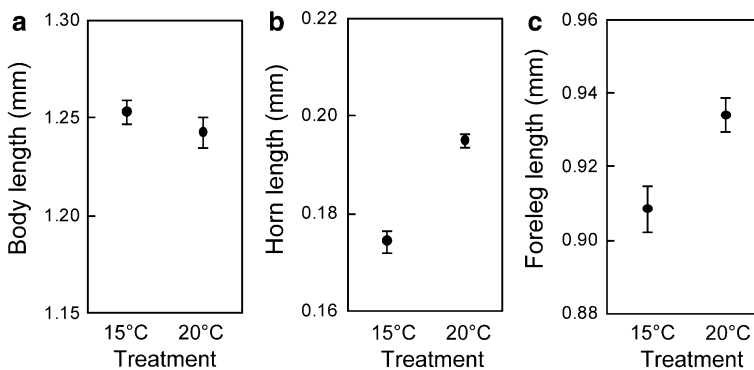
Fig. 6 Average lengths of body (**a**), horn (**b**) and foreleg (**c**) of soldiers produced by mother aphids reared at 15 and 20 °C. *Error bars* denote SE

Another factor, weapon size–related mortality of soldiers, could potentially contribute to create temporal variation in the soldiers' weapons. If predators selectively eliminate the soldiers with short horns and forelegs, the average size of the soldiers' weapon in later season is longer than early season as we observed. Because we have no knowledge about relationship between soldier's characteristics and vulnerability, it is unclear whether such hypothetical size-related mortality operates. However, it is to be noted that even if such possible this process operated, it does not fully account for the temporal changes in size distribution. In all populations, the range of morphological variation obviously differed between June and August (Fig. 7). Although some proportion of soldiers in all populations had very long horns and forelegs in August, no soldiers had such very long horns and forelegs in June. For example, soldiers with 0.20–0.23 mm of horn length existed in August, but not in June in population A (Fig. 7a). Because size-related mortality itself does not expand range of trait distribution (i.e., directional selection only reduces range of trait distribution) (Endler 1986), such discrepancies of distribution ranges between months were not caused by size-related mortality alone.
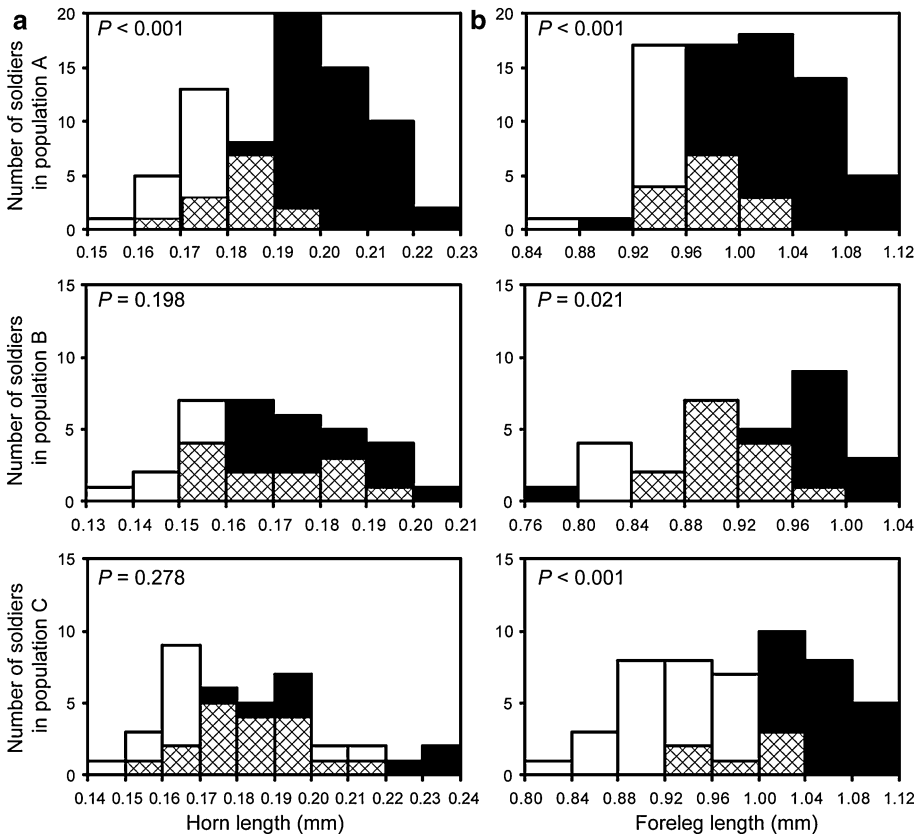
Fig. 7 Frequency distributions of horn length (**a**) and foreleg length (**b**) of soldiers in June and August. The *white bars* show June values, and the *black bars* August values. Trait distributions overlapping between June and August are indicated by *shading*. Statistical differences in the distributions between June and August, indicated by *P values*, were determined by the Fisher's exact test

In conclusion, aphid mothers apparently produce powerful soldiers just in response to the environmental temperature before the coming of "dangerous time" as a plastic strategy that can explain the mechanism of the spatiotemporal variation in soldier morphology in natural populations. Although, as Pike and Foster (2008) pointed out in their recent review of the ecology of altruism in aphids, variable predation risk exerts variable selective effects on defensive morphological traits, they reported no empirical evidence for a plasticity strategy in the morphological traits of soldiers. Therefore, our experimental results may be the first evidence of the existence of an adaptive strategy regarding the morphological traits of soldiers in eusocial aphids. Further study is needed to understand the defensive strategy of the eusocial aphids comprehensively. We need to reveal, in particular, factors affecting soldier production in *C. japonica*. The combination of the changes in soldier production and the changes in soldiers' armature is important defensive strategy in Hormaphididae species that soldier protects colony mates with morphological traits (e.g., forelegs, horns, and stylets). The relationship between soldier production and soldier armature may be estimated by comparing number and armature of soldiers that were produced by mother aphids which had been reared at different temperature.

Phenotypic plasticity of prey species has been well studied in many taxonomic groups by evolutionary ecologists (Tollrian and Harvell 1999; Agrawal 2001; Kishida et al. 2010). The expression of a defensive phenotype depending on predation risk (i.e., an inducible defense) is one type of adaptive phenotypic plasticity (Tollrian and Harvell 1999). Although a defensive phenotype may be an effective defense against predators, it is typically accompanied by some fitness cost (e.g., energetic resource allocation costs) (Lively 1986b; Pettersson and Brönmark 1997; Van Buskirk and Relyea 1998; Trussell 2000). Thus, induction of a defensive phenotype only when it is needed can be a cost-saving strategy, and, therefore, the ability to induce a defensive phenotype can itself be a target of natural selection (Van Buskirk and Relyea 1998; Kishida et al. 2007). In *C. japonica*, soldiers with larger weapons may be better defenders than those with smaller weapons. On the other hand, in the absence of predators, production of soldiers with larger weapons would likely be an inefficient investment of aphid mothers and detrimental to colony growth, because large soldiers are probably more costly for aphid mothers to produce. Thus, it is probably advantageous for colony growth that mother aphids balance the allocation of their limited resources between the production of soldiers with larger weapons and the production of reproductive individuals according to the level of predation risk.

Future studies should confirm the benefits and costs of producing soldiers with large weapons. The benefits may be estimated by comparing components of colony fitness in the presence of predation pressure between colonies which had been reared at different temperatures. We expect that colonies consisting of soldiers with large weapons that had been produced in 20 °C persist longer than those consisting of soldiers with small weapons that had been produced in 15 °C. Costs of production of soldiers with large weapons can be estimated by comparing components of colony fitness in the absence of predation pressure. For example, growth rate of the aphid colony that experienced 15 °C environments may be higher than those that experienced 20 °C in the predator-free situation. Furthermore, anatomical approach could provide good evidence of the costs of production of soldiers with large weapons. The number of embryos in ovariole of aphid mothers that experienced 20 °C environment may be fewer than those that experienced 15 °C, since embryos of soldiers with larger weapons could be larger than those of soldiers with smaller weapons.

Theory predicts that prey organisms adopting an inducible defense strategy should utilize reliable signals, indicating the emergence of predation risk for optimal expression of

their costly defenses (Tollrian and Harvell 1999). While many prey species use cues from the predator itself as signals, several species use indirect biotic (e.g., alarm cues emitted from injured conspecifics) or abiotic signals (e.g., environmental temperature) (Tollrian and Harvell 1999; Kishida et al. 2010). An abiotic environmental state may be a suitable signal if predator emergence highly correlates with the environmental state. However, little evidence is available for the plastic induction of defensive traits in response to abiotic signals. To our knowledge, the known examples are limited taxonomically to aphids (Akimoto 1992, Pike et al. 2004) and daphnids (Yurista 2000; Kappes and Sinsch 2002). For example, Yurista (2000) showed that longer spines and helmets, thought to be defensive traits, were induced in a population of *Daphnia lumholtzi* (Sars.) (Crustacea: Cladocera) in a certain temperature range. Plastic production of soldiers with large weapons in *C. japonica* may be a similar example. Abiotic signal–induced defensive strategies may be more widespread in nature than previously thought.

Although researchers have previously studied spatiotemporal variation in aphid soldier abundance, they have not focused on spatiotemporal trait variation in individual soldiers and its ecological implications (Tanaka and Ito 1994; Ito et al. 1995; Shibao 1999; Shingleton and Foster 2000; but see Pike and Foster 2008). Ecologists have become aware of the importance of trait variation among individuals for understanding population and community dynamics and trait evolution (Agrawal 2001; Werner and Peacor 2003; Miner et al. 2005; Kishida et al. 2009a, 2011; Mougi and Kishida 2009; Mougi et al. 2011). Therefore, investigations into trait variation in individuals from the perspective of phenotypic plasticity can provide insight into the adaptive nature of organisms and the dynamic nature of community assemblages, and examination of this aphid predator–prey system from the perspective of phenotypic plasticity should provide deep insights into the evolution and ecology of eusocial aphids in nature.

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