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RESEARCH ARTICLE

Soldiers with large weapons behave aggressively against predators: correlated morphological and behavioral defensive traits in a eusocial aphid

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Abstract In social insects, intracaste variation suggests the existence of adaptive mechanisms such as polymorphism and polyphenism (i.e., subcastes) for maintaining the sophisticated social system. Here, we investigated intracaste variation patterns of a behavior (i.e., aggressiveness) and of morphology in the soldier caste of a eusocial aphid, *Ceratovacuna japonica* (Homoptera, Hormaphidinae). This species produces sterile individuals (soldiers), which have larger horns and forelegs than non-soldier reproductive individuals and which specialize in colony defense against predators. We previously showed that in midsummer, when predators are abundant, mother aphids produce soldiers with larger horns and forelegs than they do in early summer, when predators are few. However, the aggressiveness of *C. japonica* soldiers, another functional trait for colony defense, has not yet been quantified, and the relationship between the expression of this behavioral trait with that of the morphological traits has not yet been examined. Here, we experimentally showed that in midsummer soldiers behave more aggressively in response to artificial stimuli mimicking contact with a predator than they do in early summer. Furthermore, this variation correlated with the phenological variation of defensive morphology in soldier aphids. These results suggest that *C. japonica* defend their colony with not only strengthening soldier's morphological weapon but also strengthening soldier's aggressiveness when predator risk is high.

Keywords *Ceratovacuna japonica* · Prey–predator interaction · Sociality · Trait correlation

Introduction

The organization of insect societies is based on the division of labor (Wilson 1985; Robinson 1992), and the division of labor leads to specialization of individuals (i.e., castes) that engage in different tasks. Each caste maximizes the fitness of the colony through the development of specialized morphological, behavioral, and life-history traits that enable its members to perform the specialized task of that caste (Oster and Wilson 1978; Shorter and Rueppell 2012). Although the identity of a caste is based on its unique task and trait characteristics, evidence of heterogeneity among members of a caste has been growing (Stern et al. 1997; Hattori and Itino 2008; Jandt et al. 2014; Walton and Toth 2016). The presence of intracaste variation suggests the existence of an unknown adaptive mechanism such as polymorphism and polyphenism (i.e., subcastes) for maintaining the social system. Therefore, exploration of intracaste variation will deepen our knowledge of the complexities of nature of labor division of social insects and lead to insights into the evolution and maintenance of sociality (Oster and Wilson 1978; Robinson 1992; Stern et al. 1996).

In investigating intracaste variation, focusing on multiple traits rather than on a single trait might be a fruitful

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approach. In general, caste members use multiple traits when they engage in their specialized task, and the allied use of multiple traits is the basis of the elaborative work of the caste. For example, worker caste is positively disposed to engage in sacrificial labor such as nest defense and nest repair, and the use of this behavioral trait produces opportunities for them to use their specialized morphological and physiological traits (Wilson 1976; Kurosu et al. 2003; Kutsukake et al. 2009; Shorter and Rueppell 2012). Hence, investigation of the expression pattern and function of multiple traits of individual caste members is necessary to characterize intracaste variation. Therefore, we investigated intracaste variation in aggressiveness and morphology in the soldier caste of a eusocial aphid.

Ceratovacuna japonica (Takahashi) (Homoptera, Hormaphidinae) is a eusocial aphid that produces both reproductive individuals (reproductive caste) and sterile defensive individuals (soldier caste) during their parthenogenetic life stage on secondary host plants. The two castes are clearly discriminated by morphological and behavioral characteristics. Compared with reproductive caste members of the same instar, soldier caste members have much longer frontal horns and forelegs and a larger body size which they use to protect their colony from predators (Hattori and Itino 2008). When soldiers encounter a predator, they immediately attack it, to repel it and to slow its predatory behavior; in contrast, reproductive caste members do not attack predators (Hattori et al. 2013a). In wild populations, the size of the morphological weapons of *C. japonica* soldiers varies temporally (Hattori and Itino 2008; Hattori et al. 2013b). In August, when predators are abundant, soldiers in the colony have significantly longer horns and forelegs than they do in June and July, when the predation risk is lower (Hattori et al. 2013b). This temporal size variation of the soldiers' morphological weapons reflects reproductive plasticity in the mother aphid. Mother aphids produce soldiers with large weapons more frequently in response to relatively high environmental temperatures, which are a reliable signal of a more dangerous predation regime. The resulting bias toward production of soldiers with large weapons in the dangerous midsummer period can be an adaptive response of mother aphids to reduce the predation threat to their colonies, if soldiers with large weapons are better defenders than those with smaller weapons. In addition to these morphological traits, aggressive behavior of a soldier toward a predator would contribute to make the soldier a better defender of the colony. However, to date, no study has quantified the aggressiveness of *C. japonica* soldiers, and thus we have no knowledge about intracaste variation in aggressiveness in the soldier caste. Therefore, in the present study, we investigated temporal variation of aggressiveness in *C. japonica* soldiers in a wild population from early to late summer and tested whether soldiers with large weapons were more aggressive than those with small weapons.

Materials and methods

The eusocial aphid *Ceratovacuna japonica*

The eusocial aphid *Ceratovacuna japonica* is a common wooly aphid in Japan (Takahashi 1958). This species has a heteroecious (i.e., host alternating) and cyclically parthenogenetic (i.e., with an asexual phase and a sexual phase) life cycle. It uses one primary host plant, *Styrax japonica* (Ebenales: Styracaceae), and several secondary host plants (Poaceae species, e.g., *Sasa senanensis* in central Japan) (Aoki and Kurosu 1991, 2010; Hattori and Itino 2008). Reproduction in *C. japonica* is almost exclusively parthenogenetic on the secondary hosts. Here, we define a colony as an aggregation of aphid individuals on a single leaf of the secondary host *S. senanensis*. An aphid colony on a leaf persists for up to several months.

On the secondary host, *C. japonica* is preyed upon mainly by larvae of two lepidopteran species (*Taraka hamada* and *Atkinsonia ignipicta*). Although other predators such as syrphid and lacewing larvae can consume *C. japonica*, compared with the lepidopterans, their predation frequencies are much lower (Banno 1997; Hattori et al. 2013b). In Japan, *T. hamada* and *A. ignipicta* larvae feed primarily on *C. japonica*; thus, they are specialist predators of *C. japonica* (Aoki and Kurosu 2010).

Measurement of soldier aggressiveness

To quantify soldier aggressiveness, we touched the head of a soldier in selected colonies five times (at 1-s intervals) with a pair of tweezers and recorded whether the soldier exhibited attack behavior (i.e., by grasping the tweezers) or not (i.e., no response or escape). We consider this experimental procedure to be appropriate because field observations show that a soldier usually attacks a predator that touches its head and their attack behavior is triggered not only by a predator but also by various physical stimuli (e.g., contact with conspecific individuals) (Aoki and Miyazaki 1978). This test was conducted on a soldier in randomly selected colonies on a leaf of different straws in the foothills of Mt. Jyonen, Nagano, central Japan (1261 m above sea level; 36°19′N, 137°47′E) from June to August in 2011 (June, $n = 70$; July, $n = 81$; August, $n = 38$). After the trials, we collected and preserved the soldiers in 70 % ethanol for measurement of morphological traits.

Measurement of soldier morphology

We measured the horn length and the foreleg length of the soldiers as defensive morphological traits.

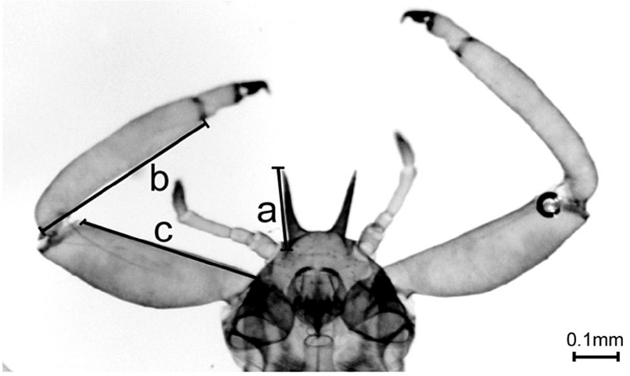


Fig. 1 Digitized image of a *C. japonica* soldier showing how (*a*): horn length and (*b*) + (*c*): foreleg length were measured on each individual

For the morphological measurements, we cleared, stained, and mounted soldier specimens on slides following the method of Kozarzhevskaya (1986). We then captured digitized images of the slide-mounted samples from the ventral side and quantified horn length and foreleg length on these digital images (Fig. 1) using Photo Measure software (Kenis Ltd., Osaka, Japan).

Statistical analysis

To examine whether the behavioral aggressiveness of the soldiers (i.e., the probability that a soldier would exhibit attack behavior in response to the five head touches, i.e., 0 times = 0, 1 time = 0.2, 2 times = 0.4, 3 times = 0.6, 4 times = 0.8, 5 times = 1) varied temporally, we used a Kruskal–Wallis test with month as the fixed factor. We then used the Wilcoxon signed rank test for post hoc comparisons of behavioral aggressiveness. Because multiple comparisons may increase the possibility of a type I error, we adjusted the significance level for each comparison by the sequential Bonferroni method (Rice 1989).

Because horn length and foreleg length were strongly and positively correlated (Pearson's correlation test, $r = 0.72$, $p < 0.001$), we calculated a composite of the two variables by conducting a principal component analysis of the correlation matrix. Because the first principal component, PC1, explained 86.20 % of the total variance and had high positive loadings for horn length and foreleg length (eigenvalue = 1.72, $\chi^2 = 139.60$, $p < 0.001$), PC1 is a good indicator of size of these morphological weapons. Hence, we defined PC1 as the composite variable "weapon size".

Because the behavioral aggressiveness of the soldiers showed large variation (see Results), we performed multiple ordinal logistic regression analyses to determine the factors explaining this variation. Here, weapon size, month of year (index of temporal environmental change), and their interaction were the predictor variables. Moreover, because the results of the analysis revealed that weapon size better explained behavioral aggressiveness than month of year, we further performed ordinal logistic regression analyses of the variation within each month using weapon size as the predictor variable to examine whether soldiers with larger weapons behaved more aggressively than those with smaller weapons. Statistical significance of the logistic regression analyses was evaluated by calculating Wald's Chi-square values. All analyses were performed with the JMP v.9.0.0 statistical package (SAS Institute).

Results

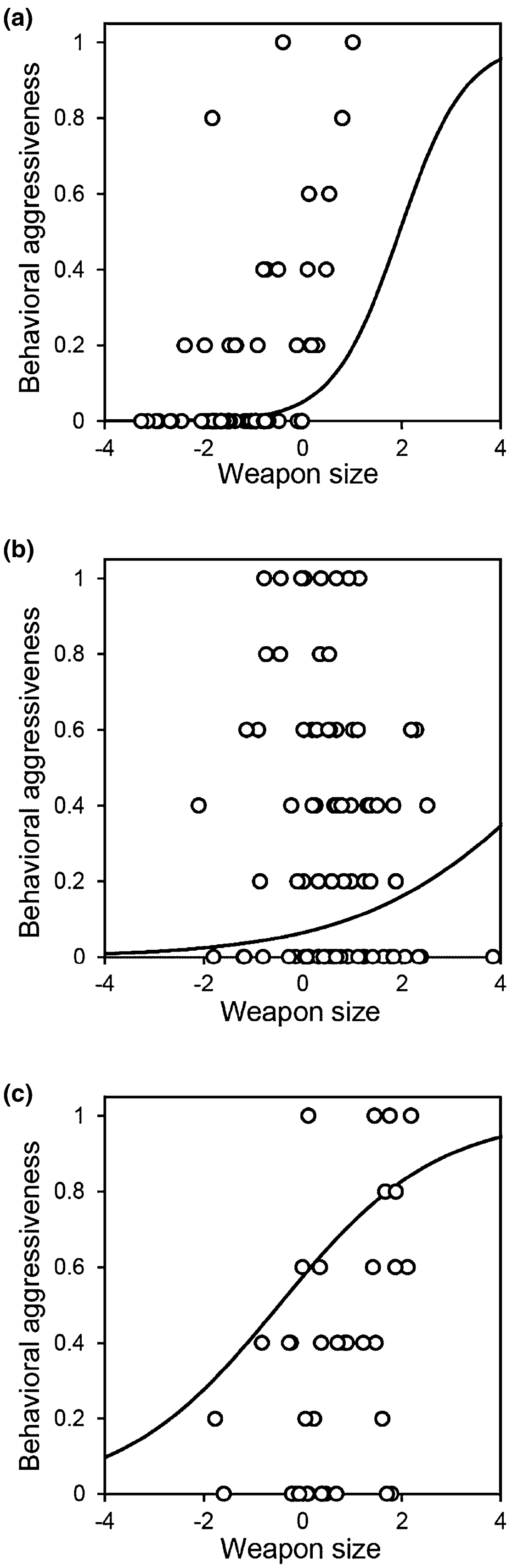
Behavioral aggressiveness varied between 0 and 1 among soldiers (Fig. 2), and mean (±SE) behavioral aggressiveness was 0.27 ± 0.23. Soldier's behavioral aggressiveness varied temporally (June, mean ± SE = 0.15 ± 0.03; July, 0.33 ± 0.04; August, 0.36 ± 0.05; $\chi^2 = 17.32, p < 0.001$). Soldiers in July and August showed over twice as much aggressiveness as soldiers in June (June vs July: $z = 3.65$, $p < 0.001$; June vs August: $z = 3.53$, $p < 0.001$) but aggressiveness did not differ between July and August ($z = 0.52$, $p = 0.60$).

The multiple ordinal logistic regression analysis considering month (index of temporal environmental change) and weapon size, and their interaction as the predictor variables showed that behavioral aggressiveness was not influenced by month (Wald's $\chi^2 = 0.74$, $p = 0.69$) or the interaction between PC1 and month (Wald's $\chi^2 = 2.90$, $p = 0.23$), but it was significantly influenced by PC1 (Wald's $\chi^2 = 18.19, p < 0.001$).

Furthermore, ordinal logistic regression analyses of the dataset of each of the 3 months showed that behavioral aggressiveness was significantly influenced by weapon size in any month (June: Wald's $\chi^2 = 19.38$, $p < 0.001$; July: Wald's $\chi^2 = 5.87$, $p = 0.015$; August: Wald's $\chi^2 = 7.79$, $p = 0.005$). These results indicate that soldiers with large weapons behave more aggressively than those with small weapons (Fig. 2), and they also suggest that temporal variation of environment by itself (e.g., environmental temperature) is less likely to influence the soldiers' behavioral aggressiveness.

Discussion

In eusocial aphids, members of the soldier caste are sterile but they contribute to colony fitness by lowering predation pressure on the reproductive caste (Aoki et al. 1981; Kutsukake et al. 2004; Hattori et al. 2013a). When soldiers detect or encounter predators in the colony, they immediately begin to attack the predators. Because attacked



◀**Fig. 2** Relationship between soldiers' defensive morphology (PC1 as an index of soldier's weapon size) and behavioral aggressiveness (probability of attack behavior in response to five artificial stimuli) in **a** June ($n = 70$), **b** July ($n = 81$), and **c** August ($n = 38$). *Lines* indicate the predicted correlation between weapon size and behavioral aggressiveness obtained by ordinal logistic regression analysis

predators reduce their foraging activity, not only the distinctive morphological traits (weapons) of the soldiers but also their aggressiveness can be important for colony defense. Thus, by investigating the pattern and cause of intraspecific and interspecific variation in soldiers' behavioral and morphological traits, we can deepen our understanding of the adaptive strategy of this eusocial aphid. Interspecific variation of aggressiveness and weapons of eusocial aphids was studied by Shingleton and Foster (2001), who reported that soldiers of *Pseudoregma nicolaiae* have larger weapons (i.e., forefemur length and foretibia length) and behave more aggressively than *P. sundanica* soldiers. *Pseudoregma sundanica* is obligatorily tended by ants, which defend the aphids from natural predators (Shingleton and Foster 2000), whereas *P. nicolaiae* is not tended by ants (Shingleton and Foster 2001); thus, *P. nicolaiae* colonies may have a greater need to defend themselves. Therefore, the interspecific correlation between morphology and behavior might be caused by differential predation pressure (Shingleton and Foster 2001). Here, we experimentally showed that in July and August *C. japonica* soldiers more frequently exhibited aggressive behavior in response to artificial stimuli mimicking contact with a predator than they did in June. This temporal variation in aggressiveness was well explained by individual variation in the weapon size (i.e., horn and foreleg length) of soldiers. That is, soldiers with large weapons, which are common in midsummer, are more aggressive than those with small weapons, which are common in early summer.

Concurrent changes in the behavioral and morphological traits of *C. japonica* soldiers from June to August may be an expression of a sophisticated defensive strategy of this species. Predation pressure on *C. japonica* aphids changes greatly from early to late summer (Banno 1997; Hattori et al. 2013b). Although the abundances of the specialist predators of this species (larvae of *Taraka hamada* and *Atkinsonia ignipicta*) are very low in early summer (June), they increase drastically from July to August (Banno 1997; Hattori et al. 2013b). Thus, *C. japonica* populations are exposed to more intensive predation pressure in midsummer than in early summer (Banno 1997). To cope with this temporal change in the predation regime, *C. japonica* seems to follow a plastic reproductive strategy. Hattori et al. (2013b) showed that mother aphids produce soldiers with large weapons in response to environmental temperature, which reliably signal the coming of the predator-abundant period (i.e., under typical July temperature conditions, mother aphids more frequently produce soldiers with large weapons). The fact that aggressive behavior is positively correlated with large morphological weapons strongly suggests that mother aphids produce more aggressive soldiers in the dangerous period as well.

This plausible reproductive plasticity of mother aphids can be adaptive if more aggressive soldiers contribute more to colony success than less aggressive soldiers in the dangerous period, but production of less aggressive soldiers is less costly than production of aggressive soldiers in the less dangerous period. Because *C. japonica* soldiers lower the foraging activity of predators by aggressively attacking them (Hattori et al. 2013a), an aggressive soldier with large weapons is likely to be a better defender than a less aggressive soldier with small weapons, and production of aggressive soldiers with large weapons is likely to be more beneficial in the dangerous predator-abundant period.

On the other hand, when the predators are few, it may be inefficient for aphid mothers to invest in the production of aggressive soldiers with large weapons, which are probably more costly to produce and maintain. For example, *C. japonica* soldiers sometimes attack siblings by mistake (Carlin et al. 1994; Hattori personal observation); thus, high soldier aggressiveness might negatively affect colony fitness through its detrimental effect on the reproductive aphids. Moreover, the detrimental effect would plausibly be greater when the soldiers have large weapons than when they have small weapons. In addition, the maintenance of behavioral aggressiveness may have a physiological cost.

Several biogenic amines that relate to variations in behavioral aggressiveness (e.g., octopamine, dopamine, and serotonin) have been identified in arthropod species, including social insects (Kravitz 1988; Sneddon et al. 2000; Stevenson et al. 2000; Hunt 2007). Therefore, mother aphids must invest more resources in the embryo of an aggressive soldier than in one of a less aggressive soldier. Thus, if the ecological and physiological costs are high, when predators are scarce the production of less aggressive soldiers rather than aggressive soldiers can be adaptive. A future study should compare colony-level fitness between a colony that has aggressive soldiers with large weapons and one that has less aggressive soldiers with small weapons. Such a study would significantly advance our knowledge about the ecology and evolution of sociality in this species and also give general insights into the evolution of correlated morphological and behavioral defenses in animals.

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