

Eggs of a Eusial Aphid's Predator are Protected Against Attacks by Aphid Soldiers

by

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ABSTRACT

Predators generally have traits that enable them to efficiently capture their prey and thus improve their survival. Natural selection should also favor traits of predators that improve the survival rate of their eggs, which are immobile and incapable of active resistance. We hypothesized that eggs of *Atkinsonia ignipicta*, a specialist predator of the eusocial aphid *Ceratovacuna japonica*, exhibit a defensive trait against aphid soldiers. We found that the hatchability of *A. ignipicta* eggs did not differ significantly between the experimental treatments with and without soldiers, which suggests that the eggs have a defensive trait that protects them from soldier aphids. Moreover, although the soldiers occasionally exhibited attack behavior when they encountered an egg, they did not continue the attack. We have observed a similar interruption of attack behavior by soldiers that attacked their aphid siblings by mistake, suggesting that the eggs may chemically mimic the soldiers' siblings. This study thus provides evidence for adaptation in a specialist predator of a eusocial aphid.

Key words: antagonistic adaptation, *Atkinsonia ignipicta*, *Ceratovacuna japonica*, eusocial aphid, predation, predator–prey interaction

INTRODUCTION

Interspecific interaction is one of the most important driving forces of organismal evolution (Ehrlich & Raven 1964; Vermeij 1994; Philipp *et al.*

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2006). The interspecific interaction between predator and prey is antagonistic (Slobodkin 1980; Kishida *et al.* 2006), and numerous studies have investigated adaptation in interacting predators and prey. Many have focused on the defensive traits of prey species (Lively 1986; Dodson 1989; Petersson & Brönmark 1997; Agrawal 1998; Kishida & Nishimura 2004; Toju & Sota 2006; for a review see Tollrian & Harvell 1999), and others have examined how the attack traits of a predator enable it to efficiently capture its prey and thus improve its survival (Abrams 2000; Michimae & Wakahara 2002; Toju & Sota 2006). Investigations of reciprocal adaptation between prey and predator can not only deepen our understanding of adaptive evolution but also of the evolution of biodiversity (Thompson 2005).

The moth larva *Atkinsonia ignipicta* (Butler) (Lepidoptera: Stathmopodidae) is a specialist predator of the eusocial aphid *Ceratovacuna japonica* (Takahashi) (Homoptera: Hormaphidinae) (Morimoto & Shibao 1993). Individuals of the defensive caste of *C. japonica*, the soldiers, have long horns and forelegs that they use to protect the aphid colony against predators (M. Hattori & Itino 2008). When the soldiers encounter a predator, they grasp it with their forelegs and then pierce it with their frontal horns, sometimes killing it (Hattori *et al.* in prep).

Females of *A. ignipicta* lay eggs directly in aphid colonies (Fig. 1; Hattori personal observation). Therefore, the fitness of eggs of *A. ignipicta* without any defensive trait against aphid soldiers would be decreased when soldier aphids were present. In fact, soldiers of *Pseudoregma bambucicola* (Takahashi) (Homoptera: Hormaphididae) (Ohara 1985) and *Ceratovacuna lanigera* (Zehntner) (Homoptera: Hormaphidinae) (Aoki *et al.* 1984) attack and crush the eggs of their generalist predators that are laid on or near their colonies. These observations suggest that natural selection might favor traits that improve the survival rate of the eggs, which are immobile and incapable of active resistance. Indeed, some predators display an oviposition behavior that improves the survival rate of their eggs (Ohara 1985; Arakaki 1992). Thus, we hypothesized that *A. ignipicta* eggs, which are laid directly in the center of a *C. japonica* colony, might have a defensive trait that protects them against the soldier aphids. Therefore, we examined whether the hatchability of *A. ignipicta* eggs is influenced by the presence of soldiers.



Fig. 1. Photograph of the eggs of the predatory mite *A. ignipicta* laid on a *S. senanensis* leaf (among the spiny trichomes) in the center of a colony of the eusocial aphid *C. japonica*.

MATERIALS & METHODS

Atkinsonia ignipicta

The specialist predator of *C. japonica*, *A. ignipicta*, is widely distributed in Japan from Kyushu to Hokkaido (Moriuti 1982). Adults of *A. ignipicta* are abundant during June and July, when they lay their yellow, barrel-shaped eggs in aphid colonies (Fig. 1). Immediately after they hatch out, *A. ignipicta* larvae attempt to prey on nearby aphids. If they are successful, they then spin silken nests in the aphid colony, where they cannot be attacked by the soldier aphids, occasionally coming out to prey on the aphids.

Ceratovacuna japonica

The eusocial aphid *C. japonica*, which is widely distributed in Japan (M. Hattori personal observation), has a heteroecious (i.e., host alternating) and cyclically parthenogenetic (i.e., an asexual phase and a sexual phase) life history. It has one primary host, *Styrax japonica* (Sieb. et Zucc.) (Ebenales, Styracaceae), and several secondary hosts (in central Japan, Poaceae species, e.g., *Sasa senanensis* [Rehd.] [Poales, Poaceae]) (Aoki & Kurosu 1991, 2011). Although this species has a complete life cycle and on its secondary hosts, it sometimes produces winged sexuparae, it has merely been observed on its primary host (Carlin *et al.* 1994). Here, we define a colony as an aggregation of aphid individuals on a single leaf of the secondary host *S. senanensis*. Such a colony can persist for up to several months. Each colony has three morphologically distinctive castes: young reproductive adult females, which produce nymphs or soldiers parthenogenetically; and sterile soldiers. Soldiers are first

instar nymphs, and they do not develop into second instar nymphs (Aoki *et al.* 1981). Soldiers have longer horns and forelegs than non-soldier aphids of the same instar (Hattori & Itino 2008), and they protect their colony from predators (Hattori *et al.* in review).

The primary predators of *C. japonica* are larvae of *Taraka hamada* (Druce) (Lepidoptera: Lycaenidae) and of *A. ignipicta*, which prey exclusively on *C. japonica* (Morimoto & Shibao 1993). Although other potential predators such as Syrphidae and Chrysopidae species are able to prey on *C. japonica*, they are seldom seen on *C. japonica* colony (Hattori personal observation).

The hatchability of *A. ignipicta* eggs in the presence of soldiers

To test whether *A. ignipicta* eggs have any defensive trait that protects them against soldiers of *C. japonica*, we compared hatching rates of eggs placed in dishes with and without soldier aphids. We designed three treatments, each with different numbers of non-soldier and soldier aphids (Fig. 2)

We haphazardly collected *A. ignipicta* eggs ($n = 50$) and 30 aphid colonies from a wild aphid population on Mt. Nabekanmuri, Nagano, central Japan ($36^{\circ}16'56''\text{N}$, $137^{\circ}49'35''\text{E}$) in July 2009. This aphid population inhabits the edges of a deciduous forest, where the secondary host plant *S. senanensis* is abundant.

After collecting the eggs, we returned to the laboratory and immediately introduced the eggs and aphids into petri dishes, mixing the aphid individuals from the 30 aphid colonies. In the course of these manipulations, we observed

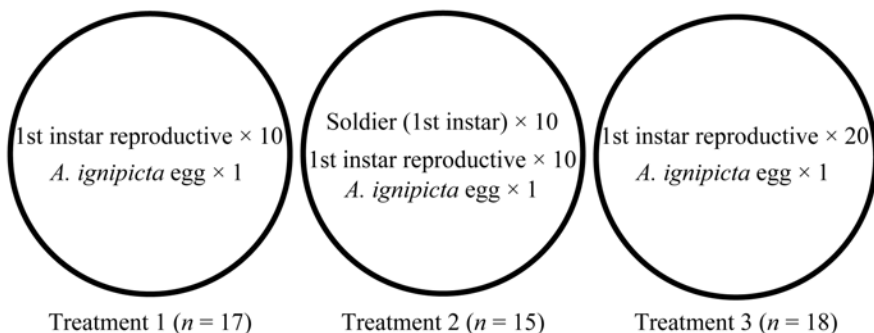


Fig. 2. The design of the egg hatching experiment. Each petri dish was 3.5 cm in diameter. In treatments 1 and 2, the number of non-soldier first instar aphid nymphs was equal. In treatments 2 and 3, the total number of aphids (non-soldier first instar nymphs plus soldiers) was equal.

Table 1: Numbers of hatched and dead *A. ignipicta* eggs among the three treatments.

	<i>n</i>	hatched	parasitized	moldy
treatment 1 (thout soldiers)	17	13	1	3
treatment 2 (th soldiers)	15	12	3	0
treatment 3 (thout soldiers)	18	15	2	1

no confounding events such as soldiers attacking conspecifics.

We observed the eggs at 24-h intervals for 1 week or until they hatched. Also, every 24-h, we removed all aphid individuals from the petri dishes and introduced newly collected aphid individuals from the same population to the petri dishes according to treatment (Fig. 2).

Statistical analysis

We compared the hatching rate of the eggs among the treatments by using Fisher's exact test (JMP v. 9.0.0 statistical package, SAS Institute).

RESULTS

In every treatment, the hatching rate was high (Table 1), and hatchability was not significantly different among treatments ($df = 2$, $\chi^2 = 0.26$, $P > 0.05$). Some eggs that did not hatch gradually turned black, and two individuals of a parasitic wasp, *Trichogramma* sp., emerged from each of these eggs. Other eggs gradually became moldy and distorted in shape (Table 1).

When the soldiers encountered an egg, they rarely showed attack behavior toward it. Those that initially showed attack behavior did not continue in their attack, but immediately stopped displaying the behavior.

DISCUSSION

The high hatching rate of the *A. ignipicta* eggs in the treatment with soldier aphids suggests that *A. ignipicta* has some defensive trait that protects it against soldiers of *C. japonica*. This result contrasts markedly with the findings of previous studies that soldiers of other social aphid species often crush the eggs of generalist predators (*P. bambucicola*, Ohara 1985; *C. lanigera*, Aoki *et al.* 1984). At present, we do not know whether *C. japonica* soldiers crush the eggs of their generalist predators. Future studies should investigate this possibility.

Although we occasionally observed attack behavior of soldiers toward eggs of *A. ignipicta*, the soldiers did not persist but immediately stopped attacking the eggs. One of us has observed a similar interruption of attack behavior by soldiers that attacked one of their siblings by mistake (M. Hattori personal observation). These observations suggest that the eggs of *A. ignipicta* may chemically mimic the aphid. For example, the cuticular hydrocarbons (CHCs) of the eggs may be similar to those of the aphids, just as myrmecophilous insects produce CHCs that mimic those of ants so that they can penetrate the ant colony (Vander Meer *et al.* 1989; Akino *et al.* 1999; Hojo *et al.* 2009). Moreover, a similar chemical mimicry has been observed in predators of ant-tending aphids (Lohman *et al.* 2006). A future study should compare the CHCs of *A. ignipicta* with those of *C. japonica* nymphs.

Most studies of predator–prey interaction in social aphids have focused on the morphology and function of the soldier’s defensive traits (Kutsukake *et al.* 2004; Hattori & Itino 2008; Hattori *et al.* in review). In contrast, even though it has been suspected that predators of eusocial aphids have various anti-soldier traits (Arakaki & Yoshiyasu 1988; Aoki & Kurosu 1992; for a review see Stern & Foster 1996), whether a predator’s defensive traits can protect it from soldiers has not been investigated quantitatively. Further study of the defensive traits of predators will deepen our understanding of not only reciprocal adaptation in predator–prey interactions but also of the evolutionary stability of eusociality in aphids.

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