Density triggers soldier production in a social aphid

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For evolution and maintenance of the social system of insect colonies, investment in the sterile caste should be adequately controlled in response to environmental cues. Recent developments using artificial diet rearing techniques have revealed an underlying mechanism of caste control in a gall-forming aphid, *Tuberaphis styraci*, which has a soldier caste in the second instar. Statistical analyses of field-collected galls detected a significant positive correlation between aphid density and soldier proportion in the natural colonies of *T. styraci*. Artificial diet experiments showed that soldiers are produced under crowded conditions. Detailed experiments demonstrated that soldiers are produced in a density-dependent manner rather than in a colony size-dependent manner. From these results, it was concluded that aphid density is the crucial cue that triggers soldier production in *T. styraci*. This study provides, to our knowledge, the first experimental demonstration of an environmental factor involved in aphid soldier differentiation.

Keywords: *Tuberaphis styraci*; social aphid; soldier differentiation; density effect

1. INTRODUCTION

In colonies of highly social insects, some individuals are engaged in reproduction, whereas others produce few or no offspring and comprise specialized castes that are characterized by distinct behavioural and morphological traits for altruistic functions. Morphologically and reproductively differentiated castes are well known in social groups such as ants, bees, wasps and termites (Wilson 1971), but such castes have also evolved in less-studied groups like aphids (Aoki 1977).

Some aphids are known to produce individuals that altruistically sacrifice their own reproduction to the benefit of their colony mates (Stern & Foster 1996). Such individuals are called 'soldiers' because their primary social role is defence, although some soldiers also play a non-defensive altruistic role: gall cleaning (Aoki & Kurosu 1989; Benton & Foster 1992). In highly social aphids, soldiers are morphologically differentiated from the normal nymphs and unable to grow, constituting a sterile caste.

Giving up their personal reproductive potential, soldiers ensure transmission of their gene copies only by contributing to the success of their colony mates. Production of

soldiers improves colony success through their altruistic tasks such as defence and housekeeping, at the expense of intrinsic growth rate of the colony. Under the condition of the trade-off between reproduction and altruistic functions, there should be an optimal investment in soldiers in a particular environment (Akimoto 1996; Stern & Foster 1996). Because environmental factors such as predation, competition and available resources may fluctuate, the optimal soldier ratio is predicted to shift with time. Therefore, mechanisms of controlling the soldier investment in response to external cues are important for the maintenance of the social system. It has been suggested that soldier production may be influenced by predator abundance (Shibao 1998), ant attendance (Shingleton & Foster 2000), host plant condition (Sakata *et al.* 1991), gall shape (Stern *et al.* 1994), clonal mixing (Abbot *et al.* 2001) and other factors. However, no studies, to our knowledge, have unequivocally identified direct environmental cues that control aphid soldier production.

Tuberaphis styraci is a social aphid that forms large coral-shaped galls on the tree *Styrax obassia*. In the galls, adult females parthenogenetically produce monomorphic first instar nymphs. When they molt into second instar, two distinct morphs, normal second instar nymphs and soldiers, are produced. Normal second instar nymphs grow to adult and reproduce, whereas soldiers neither grow nor reproduce but are specialized for altruistic tasks, e.g. colony defence and housekeeping (Aoki & Kurosu 1989, 1990). Recently, we developed an artificial diet rearing technique for *T. styraci* (Shibao *et al.* 2002), which facilitated experimental approaches to the mechanism of soldier differentiation in the aphid social system.

Here, we demonstrate that, in *T. styraci*, aphid density is the crucial cue that triggers soldier production, on the basis of data from natural galls and artificial diet experiments.

2. METHODS

(a) *Social structure in natural galls*

Sixteen second year galls of *T. styraci* were collected from *S. obassia* in central Japan from June to September 2001 (table 1). The following traits were examined for each gall: gall diameter, gall height, gall inner surface area, number of exit holes, colony size (total number of aphid individuals), soldier proportion (number of soldier individuals per colony size), aphid density (colony size per gall surface area) and number of predators. To obtain the inner surface area of irregularly shaped galls, they were cut into small pieces, and the areas of the pieces were measured and summed. To estimate factors related to soldier proportion, regression analyses were performed using the above traits as explanatory variables. Because these variables may be correlated with each other, we also adopted a stepwise regression procedure to identify significant variables on account of multicollinearity.

(b) *Rearing technique*

We used an artificial diet rearing technique to maintain *T. styraci* in the laboratory (Shibao *et al.* 2002). Unwinged adult aphids taken from the field-collected galls were transferred to the artificial diet, and their progeny born on the diet were used for experiments. Feed was replaced every third day to prevent accumulation of excreted honeydew and to minimize possible microbial contamination. All experiments were carried out at 20 °C and under a 16 L : 8 D photoperiod. Aphids originating from the same gall were randomly divided into groups and used for the experiments described below. In some of the experiments, to obtain a sufficient number of replicates, we performed several rounds of the experiments using insects from several galls.

(c) *Experiment 1: isolation versus crowding*

Newly emerged unwinged adult aphids were reared either in isolation or in groups of 10 or 20 individuals per chamber. After a month of maintenance, instar, morph and number of the offspring in each

Table 1. Ecological parameters from 16 natural galls of *Tuberaphis styraci*.

gall no.	date	gall size			social structure		predator abundance		
		diameter and height (mm)	inner surface area (mm ²)	no. of exit holes	colony size	% soldiers	aphid density within gall	no. of lacewing larvae	no. of pyralid larvae
1	6 Jun 2001	52.6 × 30.0	3539	13	389	16.7	0.11	0	0
2	19 Jun 2001	79.1 × 53.7	11 325	46	1263	30.6	0.11	0	0
3	27 Jun 2001	72.9 × 53.8	14 388	49	2848	38.9	0.20	0	0
4	27 Jun 2001	126.3 × 69.1	23 925	36	3326	35.0	0.14	0	0
5	11 Jul 2001	125.1 × 73.8	28 375	60	7707	32.2	0.27	1	0
6	11 Jul 2001	90.7 × 39.8	10 025	42	2812	36.6	0.28	0	0
7	26 Jul 2001	107.8 × 39.6	16 725	34	5770	44.4	0.34	0	0
8	26 Jul 2001	99.1 × 50.6	19 525	43	7456	48.2	0.38	3	0
9	14 Aug 2001	121.3 × 89.1	30 513	73	15 820	33.7	0.52	0	0
10	14 Aug 2001	98.5 × 58.5	20 538	35	14 846	34.8	0.72	1	0
11	28 Aug 2001	68.6 × 45.1	7313	42	10 715	61.0	1.47	4	1
12	28 Aug 2001	70.6 × 36.6	4238	25	6183	69.1	1.46	0	0
13	28 Aug 2001	86.9 × 42.2	15 700	65	9367	47.7	0.60	0	11
14	28 Aug 2001	96.4 × 46.5	15 538	71	22 452	79.4	1.44	1	5
15	28 Aug 2001	78.9 × 53.7	9375	32	7570	53.7	0.81	0	1
16	18 Sep 2001	72.7 × 45.5	4775	10	3382	62.5	0.71	0	0

Figure 1. Comparison of soldier production between colonies reared under various crowding conditions. A significant difference in soldier proportion was found between the three treatments (one-way randomized block ANOVA: $F = 52.22$, d.f. = 2,27, $p < 0.0001$). Different letters (a,b) indicate a significant difference at $p < 0.05$ after the application of a sequential Bonferroni method. Sample sizes are shown in parentheses, and standard deviations are shown by vertical bars.

chamber were recorded. The proportions of soldiers (number of soldiers/number of second instar and older nymphs including soldiers and non-soldiers but excluding morphologically undifferentiated first instar nymphs) were compared between the three treatments using one-way randomized block ANOVA.

(d) Experiment 2: colony size versus density

The experimental design is shown in figure 2a. By manipulating the number of adult aphids and size of chambers, five groups of adults, 5 large (L), 5 medium (M), 5 small (S), 10M and 20L, were arranged to discriminate between the effect of density and the effect of colony size. Chamber sizes were: L (50 mm in diameter, 10 mm high, 2000 mm² surface area), M (35 mm, 10 mm, 1000 mm²), and S (25 mm, 10 mm, 500 mm²). Colony size was constant between treatments 5L, 5M and 5S, whereas density was constant between 5S, 10M and 20L. After a month of maintenance, instar, morph and number of the offspring in each chamber were recorded and the proportion of soldiers was compared across the five treatments using one-way randomized block ANOVA.

Figure 2. (a) Experimental design to discriminate between the effects of aphid density and of colony size on soldier production. If colony size is the principal factor inducing soldier production, soldier proportion is expected to be $5L = 5M = 5S < 10M < 20L$. If density is the principal factor, soldier proportion should be $5L < 5M < 5S = 10M = 20L$. (b) Comparison of soldier production between the five treatments. A significant difference in soldier proportion was found between 5L, 5M and 5S with the same colony size (one-way randomized block ANOVA: $F = 31.20$, d.f. = 2,29, $p < 0.0001$), whereas no difference was found between 5S, 10M and 20L with the same density ($F = 0.74$, d.f. = 2,21, $p = 0.4907$). Thus the soldier proportions were regarded as $5L < 5M < 5S = 10M = 20L$. Different letters (a–c) indicate a significant difference at $p < 0.05$ after the application of a sequential Bonferroni method. Sample sizes are shown in parentheses, and standard deviations are shown by vertical bars.

Table 2. Regression analysis for the effect of environmental factors on the soldier proportion.

(r , correlation coefficient of simple regression analysis; β , standardized regression coefficient of stepwise regression analysis; * $p < 0.05$; ** $p < 0.0001$; —, environmental variable excluded from the model through stepwise regression procedure.)

variable	r	β	F -value	p
gall diameter	−0.071	—	—	—
gall height	−0.188	—	—	—
inner gall surface area	−0.208	—	—	—
no. of exit holes	0.050	—	—	—
colony size	0.586*	—	—	—
density of aphids	0.857**	0.857	38.792	< 0.0001
no. of lacewing larvae	0.277	—	—	—
no. of pyralid larvae	0.459	—	—	—

model $r^2 = 0.737$, $F = 38.792$, d.f. = 1,14, $p < 0.0001$

(e) Statistics

Statistical analyses were performed using the JMP, v. 3.0 statistical package. In parametric statistical tests, percentage data were transformed into arcsine square roots and the other data were transformed into natural logarithms to satisfy normality and constant variance. All statistical tests were corrected for multiplicity by the sequential Bonferroni method.

3. RESULTS

(a) Environmental correlates with soldier proportion in natural galls

To infer the environmental factors related to soldier production, we examined 16 natural galls of *T. styraci* for the following parameters: gall diameter, gall height, gall inner surface area, number of exit holes, colony size, aphid density, number of predators (lacewing larvae and pyralid moth larvae) and proportion of soldiers (table 1). Regression analyses indicated that soldier proportion is significantly correlated with colony size ($r = 0.586$, $p < 0.05$) and aphid density ($r = 0.857$, $p < 0.001$). In stepwise regression analyses, aphid density exhibited a highly significant correlation ($\beta = 0.857$, $p < 0.0001$) with soldier proportion (table 2).

(b) Crowded conditions induce soldier production

Using an artificial diet rearing system, we investigated in the laboratory the effect of crowding. When a single adult was reared in a chamber, few soldiers were produced. By contrast, when 10 or 20 adults were maintained in a chamber, many soldiers were produced. After a month of maintenance, soldier proportion in the offspring reached ca. 50% under the crowded conditions (figure 1).

(c) Aphid density rather than colony size is responsible for soldier induction

Next, we designed a series of artificial diet experiments to differentiate which, aphid density or colony size, is the principal factor that induces soldier production (figure 2a). Soldiers were produced in a density-dependent manner rather than in a colony size-dependent manner (figure 2b). Thus, it was concluded that high density induces soldier production in *T. styraci*.

4. DISCUSSION

Because of the difficulty in maintaining social aphids in the laboratory, previous studies on aphid soldiers have

been restricted to morphological, behavioural, ecological and phylogenetic aspects (Stern & Foster 1996). The artificial diet rearing technique developed for *T. styraci* (Shibao *et al.* 2002) opened a new window to deeper understanding of the mechanisms in the aphid social system.

In the experiments using the artificial diet system, *T. styraci* produced soldiers in response to high aphid density (figures 1 and 2), which was in agreement with the regression analyses of the data from natural galls (table 2). From these results, it was concluded that aphid density is the crucial cue that triggers soldier production in *T. styraci*. This study provides, to our knowledge, the first unequivocal demonstration of an environmental factor involved in aphid soldier differentiation on the basis of laboratory experiments.

In the light of the gall formation process of *T. styraci* (Aoki & Kurosu 1989, 1990), the density-dependent soldier production appears fit for adaptive reproductive allocation in the developing aphid colonies. In a young gall with only a small number of insects, few soldiers are produced because of low aphid density, which maximizes the intrinsic growth rate of the colony and leads to prompt attainment of a large colony size when the nutritional condition of the gall is good. As the colony size increases, aphid density in the gall is quickly elevated, available resources and space for gall inhabitants are limited and increasing aphid biomass in the gall becomes attractive for predators. At this stage of colony development, a large number of soldiers are produced in response to the high aphid density, thereby meeting these ecological requirements. However, this scenario is at present hypothetical and needs to be verified by further studies on the ecological aspects of *T. styraci*.

Aphids are known for their complex life cycle and extreme polyphenism (Miyazaki 1987). In many aphid species, crowded conditions induce differentiation of winged morphs (Kawada 1987), which is reminiscent of the soldier differentiation in *T. styraci*. It is conceivable, though speculative, that some common mechanisms might underlie the differentiation of these and other aphid morphs. Photoperiod, nutritional condition, temperature and other factors also affect the differentiation of winged morphs (Kawada 1987). In addition to density, these factors may influence the soldier production in *T. styraci*.

The processes underlying the mechanisms of the aphid soldier differentiation are still to be investigated: e.g. causal agents mediating the density effect; possible involvement of hormonal control; differential gene expression associated with the soldier caste, etc. Future studies of *T. styraci* using the artificial diet system should provide insights into such intriguing aspects of the aphid social biology.

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