Juveniles and the elderly defend, the middle-aged escape: division of labour in a social aphid

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In colonies of social insects, non-random spatial positioning within the colonies may reflect division of labour and improve colony efficiency. Here, we describe a novel defence system in the colony of a gall-forming social aphid, *Quadrartus yoshinomiyai* (Nipponaphidini), where young and old defensive aphids move towards the dangerous area typically associated with a higher risk of predation, whereas the middle-aged reproductive individuals move away. Younger nymphs and post-reproductive adults of *Q. yoshinomiyai* concurrently defend against predators that intrude after their galls open. In natural open galls, both types of defenders were preferentially located around the open area vulnerable to invasion by predators, whereas reproductive individuals remained in the safer areas. In addition, when a hole was artificially made in closed galls, these morphs located themselves in similar spatial positions to the natural open galls within 12 hours. The defensive system led by oldest and youngest individuals may reflect the possibility of future reproduction for these insects, thereby optimizing colony efficiency in a seasonally changing environment, according to the reproductive values of colony members.

1. Introduction

Division of labour is a prominent feature in the colonies of social insects, where specialized individuals constitute castes to perform their various tasks. Such specialization is considered to improve the efficiency of colony function [1]. In many social invertebrates, specialized castes show non-random spatial distribution patterns that are correlated with their functional roles, suggesting optimization of colony efficiency [2–5]. However, within-group conflict also generates non-random spatial arrangement of individuals of a group. For example, in non-clonal social organisms such as eusocial Hymenoptera, dominance interactions between workers resulting from kin-selected reproductive conflict promotes the selfish movement of individual workers, thus generating a non-random spatial distribution pattern [6,7]. Thus it is difficult to distinguish whether cooperative or competitive interaction generates a spatial distribution pattern found in a colony of social organisms that reproduce sexually.

Gall-forming aphids provide useful material to study optimal spatial organization because of clonal reproduction [8]. They live in a closed space and reproduce parthenogenetically: they are thus expected to have no kin-selected conflict within a colony if there is no migration between galls (but see [9–11]). They have evolved different levels of sociality many times independently, where some colony members perform altruistic tasks at the expense of reproduction [12,13]. A previous study showed a task-related spatial distribution pattern in the gall-forming social aphid *Pemphigus spyrothecae* [14]. In open galls of *P. spyrothecae*, morphologically specialized defenders are preferentially located in the area vulnerable to predation, whereas reproductive morphs are located in safer areas. However, it has remained unclear how these different morphs change their spatial positioning to generate the task-related spatial pattern during opening of galls.
The aphid *Quadrartus yoshinomiyai* has a unique social system that consists of two distinct types of defensive individuals—young and old. This species forms a completely closed gall on its primary host. When matured, the gall opens to let winged adults migrate to its secondary host, and then two morphs protect their colony against intruding predators. Younger nymphs, which are mainly first-instar nymphs and can potentially develop into winged adults, attack predators with their styliets [15]. In addition, wingless adults, most of which are the mothers and some are the grandmothers of the winged adults (K. Uematsu 2011, unpublished data) and stopped producing the nymphs around the time of gall opening, defend the colony by sticking themselves to predators with waxy secretion [16]. Thus, gall opening is the cue to promote the temporal division of labour over reproduction. Previous studies, however, have not investigated how colonies of *Q. yoshinomiyai*, which include these two types of defenders, organize each member’s behaviour after galls open to optimize their colony function.

Here, we investigate spatial distribution pattern and its development in *Q. yoshinomiyai* colonies, in order to determine whether spatial positioning is specialized to improve efficiency of colony function. We tested the hypothesis that the colony members change their spatial positioning in tune with their shift to defensive or reproductive tasks. From this hypothesis, we made the following predictions: (i) both wingless adults and younger nymphs will aggregate around the gall entrance at which predation risk is highest; (ii) their spatial distribution pattern will change after the gall opens. We confirmed these predictions by observations of the spatial distribution in natural open galls and the movement of colony members when a hole is made in the closed galls, i.e. artificially opened galls.

2. Material and methods

*Quadrartus yoshinomiyai* forms conspicuous galls on its primary host plant *Distylium racemosum*. A gall founder forms a closed gall, where it produces viviparous offspring that develop into wingless adults [16]. The gall lasts for about fourteen months (from April to June of the next year), and inside a gall, the second and most of the third generations grow to wingless adults, and some of the third generation and almost all of the fourth generations born from the wingless adults grow to winged adults (K. Uematsu 2011, unpublished data). The closed gall matures and forms an exit hole in early to mid-April of the second year, then the winged adults escape from the open gall via the exit hole and migrate to the secondary host plant. The wingless adults and their younger offspring defend their colonies in the open galls. The galls dry up before mid-June. The mature galls were observed and collected at Shinkiba and Wakasu, Tokyo, Japan, and some were brought to the laboratory for further analysis.

(a) Spatial distribution pattern of aphids within natural open galls

Twenty galls of *Q. yoshinomiyai* each with an exit hole were collected on 10 April 2007 and 10 April 2009 (two galls from each of six trees in 2007 and four trees in 2009). Each gall was immediately removed from the tree and cut into three sections: (i) the section around the exit hole; (ii) the bottom half of the gall except around the exit hole; and (iii) the upper half of the gall. Each section was immediately preserved in 70% ethanol and brought to the laboratory. The content of each section was examined under a dissecting microscope, and the numbers of the following morphs were recorded: (i) wingless adults, (ii) winged adults, (iii) third or fourth-instar nymphs with wing buds (WB nymphs), (iv) second or third-instar nymphs without wing buds (NB nymphs), and (v) first-instar nymphs.

(b) Spatial distribution pattern of aphids within artificially opened galls

Thirty-one closed galls of *Q. yoshinomiyai* were collected from six trees at the sites and brought to the laboratory on 10 April 2009 and 7 April 2010. In the laboratory, two protuberances of the galls were marked for each gall by a polyvinyl chloride tape, a hole of 2 mm in diameter was bored on one of the protuberances, and another protuberance was used as a control. The galls were maintained at 20°C under a dark condition in an incubator. Twelve hours later, each gall was cut into two sections: (i) the section around the artificial hole, located within 5 mm of the bored hole of protuberance; and (ii) the section without an artificial hole (control), located within 5 mm of the tip of the protuberance. Each gall section was preserved and examined as described above.

(c) Data analysis

The gall sections collected from the artificially opened galls contained very few winged adults, thus the winged adults were excluded from the following analysis. All other data were checked for normal distribution by a visual inspection and the one-sample Kolmogorov–Smirnov test. Since all of them deviated from normal distribution, they were analysed with non-parametric statistics. All statistical analyses were conducted using the software R v. 2.9.2.

3. Results

(a) Spatial distribution pattern of aphids within natural open galls

In the gall section around the exit hole, the proportions of wingless adults and first-instar nymphs were significantly higher than those in either the bottom half section or the upper half section (Steel–Dwass test, for wingless adults: $p < 0.001$ in both cases; for first-instar nymphs: $p = 0.024$ versus the bottom half and $p < 0.001$ versus the upper half). By contrast, the proportions of winged adults, WB nymphs and NB nymphs were all significantly lower in the gall section involving the exit hole than in the other gall sections (Steel–Dwass test, for winged adults: $p < 0.001$ in both cases; for WB nymphs: $p < 0.001$ in both cases; for NB nymphs: $p = 0.004$ versus the bottom half and $p = 0.003$ versus the upper half; figure 1). No significant difference in the proportions of all morphs was found between the bottom half section and the upper half section ($p > 0.1$).

(b) Formation process of spatial distribution pattern during gall opening

In the gall section around the artificial hole, the numbers of wingless adults and first-instar nymphs were significantly higher than those in the control section without an artificial hole (Wilcoxon signed-rank test, $p < 0.001$ in both cases, figure 2a,b). By contrast, the number of WB nymphs and NB nymphs were significantly higher in the control section (Wilcoxon signed-rank test, $p < 0.001$ for WB nymphs and $p = 0.029$ for NB nymphs, figure 2c,d). When comparing a
after galls open, the residual reproductive value of the first-instar nymphs is much lower than other older aphids because the galls will be exposed to predation risk and nutritional decline owing to desiccation [15]. Thus, Q. yoshinomiyai colonies may optimize their group phenotype by minimizing the cost of altruistic defence at the group level, according to the reproductive values of colony members under seasonally changing environments. In addition, because they defend their colonies in quite different ways, it is of interest to investigate whether the combination of the young defenders attacking with stylets and old defenders sticking with waxy secretion has synergistic benefits for the colony fitness, which can also explain why these different types of defenders are evolutionarily maintained.

We thank William Foster, Andy Gardner, Antonio Rodrigues and anonymous referees for helpful comments. K.U. was supported by a Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists.

References


4. Discussion

We demonstrated that the morphs of the social aphid Q. yoshinomiyai exhibit non-random spatial distribution patterns within the open galls in the field. As predicted, defensive first-instar nymphs and wingless adults showed a tendency to be placed around the exit hole, whereas reproductive older nymphs and winged adults showed a tendency to be placed in a deeper area of the galls, consistent with the previous study in other social aphids [14]. In addition, our field observation and laboratory experiments revealed that defensive morphs move towards the artificial as well as the natural hole, whereas reproductive morphs move away from the holes. These results are consistent with our predictions and support the hypothesis that the spatial distribution patterns of the aphids reflect their task-related division of labour. After galls open, Q. yoshinomiyai colonies are likely to respond to the threat of predation, and optimize their defensive and reproductive strategy through spatial reorganization.

In the artificially opened galls, dynamic migration of the colony members occurred within 12 h in darkness, suggesting the presence of some recruitment mechanisms. Future studies should clarify the mechanism of the information transmission between colony members and which cues—for example, chemical or tactile ones—elicit responses of the aphids after gall opening.

This study reveals an adaptive spatial arrangement of a previously unknown type of temporal division of labour, in which the youngest and the oldest individuals move towards the dangerous areas to respond to the threat of predation and middle-aged reproductive individuals reproduce at the end of the galling season. The evolution of this social system in a clonal group can be understood as the result of adaptation at the group level as well as at the individual level [8]. After galls open, the residual reproductive value of the first-instar nymphs is much lower than other older aphids because the galls will be exposed to predation risk and nutritional decline owing to desiccation [15]. Thus, Q. yoshinomiyai colonies may optimize their group phenotype by minimizing the cost of altruistic defence at the group level, according to the reproductive values of colony members under seasonally changing environments. In addition, because they defend their colonies in quite different ways, it is of interest to investigate whether the combination of the young defenders attacking with stylets and old defenders sticking with waxy secretion has synergistic benefits for the colony fitness, which can also explain why these different types of defenders are evolutionarily maintained.

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