Self-sacrificing gall repair by aphid nymphs

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Insect galls are vulnerable to invasion by moth larvae that can tunnel into the wall. We report that nymphs of the aphid *Nipponaphis monzeni* repair their gall self-sacrificingly. When a hole was bored into their gall, many globular nymphs discharged a large amount of body fluid from their cornicles onto the gall's wound, and kneaded the fluid, which soon became viscous and eventually congealed, plastering over the hole. Having discharged the fluid, the nymphs shrivelled to approximately one-third of their original volume. Several nymphs were buried in the plaster, like 'aphid sacrifices'. This is the most elaborate social behaviour so far known among aphids.

Keywords: gall repair; social behaviour; aphid; cornicle secretion

1. INTRODUCTION

Because of their reproduction by parthenogenesis, aphids form clonal or nearly clonal colonies, where social behaviour is likely to evolve (Hamilton 1964). Two kinds of social behaviour have been found in aphids to date: colony defence and gall cleaning. Sterile or non-sterile defensive nymphs have been reported in approximately 50 species of the subfamilies Eriosomatinae and Hormaphidinae (Stern & Foster 1996; Aoki 2003). Nymphs of several gall-forming species are also known to eject honeydew, cast-off skins and dead aphids from their gall (Kurosu & Aoki 1991; Benton & Foster 1992). We have found a third kind of social behaviour in a gall-forming aphid: aphid nymphs repair their gall, sacrificing themselves in the process.

The aphid *Nipponaphis monzeni* (Hormaphidinae: Nipponaphidini) host alternates between *Distylium racemosum* (Hamamelidaceae), its primary host, and evergreen oaks such as *Quercus glauca* and *Q. myrsinaefolia*, its secondary hosts, in the southern half of Japan (Sorin 1958, 1996). Large, globular and completely closed galls (figure 1a) are formed on *D. racemosum* (Sorin 1958, 1996). Galls are still small in early spring and expand rapidly during the late spring (Sorin 1958). When the gall matures, its wall becomes up to 3.4–3.7 mm thick (Sorin 1958, 1996) and extremely hard (figure 1b). However, the gall wall is still soft while the gall is growing in April and May. During this time, galls of *N. monzeni* are vulnerable to invasion

by lepidopteran larvae that can tunnel through the wall. In fact, closed but not-so-hard galls formed by other aphid species on *D. racemosum* are often invaded by larvae of the nolid moth *Nola innocua* (Itô & Hattori 1982, 1983; Itô 1984). Defensive behaviour against artificially introduced insect larvae by aphid nymphs has been reported for five nipponaphidine species that form completely closed galls on *Distylium* trees (Kurosu *et al.* 1995; Aoki *et al.* 1999; Fukatsu *et al.* 2000). During our research on the defensive behaviour of *N. monzeni*, we noticed that its first-instar nymphs discharge a large amount of white body fluid not only onto the body of a target insect larva, but also onto the brim of a half-cut gall. This finding led us to suspect that nymphs of *N. monzeni* might repair holes bored into their gall.

2. MATERIAL AND METHODS

(a) Collections

The galls of *N. monzeni* used for laboratory observations were collected from *D. racemosum* at Shinkiba and Meguro, Tokyo, Japan, between 27 April and 16 May 2001. They were kept in cold storage (at 4 °C) until needed for experiments.

(b) Laboratory experiments

Ten galls were cut in two, and a lepidopteran larva (a sawfly larva in one case) was introduced into an open half of the cut gall. These larvae were 8.2–15.6 mm in length, and had been collected from *D. racemosum* and other plants (i.e. some were not natural enemies of *N. monzeni*). All larvae were attacked. The attacking behaviour of aphids was observed under a dissecting microscope or on a television monitor.

An additional two galls were also cut open. We lightly tapped 50 first-instar nymphs and 50 wingless adults from each gall with the blunt tip of a minute insect pin, and recorded whether they discharged body fluid within 10 taps. Many first-instar nymphs of *N. monzeni* that had and had not discharged white fluid were fixed and preserved in alcoholic formalin (ethanol : formalin : acetic acid, 16 : 6 : 1). Some of them were decapitated in 70% ethanol, dehydrated and cleared through an ethanol-xylene series, and embedded in Paraplast plus (Monoject). Serial tissue sections (5 µm thick) were prepared on a rotary microtome, mounted on siliconized microscope slides, stained with periodic acid-Schiff's reagent, and observed and photographed under a light microscope.

Fourteen galls were used for observations on gall-repairing behaviour. The galls were 35–83 mm in length ($n = 13$) and 24–56 mm in diameter ($n = 12$), and contained approximately 700–2400 live aphids apiece. The gall walls were 0.6–2.0 mm thick ($n = 13$). Using a sharp-edged tool, we bored a small hole into each gall and observed aphid behaviour under a dissecting microscope or on a television monitor until the hole was completely plastered up. Two galls were bored twice, and similar repair behaviour was observed on each occasion. After watching the repair process, we cut the gall in two, and ascertained from the inside that the plastering was complete. Later, the plaster was heated and dissolved in 10% potassium hydroxide solution, and the number of nymphs embedded in the plaster was counted.

(c) Field experiment and survey

We bored a small hole (ca. 2 mm in diameter) into a gall on a tree at Niiza, Saitama Prefecture, Japan, on 20 May 2001. The fate of the gall was followed until the gall opened naturally. We also searched for galls bored by natural enemies at Shinkiba and Meguro.

3. RESULTS

When a lepidopteran larva was introduced into an open half of a cut gall of *N. monzeni* a number of first-instar nymphs crawled onto the larva and stung it with their stylets. These nymphs were white in colour and globular in shape, their body cavities filled with numerous large globular cells (figure 2a). In attacking the intruder some of them discharged a large amount of white fluid from the cornicles (siphunculi) located near the posterior end of their back. This behaviour could also be elicited by lightly tapping the nymph's body with a fine instrument. Ninety

Figure 1. Gall of *Nipponaphis monzeni* formed on *Distylium racemosum*. (a) An entire gall, (b) a cut gall, indicating its thick wall.

Figure 2. Cross-section of the abdomen of the first-instar nymph of *Nipponaphis monzeni*. (a) A nymph before discharging body fluid. The body cavity is filled with many large globular cells. (b) A shrivelled nymph that has discharged body fluid, with the dorsoventral muscles contracted. Most of the globular cells have been discharged. Scale bars, 50 μ m. Abbreviations: dvm, dorsoventral muscle; gut, gut; lgc, large globular cell; myc, mycetome; ov, ovariole.

out of 100 first-instar nymphs discharged the fluid within 10 taps, while none of 100 wingless adults discharged the fluid. Having discharged the fluid, the nymphs shrivelled to approximately one-third of their original volume; most of the globular cells had been discharged out of the body with the fluid (figure 2b). Some nymphs also discharged the fluid onto the brim of the cut gall.

We bored a small hole into a gall of *N. monzeni* and observed aphid behaviour under a dissecting microscope. Immediately after the hole was made a number of first-instar nymphs gathered around the hole and discharged the fluid onto the damaged area (figure 3a). Because many nymphs participated, one after another, the volume of fluid increased rapidly (figure 3b). Using their legs and rostrum to knead the fluid, which soon became viscous and congealed (figure 3c), the nymphs skillfully plastered up the hole (figure 3d). Holes (ca. 1.3–4.5 mm in diameter) made by us were completely closed within 5–93 min (mean of 36 min, $n = 16$). In all cases ($n = 13$) where we later examined the contents of the plaster, a number of nymphs (range of 1–13, mean of 6.5) had been buried in the plaster, like ‘aphid sacrifices’. In addition a few nymphs were left outside the gall (figure 3d), and they would be likely to die under natural conditions.

In a field experiment in which we bored a hole in a gall on its host tree, the hole was plastered up as in the experiments in the laboratory, and the gall survived to produce alates. On 23 November 2001 the gall was found with an exit hole characteristic of departing alates (see Sorin 1958, 1996), and still contained many aphids. The

hole bored by us was completely closed up with plant tissue developed from around it.

At Meguro, a lepidopteran larva was found in the process of boring a hole in a gall of *N. monzeni* on 27 April 2001. We reared the larva in the laboratory to the adult moth, which turned out to be the polyphagous tortricid *Archips fuscocupreanus*. In addition, we found galls in the field bearing the scars of repaired holes. For instance, a gall collected at Shinkiba on 16 May 2001 had three holes that had already been plastered up. Two of them were almost round and ca. 1.0 mm and 1.1 mm in diameter. The remaining one was elliptical and 3.0 mm and 1.7 mm in major and minor axes. Silken threads remained covering over two of the holes on the gall surface suggesting that a lepidopteran larva had been there. Another gall collected at the same place on 19 May 2001 had an elliptical hole (1.6 mm and 0.6 mm in major and minor axes) that had been plastered up.

4. DISCUSSION

There is no doubt that the gall-repairing behaviour of *N. monzeni* is an adaptation against invasion by lepidopteran larvae. The original function of aphid cornicles is for defence against predators. When an aphid is attacked by a predator such as a coccinellid larva, the aphid produces a droplet of a waxy secretion from one of its cornicles or both, and tries to daub the secretion onto the head of the predator (Edwards 1966), often with the release of alarm pheromone that alerts nearby aphids (Dixon 1998). *Nipponaphis monzeni* also uses the cornicle secretion in

Figure 3. Gall repair by first-instar nymphs of *Nipponaphis monzeni*. (a) Several nymphs gather around a hole bored into their gall. One of them is seen positioning its abdomen to discharge body fluid from its cornicles. (b) Discharged white fluid accumulating. Nymphs knead the fluid with their legs and rostrum. (c) The hole is almost filled with 'plaster'. A nymph is seen adding a large droplet of body fluid. (d) The hole is completely closed. Four nymphs are left outside.

another way, to plaster up a hole bored into their gall. We suspect that the secretion might also function to cure the gall's wound, because the wound in the gall used for the field experiment was found cured six months later. N. Pike and W. Foster (personal communication) have recently found that a hole bored into the immature gall of the poplar aphid *Pemphigus spyrothecae* is closed, but this repair takes the form of compensatory growth of plant tissue from the undamaged part into the damaged part. However, Aoki & Kurosu (1991) excised a piece of tissue (a few cubic centimetres) from a gall of the aphid *Ceratoglyphina styracicola*. The large wound was cured almost completely, probably due to growth from the damaged part. It will be interesting to know whether aphids of *C. styracicola* or other gall-forming species use their cornicle secretion for this purpose.

Because at least some first-instar 'plasterers' of *N. monzeni* die in the process of gall repairing they are no doubt self-sacrificial altruists. We have not determined whether all plasterer nymphs die after discharging the body fluid or whether they are a morphologically distinct, sterile caste. Despite this uncertainty, we think that the gall repair of *N. monzeni* is the most elaborate social behaviour so far known among aphids, and may be comparable to the nest-making behaviour of social Hymenoptera and Isoptera.

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