

Edward B. Mondor · Jay A. Rosenheim ·
John F. Addicott

Predator-induced transgenerational phenotypic plasticity in the cotton aphid

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Abstract Transgenerational phenotypic changes, whereby offspring have an altered trait or a distinct alternate phenotype, frequently occur in response to increased maternal predation risk. The cotton aphid, *Aphis gossypii* (Glover), is unique, however, as offspring consist of four distinct phenotypes (“normal” light green apterae, “normal” dark green apterae, “dwarf” yellow apterae, and alatae), all with divergent life history patterns and resulting population dynamics. Here, we show that increased predation risk induces transgenerational phenotypic changes in cotton aphids. When exposed to search tracks from larval or adult convergent ladybird beetles, *Hippodamia convergens* Guérin-Méneville, cotton aphids produced greater numbers of winged offspring. In a subsequent experiment, apterous and alate individuals on clean plants were found to have primarily normal and dwarf offspring, respectively. We suggest that elevated predation risk may cause phenotypic changes in aphids over multiple generations, resulting in a more precipitous decline in herbivore populations than could be explained solely by increased predation rates.

Keywords Inducible defence · Polyphenism · Predation risk · Trait-mediated effects · Wing induction

E. B. Mondor (✉)
Department of Entomology, University of Wisconsin,
Madison, WI, 53706, USA
e-mail: mondor@entomology.wisc.edu
Tel.: +1-608-2624319
Fax: +1-608-2623322

J. A. Rosenheim
Department of Entomology, University of California,
Davis, CA, 95616, USA
e-mail: jarosenheim@ucdavis.edu

J. F. Addicott
Department of Biological Sciences, University of Calgary,
Calgary, AB T2N 1N4, Canada
e-mail: jaddicot@ucalgary.ca

Introduction

Many plants and animals exhibit intra-generational phenotypic changes to reduce herbivory or predation risk, respectively (Tollrian and Harvell 1998). Inducible defensive structures such as thorns, trichomes, and anti-feedant compounds are commonly produced by plants when fed upon by herbivores (Karban and Baldwin 1997; Agrawal et al. 1999). Examples of phenotypic changes in animals in response to predators or their cues include accelerated egg hatch rates and deeper tail fins in anuran larvae (Van Buskirk and McCollum 2000; Chivers et al. 2001), altered body shape in ciliates (Kusch 1993; Kolaczyk and Wiackowski 1997), accelerated development, longer caudal filaments, and heavier exoskeletons in mayfly larvae (Peckarsky et al. 2001; Dahl and Peckarsky 2002), and increased shell thickness in snails (Trussell 2000).

Inter- or transgenerational phenotypic changes in response to increased predation risk are more rare, but not unprecedented. When the maternal environment contains predator kairomones, offspring of the water flea, *Daphnia pulex* L., develop defensive crests and spines (Agrawal et al. 2000). Pea aphids, *Acyrtosiphon pisum* (Harris), when exposed to parasitoids (Sloggett and Weisser 2002), predators (Weisser et al. 1999; Kunert and Weisser 2003) or predator cues (Dixon and Agarwala 1999), increase production of winged offspring, so that the clone-mates can disperse to areas with lower predation risk.

Unlike other organisms studied so far, the cotton aphid, *Aphis gossypii* (Glover), a pest of agricultural plants such as cotton, melon, and cucurbits (Leclant and Deguine 1994; Blackman and Eastop 2000), exhibits extensive phenotypic plasticity. Cotton aphids do not alter the magnitude of a single trait or produce one alternate phenotype in response to changing environmental cues, but rather, offspring of a single individual can consist of any number of four distinct phenotypes, i.e. “normal” light green apterae, “normal” dark green apterae, “dwarf” yellow apterae, and alatae (Paddock 1919; Wall 1933;

Kring 1959; Wilhoit and Rosenheim 1993). Like alatae, dwarf apterae are a distinct phenotype (Watt and Hales 1996), having a body size approximately one-third of normal apterae, yellow instead of green colouration, and five compared to six antennal segments when fully developed (Takahashi 1966; Watt and Hales 1996).

Stimuli leading to the induction of alternate cotton aphid phenotypes are of great interest, as dwarf apterae have a markedly reduced intrinsic rate of increase compared to normal apterae, leading to substantially different population dynamics (Wilhoit and Rosenheim 1993; Watt and Hales 1996). Phenotypic changes in cotton aphids have focussed principally on environmental cues such as temperature (Wilhoit and Rosenheim 1993), host plant species (Watt and Hales 1996), and host plant quality (Nevo and Coll 2001). When natural enemies become increasingly abundant in cotton fields (i.e. mid-summer), however, both alate and dwarf phenotypes become more numerous (Wilhoit and Rosenheim 1993; Rosenheim et al. 1994; Godfrey and Rosenheim 1996). Thus, we hypothesized that cotton aphids, like pea aphids (Weisser et al. 1999), may be altering offspring phenotypes in response to increased predation risk. Cotton aphids may produce winged morphs to evade natural enemies, but as predators and parasitoids preferentially attack certain sizes (Frazer and Gill 1981; Chau and Mackauer 2001) and colours (Losey et al. 1997) of aphids, it may also be adaptive for cotton aphids to produce dwarf offspring.

Here, we show that normal, dark green cotton aphids alter offspring phenotypes under increased predation risk, i.e. when a larval or adult coccinellid (*Hippodamia convergens* Guérin-Méneville) has recently searched a host plant, by producing substantially more winged offspring. In a subsequent experiment, we then show that normal, dark green cotton aphids have primarily normal offspring, while alate individuals have primarily dwarf offspring.

Materials and methods

Insects and plants

A single cotton aphid, *A. gossypii*, clone which had been reared in the laboratory for approximately 2 years was used for the experiment. Aphids were reared as a synchronous colony under low density (<10 apterous adults per plant) to minimize the development of alate individuals. Aphids were reared on cotton, *Gossypium hirsutum* L. Maxxa, potted in standard garden soil.

Convergent ladybird beetle predators, *H. convergens*, were collected as fourth instar larvae and adults from alfalfa fields at the University of California, Davis, Calif., United States. Coccinellids were maintained in groups of 20 in 1-pint paper ice-cream containers. Beetles were fed an excess of pea aphids, *A. pisum*, every day and provided with water ad libitum.

Cotton plants were grown under greenhouse conditions: 21–38°C, 39–91% rh, and 16:8 L:D photoperiod. Plants

were watered daily and fertilized once a week with Schultz Liquid Plant Food Plus 10-15-10, (Schultz, St. Louis, Miss., United States). Plants were maintained aphid-free throughout their growth.

Experimental bioassays

In experiment one, an individual larva or adult *H. convergens*, which had been deprived of food for 24 h to elicit searching behaviour, was allowed to search a 12-day-old, two-to-three true leaf, aphid-free cotton plant for 24 h ($n=9$ and 8 larva and adult replicates, respectively). A Plexiglas tube (7 cm i.d. ×15 cm high) with a fine mesh top was placed over each plant to restrict the predator to the area of the plant. Control plants ($n=9$) were not searched but were grown, maintained, and caged under the same conditions as treatment plants. After 24 h, coccinellids were removed and a normal dark green apterous adult cotton aphid was placed on each plant, using a fine paintbrush. Plants were not altered in any way prior to aphid introduction, thus plants may have contained multiple predator search cues (e.g. search track compounds, faeces, etc.) Tubes were subsequently replaced and the aphids were allowed to produce offspring over 4 days. After 4 days, the adult aphids were removed and the progeny allowed to mature for 6 days. We chose this time period as cotton aphids require 8–10 days to reach adulthood under these rearing conditions (Wilhoit and Rosenheim 1993).

In experiment two, we compared offspring production by normal apterous and alate cotton aphids from our synchronous, low density rearing colony. Individual aphids were placed on 12-day-old, two-to-three true leaf cotton plants and allowed to have offspring for 3 days ($n=9$ and 12 apterous and alate replicates, respectively). Plexiglas tubes of the same size as those in experiment one were placed over each plant. After 3 days, the adult aphids were removed from the plants, and the progeny allowed to mature for 6 days.

For both experiments, plants were thoroughly examined for numbers and phenotypes of offspring, using a dissecting microscope. A small number of second generation progeny (i.e. from normals reaching maturity sooner than dwarfs) were easily distinguished from first generation adults, on the basis of their extremely small size (Watt and Hales 1996), and were not included in the aphid counts. To objectify aphid colour, apterous aphids were sorted into categories and compared to standardized colour plates, in the Methuen handbook of colour (Kornerup and Wanscher 1967). Aphids deviating slightly in colour from their counterparts, which were few due to high host plant quality and low density conditions, were included in counts of the closest matching colour. Normal light green, normal dark green, and dwarf yellow phenotypes most closely match plate 1 B8 (Munsell renotation: 1GY, 7.9/10.6), plate 28 F6 (Munsell renotation: 7GY, 3.3/2.5), and plate 2 A4 (Munsell renotation: 8.5Y, 9.0/4.5), respectively, in the Methuen handbook of colour (Korner-

up and Wanscher 1967). Alates, which were always the normal dark green colour or darker, were easily separated by the presence of wings or wing buds. Experimental conditions for both experiments were 20–22°C, 75–85% rh, and 16:8 L:D using two 40-W cool-white fluorescent bulbs and one 40-W wide-spectrum fluorescent bulb.

Statistical analysis

MANOVAs, using JMP IN 4.0.4, were used to analyse the data from both experiments (SAS Institute 2001). We chose to use MANOVA, thereby incorporating multiple dependent variables (i.e. the numbers of each offspring phenotype) into a single model. For the first experiment, the main factor was natural enemy search cue (no predator vs larval coccinellid search cues vs adult coccinellid search cues). For the second experiment, the main factor was adult phenotype (apterous vs alate). Dependent variables for both experiments were the numbers of normal light green, normal dark green, dwarf yellow, and alate aphids, transformed ($x' = \sqrt{x + \sqrt{x + 1}}$), to normalize the data (Zar 1984). Post hoc tests were conducted using Tukey's test, $P < 0.05$ (SAS Institute 2001).

Results

Cotton aphids exposed to cues from a coccinellid larva or adult produced similar numbers of offspring as aphids on "clean" control plants (predator treatment; $F_{2,23} = 2.70$, $P = 0.089$). Aphids did, however, produce different numbers of morphs (offspring phenotype; $F_{2,46} = 20.45$, $P < 0.0001$), but more importantly, aphids produced different numbers of morphs among the three natural enemy treatments (natural enemy search cue \times offspring phenotype; $F_{4,46} = 5.27$, $P = 0.0014$; Fig. 1). While the number of light green and dark green apterae did not differ between treatments, aphids exposed to search cues from either a larval or adult *H. convergens* produced around three times more alate offspring than aphids did on clean plants (Fig. 1). Surprisingly, aphids did not produce dwarf offspring on either clean or recently searched plants.

In the second experiment, individual apterous and alate cotton aphids on clean plants produced similar numbers of offspring (adult phenotype; $F_{1,19} = 2.10$, $P = 0.16$), but not equal numbers of morphs (offspring phenotype; $F_{3,57} = 8.03$, $P = 0.0001$). Offspring of apterous and alate adults consisted of vastly different numbers of morphs (adult phenotype \times offspring phenotype; $F_{3,57} = 13.67$, $P < 0.0001$; Fig. 2). Apterous had primarily light and dark green normal offspring, while alatae had primarily dwarf offspring (Fig. 2). Unlike the first experiment, normal apterous adults did produce dwarf offspring, albeit in extremely low numbers.

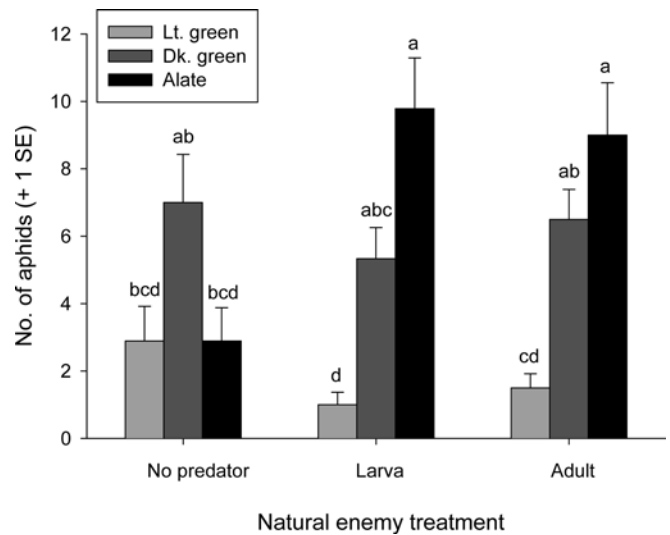


Fig. 1 Offspring phenotypes produced by normal dark green apterous cotton aphids over 4 days on a cotton plant (two-to-three leaf stage) with and without recent natural enemy (*Hippodamia convergens* larva or adult) presence for 24 h. Predator treatment \times offspring phenotype interaction; $F_{4,46} = 5.27$, $P = 0.0014$. Bars with different letters are significantly different (Tukey's test, $P < 0.05$)

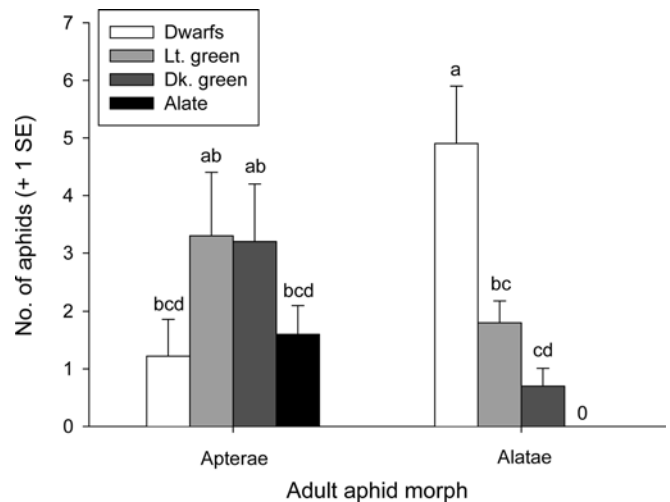


Fig. 2 Offspring phenotypes produced by normal dark green apterous and alate cotton aphids over 3 days on a cotton plant (two-to-three leaf stage). Adult phenotype \times offspring phenotype interaction; $F_{3,57} = 13.67$, $P < 0.0001$. Bars with different letters are significantly different (Tukey's test, $P < 0.05$)

Discussion

We are only beginning to understand how increased predation risk results in transgenerational phenotypic changes (Agrawal et al. 2000; Weisser 2001). In general, as predation risk increases, relatively sessile organisms tend to produce better defended offspring (Agrawal et al. 2000), while more mobile organisms produce offspring with behavioural and morphological adaptations to promote defence and/or emigration (Weisser 2001). Thus, as transgenerational polyphenisms have been observed in terrestrial and aquatic animals as well as

plants, transgenerational effects may be more common in nature than previously believed.

In this paper, we have shown that normal apterous cotton aphids produce significantly more winged offspring when either a larval or adult coccinellid has recently searched a host plant. Thus, large numbers of alate cotton aphids observed during the summer (Wilhoit and Rosenheim 1993) may be a direct result of increased predation risk. Dwarf cotton aphids were not produced in response to the level of predation risk experienced by aphids in our experiments, however, an increase in natural enemy density or searching duration may induce dwarf production. Subsequently, we found that alate individuals have predominantly dwarf offspring. Thus, if predator-induced alates are physiologically similar to density-induced alates, the subsequent generation could consist largely of dwarf offspring, as has been observed under field conditions (Wilhoit and Rosenheim 1993).

Predator-induced alate production would be adaptive, even if alate individuals have a lower intrinsic rate of increase than apterous individuals (Dixon 1998), to ensure that some clone-mates disperse to relatively enemy-free space (Weisser et al. 1999). The adaptive significance of dwarf offspring production by alates (Watt and Hales 1996; Mondor, unpublished data), however, is debatable. Dwarfs have a lower intrinsic rate of increase than do normal morphs (Wilhoit and Rosenheim 1993; Watt and Hales 1996) and dwarfs on high quality host plants take one to two generations to again produce normal apterae (Kring 1959). Thus, higher rates of survival from predators, parasitoids, or other environmental factors would be required to offset the fitness costs experienced by the clone over three to four generations. While predation rates on different phenotypes have not been evaluated, preliminary evidence suggests that dwarfs may be less susceptible to parasitism. Numbers of parasitoid mummies in aphid colonies were found to increase with increasing numbers of dark green apterae, but decrease with increasing numbers of dwarf apterae (Mondor et al., unpublished data). Further experimentation is required to determine the fitness costs and benefits of producing dwarf offspring.

The proximate mechanisms leading to transgenerational phenotypic changes in aphids are also not well-understood (Weisser 2001). As pea aphids alter offspring phenotypes in response to the direct searching of a wide range of natural enemies (Weisser et al. 1999; Sloggett and Weisser 2002; Kunert and Weisser 2003), it has been suggested that a “pseudo-crowding effect” may underlie this phenomenon (Sloggett and Weisser 2002). That is, aphids crawling back onto plants after being knocked off by predators may experience a stimulus similar to that of crowding, thereby resulting in greater alate production. However, phenotypic changes in pea aphids have also been observed solely in response to search cues from coccinellid larvae (Dixon and Agarwala 1999). In our experiments, aphids never directly encountered predators but only encountered search cues from larval and adult coccinellids, thus pseudo-crowding between adults is

unlikely to be the mechanism resulting in aphid transgenerational polyphenisms. It is more likely that changes in individual behaviour (e.g. more time walking on-plant or time spent off-plant), in response to natural enemy search cues may simulate reduced nutrient uptake similar to that of crowded conditions. This hypothesis remains untested, however, as we did not assess aphid activity in the different treatments.

We also recognize that interspecific interactions other than predator-prey interactions may influence aphid wing induction. Predator-induced polyphenisms were thought unlikely to exist in facultative or obligate ant-tended aphid species (Dixon and Agarwala 1999), however, cotton aphids, including this clone (Mondor, unpublished data), are facultative ant-tended. Ants frequently reduce wing-induction responses in aphids (El-Ziady and Kennedy 1956; Johnson 1959; Kleinjan and Mittler 1975). Thus, as increasing aphid density and predation risk may act in concert to promote wing-induction (Müller et al. 2001; Kunert and Weisser 2003) ants may be able to negate these polyphenisms. More research is required into the trade-offs between both predator- and mutualist-induced polyphenisms, and the resulting transgenerational effects.

In conclusion, predator-induced transgenerational phenotypic plasticity in cotton aphids has the potential to substantially alter aphid population dynamics. Aphid populations may decline directly due to predation, but may also decline indirectly through the emigration of winged individuals to neighbouring areas and the subsequent production of large numbers of dwarf apterae. Thus, the effects of predators on cotton aphid phenotypic expression may result in a more precipitous decline in aphid populations than would be expected solely from increased predation rates.

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