Alarm pheromone induces a transgenerational wing polyphenism in the pea aphid, *Acyrthosiphon pisum*

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**Abstract:** In response to increased predation risk, many organisms exhibit transgenerational polyphenisms whereby offspring have behavioural and (or) morphological adaptations to avoid natural enemies. The mechanisms underlying altered phenotypic expression, however, are not well understood. Aphids commonly exhibit a transgenerational wing-induction polyphenism in response to predators and parasitoids, but the stimuli inducing winged offspring production have not yet been identified. As aphids commonly emit the alarm pheromone *(E)-ß-farnesene* (EBF) when physically attacked, this compound is a reliable signal of increased predation risk for asexual conspecifics. Here we show that maternal detection of EBF induces a transgenerational wing polyphenism in offspring of the pea aphid, *Acyrthosiphon pisum* (Harris, 1776). In response to 50, 500, or 5000 ng of EBF vapor, aphids responded with 2.5-, 5.0-, and 6.0-fold increases in winged offspring production, respectively. Thus, alarm pheromone may alter aphid transgenerational phenotypic expression, thereby influencing aphid – natural enemy dynamics.

**Introduction**

Transgenerational phenotypic plasticity, whereby organisms alter the phenotypes of their offspring in response to increased risk of attack, is becoming increasingly recognized (Weisser 2001; Agrawal 2002). Despite its prevalence, the mechanisms underlying these phenotypic changes are not well understood (Weisser 2001; Agrawal 2002). A better understanding of the proximate mechanisms underlying altered phenotypic expression would contribute greatly to our ability to predict herbivore – natural enemy dynamics (Kusch 1998; Werner and Peacor 2003).

Pea aphids, *Acyrthosiphon pisum* (Harris, 1776), exhibit a transgenerational wing polyphenism whereby adults produce winged offspring in response to ladybird beetles (Weisser et al. 1999), hoverflies (Kunert and Weisser 2003), lacewings (Kunert and Weisser 2003), and hymenopteran parasitoids (Sloggett and Weisser 2002). Winged morphs have a longer development time, lower fertility, and a shorter life span than aperous morphs (Dixon 1998), but they have the ability to disperse to new host plants, thus forming new colonies in relatively enemy-free space (Dixon 1998). While the costs and benefits of producing winged phenotypes have been the

**Résumé :** En réaction à un risque accru de prédation, plusieurs organismes présentent des polyphénismes transgénérationnels par lesquels les rejetons possèdent des adaptations comportementales et (ou) morphologiques pour éviter les ennemis naturels. Les mécanismes qui sous-tendent cette expression phénotypique altérée sont, cependant, mal connus. Les pucerons présentent souvent un polyphénisme transgénérationnel d’induction des ailes en réaction aux prédateurs et aux parasitoïdes, mais les stimulus qui induisent la production de rejetons ailés restent à identifier. Comme les pucerons émettent couramment la phéromone d’alerte *(E)-ß-farnésène* (EBF) lors d’attaques physiques, ce composé est un signal fiable d’un risque accru de prédation chez les individus asexués de la même espèce. Nous démontrons ici que la détection de l’EBF par la mère induit un polyphénisme alaire transgénérationnel chez les rejetons du puceron du pois, *Acyrthosiphon pisum* (Harris, 1776). Les pucerons augmentent par des facteurs respectifs de 2,5, 5,0 et 6,0 la production de rejetons ailés en réaction à des vapes d’EBF de 50, 500 et 5000 ng. La phéromone d’alerte peut ainsi modifier l’expression phénotypique transgénérationnelle chez les pucerons, influençant alors la dynamique entre les pucerons et leurs ennemis naturels.

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subject of increasing investigation, the proximate mechanisms underlying this wing-induction response are not known.

(\(\text{E}\))-\(\beta\)-farnesene (EBF) is an alarm pheromone in over 19 genera of aphids, including that of the pea aphid (Kislow and Edwards 1972; Mondor et al. 2000), though some taxa use alternate compounds for alarm signaling (Nault and Phelan 1984). When attacked by a natural enemy, aphids often emit alarm pheromone, causing colony mates to stop feeding, walk from the site, and (or) fall off the plant in an attempt to escape (Nault et al. 1973; Pickett et al. 1992). Since aphids commonly emit EBF only when attacked (Nault et al. 1973), this pheromone is a reliable signal of increased predation risk for asexual conspecifics (Mondor and Roitberg 2004). EBF is also a good candidate compound to function as a proximate signal for wing induction in aphids, as only aphids with intact antennae exhibit wing-induction responses (Kunert and Weisser 2005). Furthermore, topical applications of EBF have been demonstrated to alter aphid development (van Oosten et al. 1990). Aerial exposure to this compound may also influence wing-induction responses, though studies have been equivocal owing to high dosages and uncontrolled density effects (Gut et al. 1987).

Here we test the hypothesis that the aphid alarm pheromone, EBF, is capable of inducing transgenerational phenotypic changes in pea aphids. By exposing maturing aphids to increasing concentrations of EBF vapor, we assess the degree to which augmented levels of aphid alarm pheromone alter the phenotypic expression of aphid offspring.

**Materials and methods**

**Insects and plants**

Pea aphids (\(\text{A. pisum}\)) were of a single, asexual lineage initially collected from red clover, \(\text{Trifolium pratense}\), in Wisconsin, USA. This pink clone was reared in the laboratory on broad bean, \(\text{Vicia faba}\), ‘Broad Windsor’, at 22 °C under a 16 h light (L): 8 h dark (D) photoperiod and low-density conditions, so that winged individuals were infrequently produced, for approximately 2 years. For the experiment, 32 broad bean plants (two plants per 10 cm pot, \(n = 16\) pots) were grown in Scotts potting soil (Scotts Company, Marysville, Ohio) at 23 °C, 16 h L: 8 h D, and watered daily.

**Experimental setup**

When plants were 2 weeks old (ca. 20 cm tall), two 5-day-old apterous aphids were placed in each pot and pots were then covered by a fine mesh sleeve. Upon reproducing, the two parental aphids were removed along with all but two offspring. These \(F_1\) offspring matured for 5 days (4th instar), and then pots were randomly divided into four equal groups and a single dose of one of four amounts (0, 50, 500, or 5000 ng) of EBF in the vapor phase was applied to each pot. The control group (0 ng of EBF) was exposed to the vapor of 5 \(\mu\)L of pure hexane (Fisher Scientific, Fair Lawn, New Jersey). For the treatment groups, pure EBF (Bedoukian Research, Inc., Danbury, Connecticut) was diluted with hexane to obtain 10, 100, and 1000 ng of EBF/\(\mu\)L. Aphids were exposed to 5 \(\mu\)L of solution, i.e., 50, 500, or 5000 ng of EBF. For all groups, solutions were pipetted onto 2 cm \(\times\) 2 cm pieces of filter paper and placed on a broad bean leaf approximately mid-plant. The filter paper remained in the pot during adult and progeny maturation. The two 4th-instar \(F_1\) aphids were given only this single dose of EBF during the experiment.

Aphids were exposed to EBF during the 4th instar because previous studies have shown that pre-reproductive aphids are capable of altering their reproductive output more than adults (Mondor and Roitberg 2003). We chose to use 50 and 500 ng of EBF in our experiment because these are biologically relevant amounts approximating those given off by 1 and 10 aphids, respectively, through cornicle droplets and body tissue damage following attack by natural enemies (Hedin et al. 1991; Mondor et al. 2000; Acar et al. 2001). We incorporated a 5000-ng EBF treatment in our experiment to discern whether extreme doses of alarm pheromone are required to evoke wing-induction responses.

The treatment that each pot received was determined randomly, using a random number generator (JMP IN 4.0.4, SAS Institute Inc. 2001). To minimize possible pheromone diffusion between treatments, replicates of each treatment were placed near each other in an environmental chamber. To control for any bias in pot location, however, we also randomized the location of each treatment group in the chamber.

After reproducing for 4 days, the two \(F_1\) adults were removed and the \(F_2\) offspring were allowed to mature. After the \(F_2\) generation had reached maturity, mesh bags were removed and proportions of winged phenotypes were counted. A small number of second-generation progeny (i.e., the \(F_3\) generation), from a few aphids reaching maturity, were easily distinguished on the basis of their small size (<2 mm) compared with that of adults (>4 mm) and were not included in the aphid counts.

**Statistical analyses**

A one-way analysis of covariance was performed, using JMP IN 4.0.4, to determine whether increasing levels of EBF induced greater proportions of winged offspring. The main factor was the amount of EBF (0, 50, 500, or 5000 ng). Aphid density per plant was incorporated into the analysis as a covariate to control for density-induced winged offspring production. The dependent variable was the proportion of winged offspring on each plant. To normalize the data, the dependent variable was transformed \((x' = \arcsin(\sqrt{x}))\) (Zar 1984). A Tukey–Kramer post hoc test was used to determine pairwise differences among EBF concentrations (SAS Institute Inc. 2001).

**Results**

Increasing amounts of EBF vapor altered offspring phenotypic expression in a relatively linear manner \((F_{[3,11]} = 6.85, P = 0.0072;\) Fig. 1). Aphids exposed to 50, 500, or 5000 ng of EBF produced 2.5, 5.0, and 6.0 times more winged offspring, respectively, than the control group (0 ng of EBF). The covariate, aphid density, was marginally significant, as aphids were more likely to produce greater numbers of winged morphs at the increasing but, overall, low densities in our experiment \((F_{[3,11]} = 3.66, P = 0.082)\). Thus, aphid alarm pheromone does induce a transgenerational
wing-induction polyphenism in pea aphids. Furthermore, increasing aerial concentrations of EBF result in larger proportions of winged offspring.

**Discussion**

Transgenerational polyphenisms are becoming increasingly recognized in both plants and animals (Agrawal et al. 1999). To better understand these phenomena and to improve our predictions of population dynamics (Lloyd and Lushai 1999), we require a more comprehensive understanding of the mechanisms underlying these phenotypic changes. As aphid alarm pheromone is commonly emitted only when an individual is physically attacked (Naught et al. 1973), EBF provides a reliable cue of increased predation risk for asexual conspecifics (Mondor and Roitberg 2004). Here, we confirm our hypothesis that exposure of maternal aphids to EBF culminates in increased winged offspring production.

Aphids exposed to 500 or 5000 ng of EBF produced 5.0 and 6.0 times more winged offspring, respectively, than aphids exposed to pure hexane. Five hundred nanograms would be given off if multiple aphids (approximately 10) were attacked by a predator (Hedin et al. 1991; Acar et al. 2001) and would thus be indicative of an intense colony predation sequence (Nakamuta 1983). Though the result was nonsignificant, we believe it to be biologically significant that aphids exposed to only 50 ng of EBF, an amount approximately equal to that given off by one attacked aphid, produced 2.5 times more winged offspring than controls. Pheromone emissions, even at small levels, may interact with other stimuli such as conspecific density or host plant quality (Müller et al. 2001; Kunert and Weisser 2003) to further influence phenotypic expression.

While EBF alters offspring phenotypes, the proximate physiological mechanism by which EBF induces this transgenerational polyphenism is still not understood. Induction of winged offspring production may be related to the observed intraclonal genetic variation among aphid phenotypes (Lushai et al. 1997). In response to adverse conditions such as predator-induced alarm pheromone emissions, it is possible that genes may be switched on or off, thereby altering the phenotypic expression of developing offspring (Lushai et al. 1997). Knowledge of the degree to which EBF induces phenotypic changes among aphid taxa, especially those that do not use EBF as an alarm pheromone (Naught and Montgomery 1979), and the extent to which structurally related compounds such as the nor-farnesenes (Nishino et al. 1976) induce phenotypic changes in offspring would provide a wealth of information regarding the physiological mechanism underlying this phenomenon.

It is important to note, however, that mechanisms other than the detection of EBF must also lead to increased wing-induction responses in aphids. Aphid wing polyphenisms have been observed in response to both natural enemies (Weisser et al. 1999; Sloggett and Weisser 2002; Kunert and Weisser 2003) and their search cues alone (Dixon and Agarwala 1999; Mondor et al. 2004, 2005). Since aphids exposed to natural enemy search cues would not receive alarm pheromone emissions, multiple stimuli must result in commonly observed wing-induction responses. We are currently examining whether reduced nutrient acquisition, through altered feeding patterns, is the common mechanism underlying aphid wing induction.

Because aphid alarm pheromone induces a transgenerational polyphenism, it is clear that herbivores can show both direct and indirect responses to an increased risk of attack from natural enemies (Werner and Peacock 2003; Schmitz et al. 2004). Since wing polyphenisms and polymorphisms exist in numerous insects (Zera and Denno 1997), the dynamics of many species may be better understood by identifying the mechanisms influencing the production of winged versus unwinged morphs.

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**References**


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