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Adaptation of Defensive Strategies by the Pea Aphid Mediates Predation Risk from the Predatory Lady Beetle

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Abstract Within a species, individual animals adopt various defensive strategies to resist natural enemies, but the defensive strategies that are adopted in response to variations in predation risk are poorly understood. Here, we assessed consecutive foraging processes on cohorts of two biotypes (green and red) of the pea aphid, *Acyrthosiphon pisum,* by the predatory lady beetle *Propylea japonica*, to investigate the adaptive mechanism underlying the defensive strategy. We observed the behavioral responses of individuals (continue feeding or escape, i.e., walk away or drop off from initial feeding site), simultaneously quantified the amount of alarm pheromone, (*E*)-β-farnesene (EβF) released from cohorts using gas chromatography-mass spectrometry (GC-MS), and recorded the foraging times of predators in intervals. The results indicated that: (1) the anti-predator responses differed markedly between biotypes and among the stages of the consecutive foraging processes. (2) Few green cohorts tended to release EβF during the first foraging; those that did released only a low dose that did not increase the number of escapes. However, the amount of EβF rose rapidly following the second foraging process, which caused an intense escape response. In contrast, more red cohorts released greater amounts of EβF, which caused more individuals to escape from their innate feeding sites during the first foraging. During the second foraging, more red individuals tended to escape without releasing EβF in greater quantities. (3) The foraging time was effectively shortened in each biotype cohort that adopted diverse defensive strategies. This study of the defensive strategies of the pea aphid may contribute to understanding the intraspecific differences in aphid defense mechanisms.

Keywords Pea aphid · Alarm pheromone · (*E*)-β-farnesene · Predation risk · Natural enemy · Defensive strategy

Li-Peng Fan and Fang Ouyang contributed equally to this work.

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Introduction

Environmental pressures influence the evolution of species through natural selection, which increases species diversity (Dall et al. 2004; Dingemanse and Reale 2005), and predation risk has been implicated as the major selective force in the evolution of various morphological and behavioral characteristics of prey species (Balog and Schmitz 2013b; David et al. 2014). Under different levels of predation pressure, individuals in a population evolve a variety of intraspecific phenotypes and specialized behavioral strategies to reduce predation risk (Ninkovic et al. 2013). The feeding strategies (i.e., feeding on the same plant for extended periods) and poor mobility of herbivorous insects in certain species or life stages may render them defenseless and susceptible to attack by predators, thus these herbivores are inclined to develop various adaptive defensive tactics (Hermann and Thaler 2014; Buchanan et al. 2017).

The pea aphid, *Acyrthosiphon pisum* Harris (Aphididae), feeds on various Fabaceae species in temperate regions

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(Frantz et al. 2006; Peccoud et al. 2009). Different biotypes of pea aphid present a wide variety of phenotypes, including body colors, host adaptations and anti-predator behavioral tactics (Braendle and Weisser 2001; Purandare et al. 2014). The pea aphid is prey to multiple species of predatory natural enemies, such as the lacewing, *Chrysoperla carnea* (Stephens), hoverfly, *Episyrphus balteatus* (DeGeer), and various lady beetle species. Increasing evidence suggests in response to predation risk, that pea aphids exhibit various types of defensive tactics that involve both behavioral and physiological responses (Schuett et al. 2015; Boullis et al. 2017).

Defensive behaviors in aphid primarily consist of camouflaged immobility, resistance behaviors (e.g., swinging the body or kicking predators) and escape behaviors (i.e., walking away or dropping from feeding sites) (Dixon 1958), and it is generally recognized that escaping from a feeding site before contact with a predator is a direct and effective approach for reducing predation risk (Braendle and Weisser 2001; Ninkovic et al. 2013). However, there is also evidence that aphid escape inevitably results in a loss of habitat for an uncertain amount of time (Losey and Denno 1998a), and the escaping individuals face other potential risks (Dill et al. 1990; Kielty et al. 1996; Honek et al. 1998).

The release of alarm pheromone is the typical physiological response of most aphid species to attack (Vandermoten et al. 2012), and sesquiterpene (*E*)-β-farnesene (EβF) is the only effective component of the pea aphid alarm pheromone (Francis et al. 2005a). Previous investigations demonstrated that EβF functions as a warning to conspecific individuals to avoid invading predators and revealed that the emission of alarm pheromone is an important component of the holistic defensive strategy in aphid populations (Mondor and Roitberg 2004; Verheggen et al. 2009; Nault 2013; Dumont et al. 2015). Interestingly, a few controversial reports have proposed that EβF might act as a stimulus and attract predators to microhabitats (Du et al. 1998; Mondor and Roitberg 2000; Francis et al. 2004).

Great effort has been invested into improving general theories explaining predator avoidance and exploring the anti-predator mechanisms of aphids (Chau and Mackauer 1997; Braendle and Weisser 2001; Mondor et al. 2005; Foster et al. 2011). Previous studies have revealed that not all pea aphids escape from their host plant (Losey and Denno 1998b; Harrison and Preisser 2016) or continuously release alarm pheromone before contact with a predator (Verheggen et al. 2008; Joachim et al. 2013). The available evidence seems to suggest that the response tendencies of aphids are affected by multiple factors, including aphid ontogeny, predator type, host qualities and other abiotic environmental variables (Dill et al. 1990; Mondor et al. 2000; Joachim and Weisser 2013). Recent studies also appear to support the hypothesis that the predation risk likely mediates various escape behaviors and EβF release patterns (Balog and Schmitz 2013b; Schuett et al. 2015).

The remarkable differences in anti-predator behaviors between the red and green body-color morph biotypes of the pea aphid have attracted the considerable concern in recent years (Braendle and Weisser 2001; Boullis et al. 2017). The differences between the two aphid biotypes may result from the combined effects of variation in body color, nutrient utilization, symbiotic bacteria and vulnerabiity to predation risk (Balog and Schmitz 2013a; Keiser et al. 2015; Polin et al. 2015), and the internal links among these factors might determine which defense strategies are adopted by the different biotypes. Here, to explore the mechanism underlying the choice of defensive strategy by the pea aphid, we conducted a series of two consecutive foraging processes to investigate the behavioral responses of different-colored aphids to the predatory lady beetle, *Propylea japonica* Thunberg (Coleoptera: Coccinellidae), which is an important predator of aphids in agroecosystems (Fig. 1a). We

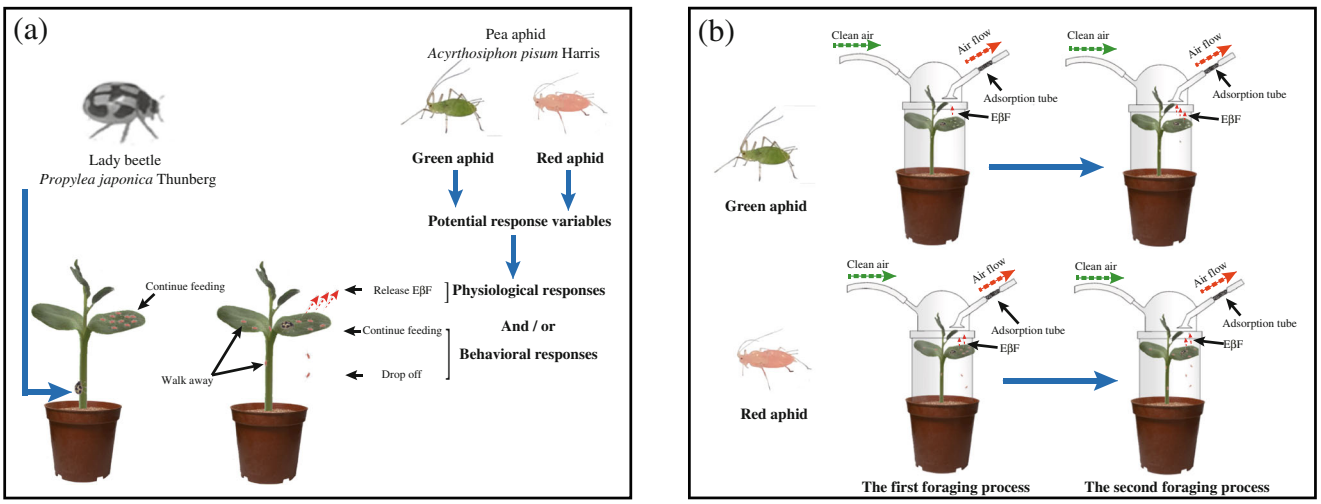

Fig. 1 Experimental design and procedures: **a** potential behavioral and physiological responses of pea aphids threatened by lady beetles. **b** behavioral and physiological responses of green and red pea aphids under two consecutive lady beetle foraging processes

recorded the foraging time at intervals and simultaneously quantified the EβF extracted from the cohorts using gas chromatography-mass spectrometry (GC-MS). Our objective was to sequentially investigate three interrelated issues: (1) the differences in the behavioral responses of aphids in green and red cohorts (continued feeding, walk away or drop from initial feeding sites under attack by *P. japonica*; (2) the quantities of EβF released from aphid cohorts; and (3) foraging time as a reflection of the risk to aphid cohorts adopting diverse defensive strategies. This investigation of defensive strategies may help us understand aphid evolution in terms of anti-predator mechanisms and provide a possible approach for improving aphid biological control.

Methods and Materials

Aphids and Lady Beetles

Two *A. pisum* color morphs were selected for this study because our preliminary work indicated that these biotypes exhibit differences in their sensitivity to artificial disturbances. The red morph pea aphids were originally collected from alfalfa hosts (*Medicago sativa* L. from Ningxia Province in northern China), and the green morph pea aphids were originally collected from pea plant hosts (*Pisum sativum* L. from Yunnan Province in southern China). The two biotypes were reared for more than 100 generations in our laboratory on the universal host plant, the broad bean, *Vicia faba* (seeds were supplied by the Biotechnology Research Institute of the Chinese Academy of Agricultural Sciences), with an average of 30 mixed-instar apterous aphids per plant under a 16 h light (22 °C) / 8 h dark (20 °C) photoperiod at 70 ± 10% relative humidity in photoclimate controlled chambers (PRX-450C, Saifu Experimental Instrument, Ningbo City, China). Under these conditions, pea aphid cohorts are unlikely to produce winged offspring (Purandare et al. 2014).

Lady beetles *P. japonica* are especially important predators of aphids, known to cause varous anti-predator responses (Francke et al. 2008; Barry and Ohno 2016). Adult *P. japonica* were collected from corn (*Zea mays* L.) in a field at the Yucheng experimental station of the Chinese Academy of Sciences in Shandong Province in northern China (116°36′E, 36°57′N) between September and October of 2014. In the laboratory, 5 pairs of lady beetles were reared per plastic cage (40 × 40 × 40 cm) and fed with the two color pea aphid biotypes in photoclimate controlled chambers that were maintained at 70 ± 10% relative humidity under a 16 h light (22 °C) / 8 h dark (20 °C) photoperiod. To prevent prey experience bias (Zarghami et al. 2014), 100 third-instar pea aphids (50 green morphs and 50 red morphs) were introduced into each cage as prey for the predators every day. In the tests, 3rd-generation adult lady beetles were used after 24 h of starvation.

Two Consecutive Predation Events

The pea aphid cohorts used for the experiment were established from the 3rd generation initiated from one apterous pea aphid of each biotype. First, a germinated broad bean seed was placed into a peat moss substrate (producer: Floragard, Germany) at a planting depth of 10 cm in a polyethylene flowerpot (13 cm diameter, 23 cm height). Approximately 10 days later, when the plant had grown to approximately 10 cm in height, a young reproductive adult aphid from the 2nd generation was transferred to a leaf on the middle-upper section of the new plant and covered with a transparent glass tube (9 cm in diameter, 15 cm in height) to restrict other aphids from feeding on the plant. After an additional 2 days (each young adult could produce approximately 6 to 8 nymphs per day in our system), the adult was removed, and the nymphs were reared to the third-instar stage. We chose to use 3rd insatr in experiments because the concentration of EβF in a droplet is highest in second- to fourth-instar pea aphid nymphs (Mondor et al. 2000). Then, 10 nymphs (at a low population density; pea aphid nymphs usually do not disperse until they develop into adults) were transferred to a new, unifested plant 2 h before introducing the predator. This 10-aphid infestation would not have affected the volatile components of the plants during the experiment (Schwartzberg et al. 2011). Next, the glass tube was carefully removed, and the broad bean with 10 aphids was placed in a pull headspace collection system. (8 cm diameter and 12 cm height with a total volume of 0.6 l) (Fig. 1b) (Tholl et al. 2006). A constant clean airflow, which was purified via passage through an activated charcoal filter, was pulled into the chamber through the inlet, and the air containing the volatiles emitted by the organisms was pulled out through the chamber outlet and drawn through an adsorbent tube (Porapak Q™,50 mg, Grace Alltech Inc., USA) connected to a vacuum pump through a Teflon® tube. The rate of the airflow passing through both the inlet and the outlet was 0.9 l / min^{-1}, which was controlled by a needle valve and measured by a flow meter. This flow rate allowed most volatiles to be purged in the minimum absorption time and minimized the interference with the aphids as much as possible. The interior position of the outlet was adjusted to 2 cm above the leaf on which the aphid cohort was feeding. After 1 h of stable aphid feeding under the ventilated conditions (Joachim and Weisser 2013), a predator that had previously been starved for 24 h was introduced into the system by placing it on the soil surface near the plant.

In order identify the avoidance strategies of each aphid biotype in response to predation risk, we set up 48 replicates per biotype and monitored behavior and alarm pheromones across two consecutive predation events within each replicate. In each replicate, we defined the phases of the two consecutive predation events as follows. The first foraging process was defined as the period from the time when the predator was introduced into

the system to when the predator captured its first aphid, and the second foraging process was the period from when the first aphid was completely consumed to when a second aphid was captured by the predator. If the predator did not capture prey within 5 min, the replicate was considered an unsuccessful foraging process and discarded (green cohort: total 51, successful replicates 48, discarded 3; red cohort: total replicates 53, successful replicates 48, discarded 5). The first EβF sampling time was defined as the period from when the lady beetle was first introduced into the system to when it contacted its first prey before consumption, and the second sampling time was from when the first aphid was completely consumed to when the predator contacted the second prey before consumption. The system remained under a state of constant ventilation during the interval between the two consecutiveforaging processes. The behavioral responses of the aphids, which had previously been described by Dixon (Dixon 1958), were observed during the two consecutive foraging processes, and the adsorbent traps were exchanged between sampling phases.

Chemical Identification and Quantification

The EβF absorbed by the Porapak Q tube was eluted with 0.5 ml hexane, and the EβF was immediately identified and quantified via coupled GC-MS on an Agilent Technologies 6890 N GC-5973 N MSD. (Agilent Technologies, Santa Clara, CA, USA). The GC was equipped with a DB-WAX column (60 m × 0.25 mm [i.d.] with a film thickness of 0.25 μm, J&W Scientific, Folsom, CA) that was used for the carrier gas with a constant flow rate of 1 ml / min, and a 5-μl sample was injected in splitless mode. The injection temperature was 230 °C; the GC-MS transfer line temperature was 230 °C; the ion source was 230 °C; and the quadrupole was 150 °C. Following injection, the column temperature was held at 50 °C for 1 min and then increased from 50 °C to 230 °C at 4 °C / min and held for 5 min. All compounds were analyzed with 70 eV nominal electron energy with selected ion monitoring (SIM) at 69, 93 and 120 (m/z), which discriminated EβF from co-eluting compounds to obtain peak integration areas within the retention time of the compound (Byers 2005). Compounds were identified by comparing their retention times with those of authentic reference standard trans-β-Farnesene (EβF, CAS: 18,794–84-8, assay ≥90%, Sigma-Aldrich, Germany) and by comparing their spectra with those of Nist02 mass spectral libraries (Rev. D.04.00, Agilent Technologies, Palo Alto, CA, USA). Compounds were quantified using four-point response curves constructed with the authentic standard, and the calibration curve was established using standard EβF hexane solutions at concentrations of 0.06, 0.30, 1.50, 8.0 and 40.0 ng•μl^{-1} via GC-MS. The calibration curve was described by the equation y = 148,830× - 193.11, $R^2 = 0.9995$, and the y response of the integral area and the x response to the amount of EβF (ng). Each concentration was tested 5 times.

Statistical Analyses

The behavioral response included three nominal independent variables (pea aphid biotype, EβF release, foraging stage), and the interaction terms (pea aphid biotype × EβF release, pea aphid biotype ×foraging stage, EβF release ×foraging stage, and pea aphid biotype × EβF release ×foraging stage) were included in the analysis. Three dependent variables (the numbers of feeding aphids remaining at, walking away or dropping from their initial feeding sites during each predation process), were analyzed by repeated measures ANCOVA (Supplementary 1). The numbers of aphids that performed different behavioral responses during each foraging process were compared using Student's t and non-parametric Kruskal-Wallis tests followed by Dunn's test and an adjustment of the p values for multiple pairwise comparisons with the Bonferroni correction. An analysis of covariance was used to test how the aphid biotype and the sampling time influenced the total amounts of EβF, and the differences in the percentages of green and red pea aphid cohorts releasing EβF during the first foraging process or during the second consecutive foraging process were analyzed using χ^2 tests. The effect of EβF release on the foraging time was tested by analysis of covariance with the number of feeding aphids and the amount of EβF as the covariates. The foraging time included a nominal independent variable (release EβF or not) and two dependent variables (the numbers of feeding aphids and the amount of EβF),

Multiple linear regressions were used to test the effect of the number of individuals that continued feeding in the aphid cohort and the total amount of EβF on the time it took the lady beetles to catch a prey item in each predation event. The partial eta squared (η^2) value was used to estimate the size of the main factors and their interaction effects. All statistical analyses were conducted using R software (version 3.4.0 - R development core team 2017).

Results

Behavioral Responses of Aphids in Consecutive Predation Events

The behavioral responses of pea aphids were significantly affected by the pea aphid biotype($F_{1,\ 195} = 23.57$, $P< 0.001$, S 1), the release of EβF ($F_{1,\ 191} = 19.06$, $P < 0.001$), as well as the foraging stage ($F_{1,\ 191} = 391.61$, $P < 0.001$).

During the first foraging process, most of the pea aphids, regardless of whether they were the green (6.3 ± 0.2) or red (5.2 ± 0.3) biotype, were prone to continue feeding on the original sites (Fig. 2a), but the number of green pea aphids that continued feeding was significantly greater than the number of red aphids ($t = 3.91$, $df = 1$, $P = 0.003$). In the presence of EβF, the number of green pea aphids that continued feeding (6.0 ± 0.5) was significantly greater than the number of red aphids (2.8

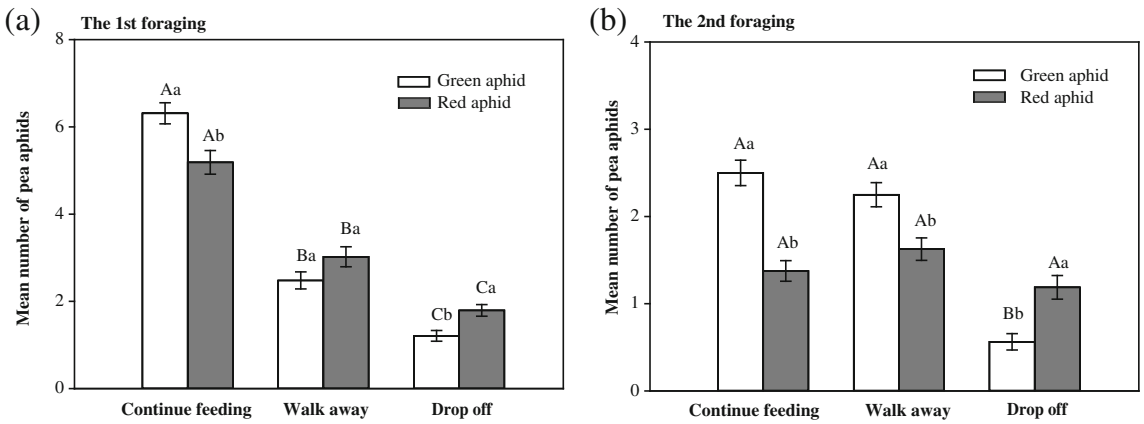

Fig. 2 Three behavioral responses (continued feeding, walking away and dropping off from thrir) between two pea aphid biotypes during (**a**) the first predation and (**b**) the second consecutive predation by a predatory lady beetle, *P. japonica*. Data are shown as the mean number ± SE. Different lowercase letters indicate significant differences between the green and red biotypes, and different uppercase letters indicate significant differences among the three behavioral responses within the same biotype

± 0.2) ($\chi^2 = 43.29$, $df = 3$, $P = 0.016$, Kruskal-Wallis, Fig. 3a), while the difference in the numbers of aphids that continued feeding between the two biotypes was nonsignificant if the first foraging process did not lead to the release of EβF ($\chi^2 = 2.80$, $df = 3$, $P = 1.00$). Number of green pea aphids that dropped off was significantly fewer than that of the red aphids (1.8 ± 0.1) ($t = 3.22$, $df = 1$, $P = 0.002$). In the presence of EβF, number of green pea aphids that dropped off (1.0 ± 0.3) was significantly fewer than the number of red aphids (2.4 ± 0.3) ($\chi^2 = 37.29$, $df = 3$, $P = 0.04$), while, if the first foraging process did not lead to the release of EβF, there was no significant difference between the two biotypes in the number of aphids that dropped off ($\chi^2 = 9.98$, $df = 3$, $P = 0.58$).

During the second foraging process, number of green pea aphids that continued feeding (2.5 ± 0.2) was significantly greater than the number of red aphids (1.4 ± 0.1) ($t = 6.00$, $df = 1$, $P < 0.001$, Fig. 2b), and the number of green pea aphids that walked away from initial feeding sites (2.3 ± 0.1) was also significantly greater than the number of red ones (1.6 ± 0.1) ($t = 3.31$, $df = 1$, $P = 0.001$). In the presence of EβF, number of green pea aphids that walked away from initial feeding sites (2.7 ± 0.2) was also significantly greater than the number of red ones (1.1 ± 0.1) ($\chi^2 = 43.64$, $df = 3$, $P < 0.001$, Kruskal-Wallis, Fig. 3b), while there was no significant difference between the two biotypes in the number of aphids that walked away from initial feeding sites if the second foraging process did not lead to the release of EβF ($\chi^2 = 5.03$, $df = 3$, $P > 0.05$). Number of green pea aphids that dropped off (0.6 ± 0.1) was significantly fewer than that of the red aphids (1.2 ± 0.1) ($t = 3.80$, $df = 1$, $P < 0.001$). If the second foraging process did not lead to the release of EβF, number of green pea aphids that dropped off (0.56 ± 0.11) was significantly fewer than the number of red aphids (0.8 ± 0.3) ($\chi^2 = 28.02$, $df = 3$, $P = 0.001$), while there was no significant difference between the two biotypes in the number of aphids that dropped off in the presence of EβF ($\chi^2 = 2.50$, $df = 3$, $P > 0.05$).

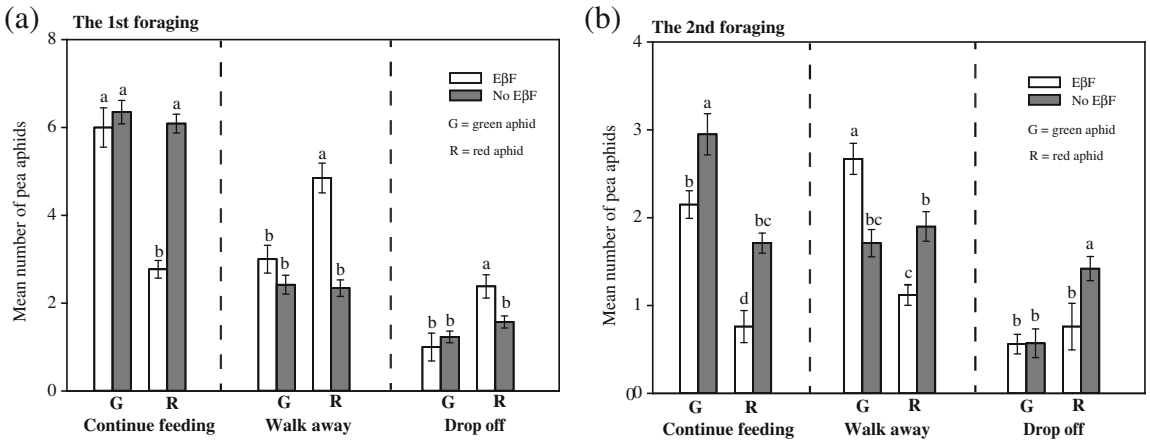

Fig. 3 The effects of EβF release on the behavioral responses of *A. pisum* during (**a**) the first and (**b**) second predation by *P. japonica*. Data are shown as the mean number ± SE. Different lowercase letters indicate significant differences in the number of responses within the same behavior

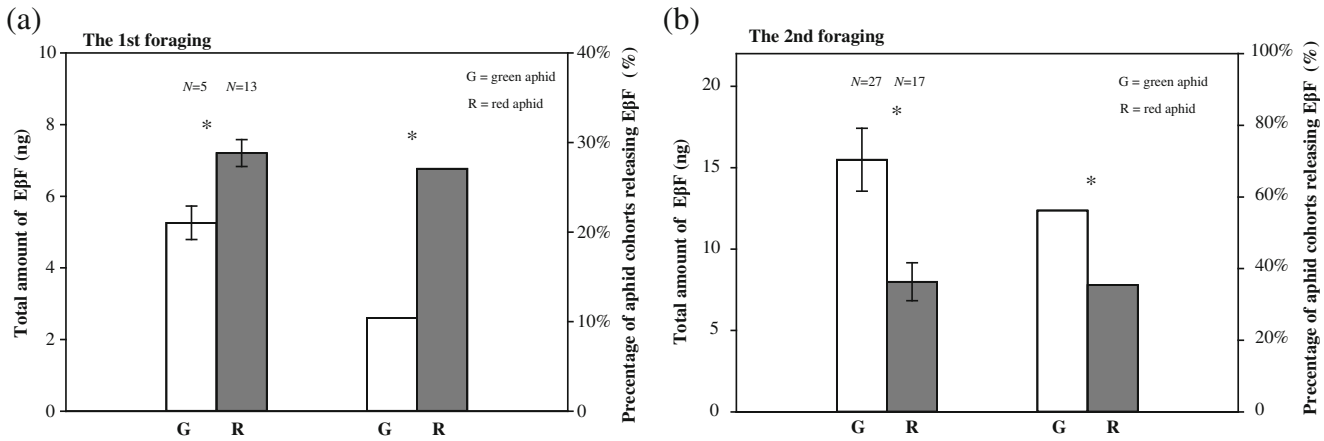

Fig. 4 Variations in the total amount of EβF and the percentage of aphid cohorts releasing EβF during (**a**) the first predation event and (**b**) the second predation event by the predator *P. japonica*. Data are shown as the mean ± SE. Asterisk indicates significant difference in the total amount of EβF (*t*-tests, $p < 0.05$) or in the percentage of aphid cohorts releasing EβF (χ^2 tests, $P < 0.05$) between green and red pea aphid cohorts

Release of Alarm Pheromone (EβF) by Aphids in Consecutive Predation Events

During the first foraging process, total amount of EβF released from red cohorts (7.2 ± 0.4 ng) was 27.1% higher than the total amount released from green cohorts (5.3 ± 0.5 ng) ($t = 2.88$, $df = 1$, $P = 0.011$, Fig. 4a). Percentage of red cohorts (27.1%) that released EβF before individuals came in contact with a predator was also 61.5% higher than the percentage of green cohorts (10.4%) ($\chi^2 = 4.38$, $df = 1$, $P = 0.036$).

During the second foraging process, total amount of EβF released from green cohorts (15.5 ± 1.9 ng) was significantly greater than that released from red cohorts (8.0 ± 1.2 ng) ($t = 14.42$, $df = 1$, $P < 0.001$, Fig. 4b). Percentage of green cohorts (56.3%) that released EβF was significantly higher than that of the red cohorts (35.4%) ($\chi^2 = 4.20$, $df = 1$, $P = 0.041$).

Comparing the releases of EβF between the first and second foaging processes, the results showed that green cohorts significantly increased both the total amount of EβF released and the percentage of cohorts releasing EβF during the second foraging process compared with the first foraging process (total amount: $t = 11.44$, $df = 1$, $P < 0.001$; percent releasing: $\chi^2 = 22.67$, $df = 1$, $P < 0.001$). In contrast, no significant changes in total amount of EβF and percent releasing were observed in red cohorts during the two foraging processes (total amount: $t = 1.70$, $df = 1$, $P = 0.10$; percent releasing: $\chi^2 = 0.78$, $df = 1$, $P = 0.38$).

Factors Affecting Pea Aphid Predation Risk

For both the pea aphid biotypes in the consecutive foraging processes, the number aphids that continued feeding had the greatest influence on foraging time (Supplementary 2). Both the first and second foraging times were significantly negatively correlated with the number of pea aphids that continued feeding (green: $R^2 = 0.233$, $p < 0.001$; red: $R^2 = 0.632$, $P < 0.001$, Fig. 5a and green: $R^2 = 0.268$, $P < 0.001$; red: $R^2 = 0.265$, $P < 0.001$, Fig. 5b, respectively).

During the firstforaging process (Fig. 6a), foraging time of the predators in the green cohorts releasing EβF (48.40 ± 8.74 s) was 36.9% shorter than the time in green cohorts without releasing EβF (76.65 ± 4.78 s) ($F_{1,\ 46} = 7.22$, $P = 0.01$). In contrast, the foraging time of the predators in red cohorts releasing EβF (72.77 ± 4.49 s) was 50.4% longer than the time in red cohorts without releasing EβF (48.40 ± 8.74 s) ($F_{1,\ 46} = 32.12$, $P < 0.001$).

During the second foraging process (Fig. 6b), foraging time of the predators in green cohorts releasing EβF (86.86 ± 4.04 s) was 36.5% shorter than the time in green cohorts without releasing EβF (138.17 ± 8.38 s) ($F_{1,\ 31} = 123.849$, $P < 0.001$) (Fig. 6b). Foraging time of the predators in red cohorts releasing EβF (90.80 ± 6.65 s) was 17.4% shorter than the time in red cohorts without releasing EβF (109.93 ± 4.53 s) ($F_{1,\ 35} = 26.050$, $P < 0.001$).

In the presence of EβF, total amount of EβF significantly influenced the time it took the predator to capture its first red prey ($F_{1,\ 182} = 7.66$, $P = 0.01$) and its second green prey ($F_{1,\ 182} = 3.45$, $P = 0.04$, Supplementary 2). The time it took the predator capture its first prey was positively correlated with the total amount of EβF (green: $R^2 = 0.049$, $P = 0.30$, red: $R^2 = 0.156$, $P = 0.001$) (Fig. 7a), and the time it took to capture the second prey was also positively correlated with the total amount of EβF (green: $R^2 = 0.188$, $P < 0.001$, red: $R^2 = 0.119$, $P = 0.03$) (Fig. 7b).

Discussion

Escape behaviors are the most important anti-predator responses of aphids (Dixon 1958), and we observed obvious discrepancies in escape behavior between the two color

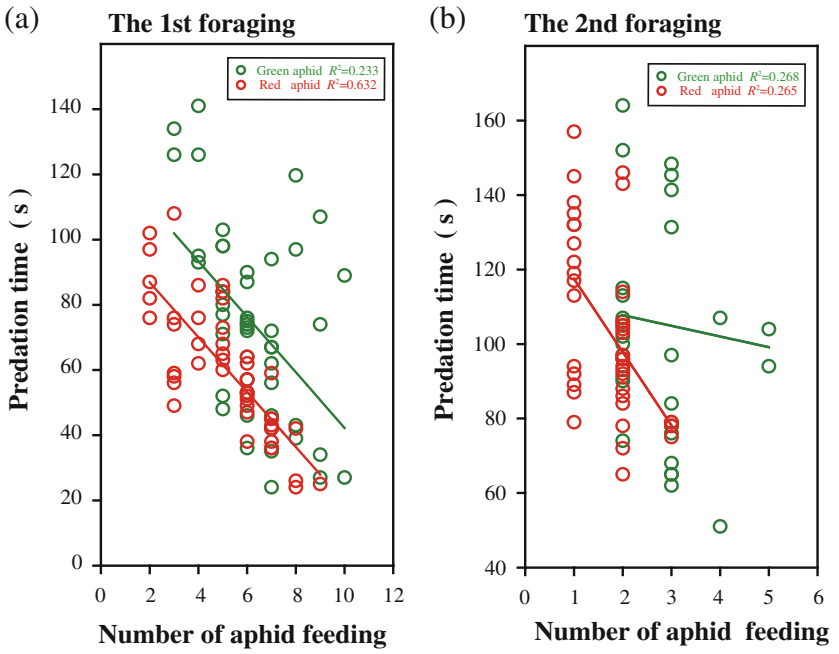

Fig. 5 Relationship between the number of aphids that continued feeding and the time required by the predator to locate its (**a**) first (regression equations: green y = 127.57–8.53×, $R^2 = 0.233$, $P < 0.001$; red y = 103.79–8.43×, $R^2 = 0.632$, $P < 0.001$) and (**b**) second (regression equations: green y = 113.51–2.87×, $R^2 = 0.268$, $P < 0.001$; red y = 137.22–19.69×, $R^2 = 0.265$, $P < 0.001$) prey in the pea aphid cohort

biotypes of pea aphids, especially in the different stages of consecutive foraging processes by a lady beetle. Most of the green individuals continued feeding and did not leave their original sites when the predator began to consume indiduals from the cohorts, but interestingly, once the first individual was caught and consumed, the remainder of individuals tended to abruptly walk away from their feeding sites. In contrast, the aphids in the red cohorts tended to immediately drop off and remain out of danger during the entire foraging process It is commonly assumed that escape behaviors might put individual aphids at a disadvantage (Chau and Mackauer 1997) because abandoning their host may cause aphids to lose their nutritional supply for an uncertain period as well as sustain other unknown injuries in the future (Byers 2005; Outreman et al. 2010). Our results indicated that disturbed red aphids were more likely to drop off the host plant, which was consistent with previous findings (Braendle and Weisser 2001; Boullis et al. 2017), and we further found that green aphids adopted a different means of escape (i.e., walking away) from the predator. Dropping off directly causes the aphids to lose their nutritional supply immediately, while the aphids that walked away from initial feeding sites might have found new feeding sites on the same plant within a short time (Chau and Mackauer 1997). An unpublished result from our other studies is that green aphids have higher nutritional requirements than red aphids, so maintaining a steady food supply is a more feasible behavioral response for green aphids until a predator enters the cohort. Thus, the predator came very

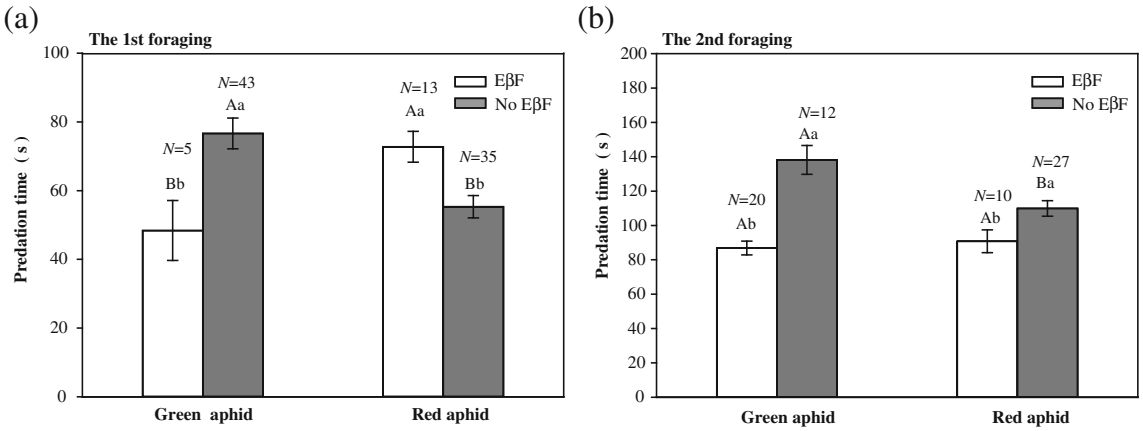

Fig. 6 The effects of EβF release on the time required for the predator *P. japonica* to locate the (**a**) first and (**b**) second prey during two consecutive predation events. Data are shown as the mean ± SE. Different uppercase letters indicate significant differences between the green and red cohorts within the time required for prey location under identical EβF release conditions, while different lowercase letters indicate significant differences between conditions with and without EβF releases within the same biotype treatment

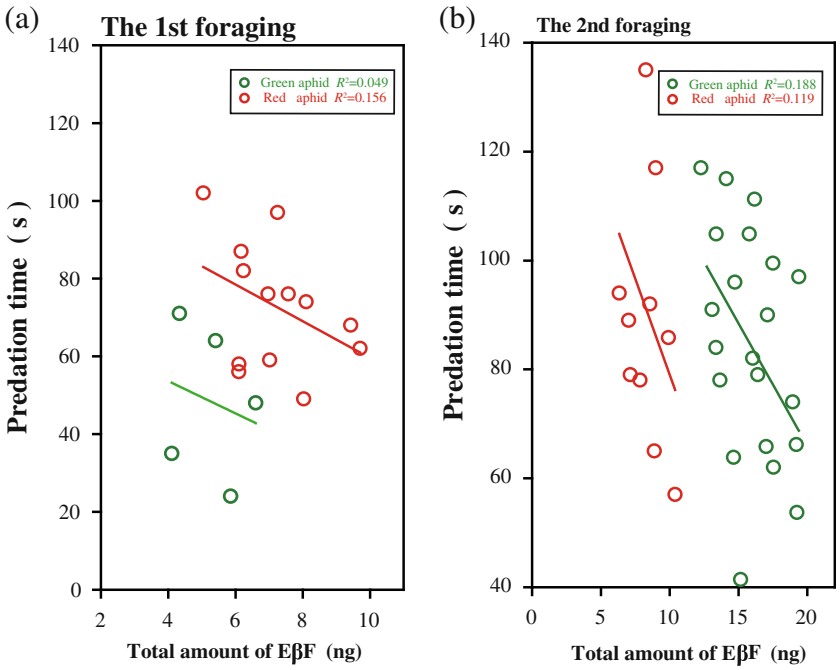

Fig. 7 Relationship between the total amount of EβF released from the pea aphid cohort and the time required by the predator to locate its (**a**) first (regression equations: green y = 70.24–4.15×, R^2 = 0.049, P = 0.30; Red y = 106.81–4.72×, R^2 = 0.16, P = 0.001) and (**b**) second (regression equations: green y = 156.00–4.50×, R^2 = 0.188, P < 0.001; Red y = 149.59–7.06×, R^2 = 0.119, P = 0.03) prey in the pea aphid cohort

close to the remaining aphids after the first predation, which may have increased the risk of detection by the predator for green aphids (Ameixa and Kindlmann 2012). Hence, walking away after perceiving signals of an immediate risk (i.e., visual cues, vibrations and chemical signals from predators) (Gish et al. 2011; Ninkovic et al. 2013) is a crucial adaptation to a change in predation risk.

The release of alarm pheromone is a typical physiological response to attacks by natural enemies in many aphid species (Verheggen et al. 2010; Vandermoten et al. 2012), and our research provides evidence that alarm pheromone release patterns (the amount and percentage of cohorts) differed between the two pea aphid biotypes during consecutive foraging processes. The red cohorts tended to continuously release alarm pheromone at a relative low dose throughout the foraging processes, and this tactic was characterized by the release of a slightly lower amount of pheromone and a stable percentage of pheromone-releasing cohorts. In contrast, green cohorts exhibited variable alarm pheromone release patterns; few green cohorts tended to release alarm pheromone before predator contact, and those that did released a lower level. However, after the first predation, both the percentage of cohorts releasing the pheromone and the amount of alarm pheromone released rose rapidly in green cohorts.

Alarm pheromone is generally considered to be a warning signal among pea aphid conspecifics (Pickett et al. 1992; Lambin et al. 1996), and it plays important roles in mediating interactions multi-trophic, especially the interactions between aphids and their natural enemies (Vandermoten et al. 2012). Although the release of EβF can benefit a cohort by providing an early warning signal, such releases are accompanied by increased physiological costs and potential predation risks (Mondor et al. 2000; Gwynn et al. 2005). The raw materials required to synthesize the triglyceride contained in EβF are difficult to obtain from food, and the EβF synthesis pathway is linked to juvenile hormone precursors. Thus, EβF synthesis may affect aphid development and offspring production (Mondor et al. 2000; Byers 2005). Moreover, some studies have suggested that releasing EβF could incur additional risks for aphid cohorts because EβF, either alone or in association with herbivore-induced plant volatiles, might attract natural enemies (Raymond et al. 2000; Hatano et al. 2008b). Consequently, there should be a selective advantage of minimizing EβF emissions to ensure that the benefits of alarm communication are higher than the costs of releasing the alarm signal itself (Gwynn et al. 2005; Joachim et al. 2013). In recent years, multiple methods have been used to analyze the release patterns of alarm pheromone when a single aphid or a cohort of aphids is being consumed by a predator (Schwartzberg et al. 2008; Joachim and Weisser 2013). Such studies have emphasized that alarm signaling varies among cohorts and that red pea aphid cohorts might not release alarm pheromone even when they are attacked by a predator (Hatano et al. 2008a; Joachim and Weisser 2013). The results of our research agree with those of these previous studies, and our findings further indicated that the alarm pheromone release patterns varied and that this is linked to variations in predation risk.

The predation risk to the prey was reflected in the foraging time (Crane and Ferrari 2016). *Propylea japonica* requires more time to locate green individuals in aphid cohorts producing a lower amount of EβF. At close range, vision plays an important role in the location of prey by lady beetles (Harmon

et al. 1998), so from this perspective, the color of green aphids, which is similar to the color of their host plants, apparently makes it more difficult for the predator to recognize and prey upon these aphids (Lambin et al. 1996; Farhoudi et al. 2014). In contrast, the red pea aphid biotype exhibits a strong visual signal in contrast to the green host leaves, so it is more likely to be caught by the predator, which implies that the predation risk for green individuals may be lower than that for red individuals before the predator invades a cohort. The foraging time of the lady beetle is negatively correlated with the prey density in an area, so escaping individuals would reduce the size of the cohort. Maintaining a minimum group size reduces the probability that a gregarious species will be found by a predator and prevents excessive death rates (Jackson et al. 2005). Therefore, the fewer aphids that continue feeding, the greater the time required by the predator to locate prey and the lower the predation risk to the remaining individuals, indicating that the predation risk of a cohort could be effectively reduced by escaping individuals.

The release of EβF did not always lead to the dispersion of individuals in the cohort, and the variable results may be due to the different amounts of EβF released and the sensitivity of the insects to EβF. The total amount of EβF released from the green cohort, which was lowest in the four tests, did not trigger a higher percentage of escape (walking away or dropping off). Previous work has indicated that the threshold dosage of EβF to trigger an escape response in 14 aphid species was between 0.02 and 100 ng (Montgomery and Nault 1977). The two pea aphid biotypes examined in the present study may also exhibit different sensitivities to EβF, which should be determined via electroantennography and olfactory tests in future research.

Our results indicated that EβF release reduced the time required for a successful predation by *P. japonica* in green aphid cohorts. Although an attraction response to EβF has been widely confirmed in parasitic wasps (Micha and Wyss 1996; Ameixa and Kindlmann 2012; Wang et al. 2015), whether aphid alarm pheromone can be used by predatory natural enemies to locate prey remains controversial (Francis et al. 2005b; Vosteen et al. 2015). Many factors, such as distance, the natural enemy species involved, herbivore-induced plant volatiles (HIPVs) and, especially, the amount of alarm pheromone, influence whether EβF can be objectively judged to be a kairomone (Nakamuta 1984; Purandare and Tenhumberg 2012; Vosteen et al. 2015). In our experiment, the time required for a successful foraging process by the predator was significantly reduced when EβF was released, indicating that the natural dose of EβF released by aphids could act as a cue for predators and help them locate prey over short distances.

In recent years, the potential use of alarm pheromone to manage aphid populations has been a controversial topic (Su et al. 2006; Dewhirst et al. 2010), but studies on the application of EβF for aphid control have made a great progress (Cui et al. 2012; Zhou et al. 2016). Our study further confirmed that EβF causes aphids in a cohort to leave their initial host plants, but they may find new host plants, which implies that the improper use of EβF might simply cause pests to spread more rapidly or widely in the field. Our study also indicated that a natural dose of released EβF may increase the probability that *P. japonica* will find prey in its microhabitats, which implies that EβF may be used as a strategy to enhance the ability of natural enemies to control pests. However, if the characteristics of the defensive strategies of aphids are not thoroughly studied, artificial releases of EβF without scientific support may result in reduced the control by natural enemies due to the dispersion of insect pests.

In summary, aphid defensive strategies, which consist of various behavioral responses and alarm pheromone release patterns, accompany predation risk dynamics. The present study reveals that the tailored measures should be adopted for the integrated pest management of different aphid species and even among different biotypes of the same species. Moreover, the phenotypic diversity of intraspecific pea aphid biotypes affects their ecological fitness and causes the differentiation of anti-predator behaviors. This investigation into the adaptive defensive strategies of aphids may contribute to the understanding of intraspecific differences in aphid defense mechanisms.

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References

Ameixa OMCC, Kindlmann P (2012) Effect of synthetic and plant-extracted aphid pheromones on the behaviour of *Aphidius colemani*. J Appl Entomol 136:292–301. https://doi.org/10.1111/j.1439-0418.2011.01638.x

Balog A, Schmitz OJ (2013a) Predation determines different selective pressure on pea aphid host races in a complex agricultural mosaic. PLoS One 8:e55900. https://doi.org/10.1371/journal.pone.0055900

Balog A, Schmitz OJ (2013b) Predation drives stable coexistence ratios between red and green pea aphid morphs. J Evol Biol 26:545–552. https://doi.org/10.1111/jeb.12070

Barry A, Ohno K (2016) Alterations in foraging behavior of Coccinella Septempunctata and Propylea japonica mediated by a novel defended prey affect their predatory potential. Entomol Exp Appl 161:31–38. https://doi.org/10.1111/eea.12486

Boullis A et al (2017) Elevated carbon dioxide concentration reduces alarm signaling in aphids. J Chem Ecol 43:164–171. https://doi.org/10.1007/s10886-017-0818-z

Braendle C, Weisser WW (2001) Variation in escape behavior of red and green clones of the pea aphid. J Insect Behav 14:497–509. https://doi.org/10.1023/a:1011124122873

Buchanan AL, Hermann SL, Lund M, Szendrei Z (2017) A meta-analysis of non-consumptive predator effects in arthropods: the influence of organismal and environmental characteristics. Oikos 126:1233–1240. https://doi.org/10.1111/oik.04384

Byers JA (2005) A cost of alarm pheromone production in cotton aphids, *Aphis gossypii*. Naturwissenschaften 92:69–72. https://doi.org/10.1007/s00114-004-0592-y

Chau A, Mackauer M (1997) Dropping of pea aphids from feeding site: a consequence of parasitism by the wasp, *Monoctonus paulensis*. Entomol Exp Appl 83:247–252. https://doi.org/10.1046/j.1570-7458.1997.00179.x

Crane AL, Ferrari MCO (2016) Uncertainty in risky environments: a high-risk phenotype interferes with social learning about risk and safety. Anim Behav 119:49–57. https://doi.org/10.1016/j.anbehav.2016.06.005

Cui LL et al (2012) The functional significance of *E*-β-Farnesene: does it influence the populations of aphid natural enemies in the fields? Biol Control 60:108–112. https://doi.org/10.1016/j.biocontrol.2011.11.006

Dall SRX, Houston AI, McNamara JM (2004) The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecol Lett 7:734–739. https://doi.org/10.1111/j.1461-0248.2004.00618.x

David M, Salignon M, Perrot-Minnot MJ (2014) Shaping the antipredator strategy: flexibility, consistency, and behavioral correlations under varying predation threat. Behav Ecol 25:1148–1156. https://doi.org/10.1093/beheco/aru101

Dewhirst SY, Pickett JA, Hardie J (2010) Aphid pheromones. In: Litwack G (ed) Vitamins and hormones: pheromones, vol 83. Vitamins and Hormones. Elsevier Academic Press, San Diego, pp 551–574

Dill LM, Fraser AHG, Roitberg BD (1990) The economics of escape behaviour in the pea aphid, *Acyrthosiphon pisum*. Oecologia 83:473–478. https://doi.org/10.1007/bf00317197

Dingemanse NJ, Reale D (2005) Natural selection and animal personality. Behaviour 142:1159–1184. https://doi.org/10.1163/156853905774539445

Dixon AFG (1958) The escape responses shown by certain aphids to the presence of the coccinellid *Adalia decempunctata* (L.) Trans R Ent Soc London 110:319–334. https://doi.org/10.1111/j.1365-2311.1958.tb00786.x

Du YJ, Poppy GM, Powell W, Pickett JA, Wadhams LJ, Woodcock CM (1998) Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. J Chem Ecol 24:1355–1368. https://doi.org/10.1023/a:1021278816970

Dumont F, Lucas E, Brodeur J (2015) Do furtive predators benefit from a selfish herd effect by living within their prey colony? Behav Ecol Sociobiol 69:971–976. https://doi.org/10.1007/s00265-015-1909-x

Farhoudi F, Allahyari H, Tabadkani SM, Gholizadeh M (2014) Prey preference of *Aphidoletes aphidimyza* on *Acyrthosiphon pisum*: effect of prey color and size. J Insect Behav 27:776–785. https://doi.org/10.1007/s10905-014-9470-4

Foster SP, Denholm I, Poppy GM, Thompson R, Powell W (2011) Fitness trade-off in peach-potato aphids (*Myzus persicae*) between insecticide resistance and vulnerability to parasitoid attack at several spatial scales. B Entomol Res 101:659–666. https://doi.org/10.1017/s0007485310000623

Francis F, Lognay G, Haubruge E (2004) Olfactory responses to aphid and host plant volatile releases: (*E*)-β-farnesene an effective kairomone for the predator *Adalia bipunctata*. J Chem Ecol 30:741–755. https://doi.org/10.1023/B:JOEC.0000028429.13413.a2

Francis F, Vandermoten S, Verheggen F, Lognay G, Haubruge E (2005a) Is the (*E*)-β-farnesene only volatile terpenoid in aphids? J Appl Entomol 129:6–11. https://doi.org/10.1111/j.1439-0418.2005.00925.x

Francis FD, Martin T, Lognay G, Haubruge E (2005b) Role of (*E*)-β-farnesene in systematic aphid prey location by *Episyrphus balteatus* larvae (Diptera : Syrphidae). Eur J Entomol 102:431–436. 10.14411/eje.2005.061

Francke DL, Harmon JP, Harvey CT, Ives AR (2008) Pea aphid dropping behavior diminishes foraging efficiency of a predatory ladybeetle. Entomol Exp Appl 127:118–124. https://doi.org/10.1111/j.1570-7458.2008.00678.x

Frantz A, Plantegenest M, Simon JC (2006) Temporal habitat variability and the maintenance of sex in host populations of the pea aphid. P Roy Soc Lond B Biol Sci 273:2887–2891. https://doi.org/10.1098/rspb.2006.3665

Gish M, Dafni A, Inbar M (2011) Avoiding incidental predation by mammalian herbivores: accurate detection and efficient response in aphids. Naturwissenschaften 98:731–738. https://doi.org/10.1007/s00114-011-0819-7

Gwynn DM, Callaghan A, Gorham J, Walters KF, Fellowes MD (2005) Resistance is costly: trade-offs between immunity, fecundity and survival in the pea aphid. P Roy Soc Lond B Biol Sci 272:1803–1808. https://doi.org/10.1098/rspb.2005.3089

Harmon JP, Losey JE, Ives AR (1998) The role of vision and color in the close proximity foraging behavior of four coccinellid species. Oecologia 115:287–292. https://doi.org/10.1007/s004420050518

Harrison KV, Preisser EL (2016) Dropping behavior in the pea aphid (Hemiptera: Aphididae): how does environmental context affect antipredator responses? J Insect Sci 16:1–5. https://doi.org/10.1093/jisesa/iew066

Hatano E, Kunert G, Bartram S, Boland W, Gershenzon J, Weisser WW (2008a) Do aphid colonies amplify their emission of alarm pheromone? J Chem Ecol 34:1149–1152. https://doi.org/10.1007/s10886-008-9527-y

Hatano E, Kunert G, Michaud JP, Weisser WW (2008b) Chemical cues mediating aphid location by natural enemies. Eur J Entomol 105:797–806. https://doi.org/10.14411/eje.2008.106

Hermann SL, Thaler JS (2014) Prey perception of predation risk: volatile chemical cues mediate non-consumptive effects of a predator on a herbivorous insect. Oecologia 176:669–676. https://doi.org/10.1007/s00442-014-3069-5

Honek A, Jarosik V, Lapchin L, Rabasse JM (1998) The effect of parasitism by *Aphelinus abdominalis* and drought on the walking movement of aphids. Entomol Exp Appl 87:191–200. https://doi.org/10.1046/j.1570-7458.1998.00320.x

Jackson AL, Brown S, Sherratt TN, Ruxton GD (2005) The effects of group size, shape and composition on ease of detection of cryptic prey. Behaviour 142:811–826. https://doi.org/10.1163/1568539054729105

Joachim C, Weisser WW (2013) Real-time monitoring of (*E*)-β-farnesene emission in colonies of the pea aphid, *Acyrthosiphon pisum*, under lacewing and ladybird predation. J Chem Ecol 39:1254–1262. https://doi.org/10.1007/s10886-013-0348-2

Joachim C, Hatano E, David A, Kunert M, Linse C, Weisser WW (2013) Modulation of aphid alarm pheromone emission of pea aphid prey by predators. J Chem Ecol 39:773–782. https://doi.org/10.1007/s10886-013-0288-x

Keiser CN, Mondor EB, Koenig W (2015) Cues of predation risk induce instar- and genotype-specific changes in pea aphid colony spatial structure. Ethology 121:144–151. https://doi.org/10.1111/eth.12325

Kielty JP, AllenWilliams LJ, Underwood N, Eastwood EA (1996) Behavioral responses of three species of ground beetle (Coleoptera: Carabidae) to olfactory cues associated with prey and habitat. J Insect Behav 9:237–250. https://doi.org/10.1007/bf02213868

Lambin M, Ferran A, Maugan K (1996) Perception of visual information in the ladybird *Harmonia axyridis* Pallas. Entomol Exp Appl 79:121–130. https://doi.org/10.1111/j.1570-7458.1996.tb00817.x

Losey JE, Denno RF (1998a) The escape response of pea aphids to foliar-foraging predators: factors affecting dropping behaviour. Ecol

Entomol 23:53–61. https://doi.org/10.1046/j.1365-2311.1998.00102.x

Losey JE, Denno RF (1998b) Interspecific variation in the escape responses of aphids: effect on risk of predation from foliar-foraging and ground-foraging predators. Oecologia 115:245–252. https://doi.org/10.1007/s004420050513

Micha SG, Wyss U (1996) Aphid alarm pheromone (*E*)-β-farnesene: a host finding kairomone for the aphid primary parasitoid *Aphidius uzbekistanicus* (hymenoptera: Aphidiinae). Chemoecology 7:132–139. https://doi.org/10.1007/bf01245965

Mondor EB, Roitberg BD (2000) Has the attraction of predatory coccinellids to cornicle droplets constrained aphid alarm signaling behavior? J Insect Behav 13:321–329. https://doi.org/10.1023/a:1007754000862

Mondor EB, Roitberg BD (2004) Inclusive fitness benefitsof scent-marking predators. P Roy Soc Lond B Biol Sci 271:S341–S343. https://doi.org/10.1098/rsbl.2004.0179

Mondor EB, Baird DS, Slessor KN, Roitberg BD (2000) Ontogeny of alarm pheromone secretion in pea aphid, *Acyrthosiphon pisum*. J Chem Ecol 26:2875–2882. https://doi.org/10.1023/a:1026402229440

Mondor EB, Rosenheim JA, Addicott JF (2005) Predator-induced transgenerational phenotypic plasticity in the cotton aphid. Oecologia 142:104–108. https://doi.org/10.1007/s00442-004-1710-4

Montgomery ME, Nault LR (1977) Comparative response of aphids to alarm pheromone, (*E*)-β-farnescene. ? Entomol Exp Appl 22:236–242. https://doi.org/10.1111/j.1570-7458.1977.tb02712.x

Nakamuta K (1984) Visual orientation of a ladybeetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), toward its prey. Appl Entomol Zool 19:82–86 https://www.jstage.jst.go.jp/article/aez1966/19/1/19_1_82/_pdf

Nault LR (2013) Aphid alarm pheromones... And now for the rest of the story. Am Entomol 59:229–239. https://doi.org/10.1093/ae/59.4.229

Ninkovic V, Feng Y, Olsson U, Pettersson J (2013) Ladybird footprints induce aphid avoidance behavior. Biol Control 65:63–71. https://doi.org/10.1016/j.biocontrol.2012.07.003

Outreman Y, Kunert G, Simon J-C, Weisser WW (2010) Ecological costs of alarm signalling in aphids. In: Kindlmann P, Dixon AFG, Michaud JP (eds) Aphid biodiversity under environmental change: patterns and processes. Springer Netherlands, Dordrecht, pp 171–181

Peccoud J, Ollivier A, Plantegenest M, Simon JC (2009) A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. PNAS 106:7495–7500. https://doi.org/10.1073/pnas.0811117106

Pickett JA, Wadhams LJ, Woodcock CM, Hardie J (1992) The chemical ecology of aphids. Annu Rev Entomol 37:67–90. https://doi.org/10.1146/annurev.en.37.010192.000435

Polin S, Le Gallic JF, Simon JC, Tsuchida T, Outreman Y (2015) Conditional reduction of predation risk associated with a facultative symbiont in an insect. PLoS One 10:e0143728. https://doi.org/10.1371/journal.pone.0143728

Purandare SR, Tenhumberg B (2012) Influence of aphid honeydew on the foraging behaviour of *Hippodamia convergens* larvae. Ecol Entomol 37:184–192. https://doi.org/10.1111/j.1365-2311.2012.01351.x

Purandare SR, Tenhumberg B, Brisson JA (2014) Comparison of the wing polyphenic response of pea aphids (*Acyrthosiphon pisum*) to crowding and predator cues. Ecol Entomol 39:263–266. https://doi.org/10.1111/een.12080

R Development Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Raymond B, Darby AC, Douglas AE (2000) The olfactory responses of coccinellids to aphids on plants. Entomol Exp Appl 95:113–117. https://doi.org/10.1046/j.1570-7458.2000.00648.x

Schuett W, Dall SRX, Kloesener MH, Baeumer J, Beinlich F, Eggers T (2015) Life-history trade-offs mediate 'personality' variation in two colour morphs of the pea aphid, *Acyrthosiphon pisum*. J Anim Ecol 84:90–101. https://doi.org/10.1111/1365-2656.12263

Schwartzberg EG, Kunert G, Röse USR, Gershenzon J, Weisser WW (2008) Alarm pheromone emission by pea aphid,Acyrthosiphon Pisum, clones under predation by lacewing larvae. Entomol Exp Appl 128:403–409. https://doi.org/10.1111/j.1570-7458.2008.00721.x

Schwartzberg EG, Boroczky K, Tumlinson JH (2011) Pea aphids, *Acyrthosiphon pisum*, suppress induced plant volatiles in broad bean, *Vicia faba*. J Chem Ecol 37:1055–1062. https://doi.org/10.1007/s10886-011-0006-5

Su J, Zhu S, Zhang Z, Ge F (2006) Effect of synthetic aphid alarm pheromone (*E*)-β-farnesene on development and reproduction of *Aphis gossypii* (Homoptera: Aphididae). J Econ Entomol 99:1636–1640. https://doi.org/10.1603/0022-0493-99.5.1636

Tholl D, Boland W, Hansel A, Loreto F, Rose USR, Schnitzler JP (2006) Practical approaches to plant volatile analysis. Plant J 45:540–560. https://doi.org/10.1111/j.1365-313X.2005.02612.x

Vandermoten S, Mescher MC, Francis F, Haubruge E, Verheggen FJ (2012) Aphid alarm pheromone: an overview of current knowledge on biosynthesis and functions. Insect Biochem Molec 42:155–163. https://doi.org/10.1016/j.ibmb.2011.11.008

Verheggen FJ, Mescher MC, Haubruge E, Moraes CM, Schwartzberg EG (2008) Emission of alarm pheromone in aphids: a non-contagious phenomenon. J Chem Ecol 34:1146–1148. https://doi.org/10.1007/s10886-008-9528-x

Verheggen FJ, Haubruge E, De Moraes CM, Mescher MC (2009) Social enviroment influences aphid production of alarm pheromone. Behav Ecol 20:283–288. https://doi.org/10.1093/beheco/arp009

Verheggen FJ, Haubruge E, Mescher MC (2010) Alarm pheromones-chemical signaling in response to danger. Vitam Horm 83:215–239. https://doi.org/10.1016/S0083-6729(10)83009-2

Vosteen I, Weisser WW, Kunert G (2015) Is there any evidence that aphid alarm pheromones work as prey and host finding kairomones for natural enemies? Ecol Entomol 41:1–12. https://doi.org/10.1111/een.12271

Wang GP, XD Y, Fan J, Wang CS, Xia LQ (2015) Expressing an (*E*)-β-farnesene synthase in the chloroplast of tobacco affects the preference of green peach aphid and its parasitoid. J Integr Plant Biol 57:770–782. https://doi.org/10.1111/jipb.12319

Zarghami S, Kocheili F, Mossadegh MS, Allahyari H, Rasekh A (2014) Prey preference and consumption capacity of *Nephus arcuatus* (Coleoptera: Coccinellidae): the influence of prey stage, prey size and feeding experience. Biocontrol Sci Tech 24:1062–1072. https://doi.org/10.1080/09583157.2014.919376

Zhou H, Chen L, Liu Y, Chen J, Francis F (2016) Use of slow-release plant infochemicals to control aphids: a first investigation in a Belgian wheat field. Sci Rep 6:31552. https://doi.org/10.1038/srep31552