



Differences in escape behavior between pea aphid biotypes reflect their host plants' palatability to mammalian herbivores

Matan Ben-Ari^{a,b}, Yannick Outreman^c, Gaëtan Denis^d,
Jean-François Le Gallic^d, Moshe Inbar^{a,*}, Jean-Christophe Simon^d

^aDepartment of Evolutionary and Environmental Biology, University of Haifa, Israel

^bDepartment of Science Education, Oranim Academic College of Education, Kiryat Tiv'on, Israel

^cUMR 1349, IGEPP (Institut de Génétique, Environnement et Protection des Plantes), Agrocampus Ouest, INRA, Université de Rennes 1, Université Bretagne-Loire, 35000 Rennes, France

^dUMR 1349, IGEPP (Institut de Génétique, Environnement et Protection des Plantes), INRA, Agrocampus Ouest, Université Rennes 1, Domaine de la Motte, 35653 Le Rheu Cedex, France

Received 24 April 2018; accepted 9 October 2018
Available online 16 October 2018

Abstract

Phytophagous insects have evolved traits that help them avoid predation risks, traits that may be affected by characteristics of the host plant. Since most phytophagous insects have narrow host ranges, we expect differences in risk avoidance between plant-specialized populations of several closely related insect lineages. To test this hypothesis, we used the pea aphid (*Acyrtosiphon pisum*), which forms a complex of about 15 biotypes, each adapted to one or a few species of legume plants (Fabaceae). We examined the differences in defensive behaviors of 38 clones from 13 distinct plant-specialized biotypes of pea aphids. We exposed mature aphids to simulated breath of a mammalian herbivore, a cue that causes part of the aphids in a colony to immediately drop off the plant to avoid incidental ingestion during mammal feeding. Dropping tendency varied substantially between biotypes (15–93% average rates). Dropping rates of a certain biotype of aphid reflected their host's palatability to mammalian herbivores, with ~80–90% rates in fodder and pasture plants and ~15–40% dropping in inedible plants. The dropping tendency showed no correlation with walking ability (tarsal & body length), nor with the tendency to escape in response to the alarm pheromone released by conspecifics in response to arthropod enemies. The specialization on a specific host plant brings with it particular selective pressures, and it seems that the palatability of the plants to mammals promotes behavioral divergence between biotypes, reinforcing diversification through ecological divergence.

© 2018 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.

Keywords: Host biotypes; Incidental ingestion; Dropping response; Mammalian herbivores; Plant edibility

Introduction

Phytophagous (plant-feeding) insects face complex selective pressures imposed by their host plants and natural enemies. In response to pressures by natural enemies,

*Corresponding author.

E-mail address: minbar@research.haifa.ac.il (M. Inbar).

phytophagous insects have evolved a wide range of effective defensive traits (e.g. physiological, behavioral, symbiont-mediated) (Gross 1993; Oliver, Russell, Moran, & Hunter 2003). Since defensive responses are usually costly (Nelson 2007) and different plant species incur different predation pressures (e.g. Ballabeni, Włodarczyk, & Rahier 2001), we expect a link between defensive responses and the intensity of specific natural enemy pressures on various plant species. We addressed this hypothesis with genetically distinct populations of the pea aphid, *Acyrtosiphon pisum*, adapted to feeding on different legume plants.

This aphid feeds in large colonies on a variety of legumes (family Fabaceae) (Van Emden & Harrington 2007) and can be a pest of legume crops. Pea aphid populations on distinct legume hosts experience considerable reproductive isolation (Peccoud & Simon 2010). This restriction in gene flow results in genetic divergence between populations, from partially isolated host races to incipient or cryptic species (Peccoud, Ollivier, Plantegenest, & Simon 2009). So far, 15 distinct biotypes (a term that applies to any degree of genetic or phenotypic divergence below or at the species level), each specialized on one or a few legume species and with their own genetic make-up have been characterized in the pea aphid complex (Peccoud et al. 2009; Ferrari, West, Via, & Godfray 2012; Peccoud, Mahéo, Huerta, Laurence, & Simon 2015).

Interestingly, in addition to plant specialization, this genetic divergence is associated with other phenotypic differences. For example, *A. pisum* populations specialized on pea, clover and alfalfa showed varying degrees of defense responses to the aphid alarm pheromone (*E*)- β -farnesene (Kunert, Belz, Simon, Weisser, & Outreman 2010), which resulted in variable responses to an arthropod enemy (i.e., predators and parasitoids). Such differences may have arisen simply via the accumulation of enough genetic differences between the biotypes (i.e. genetic drift). However, a new host plant entails a whole suit of characteristics, some of which affect the aphids' ability to avoid and withstand predation and parasitism (Hufbauer & Via 1999). Such characteristics may include plant topology, phenology, size, plant blooming and fruiting timing and the presence of other herbivorous insects (Feder 1995). The various legume plants that serve as hosts for *A. pisum* vary in many traits – size, color and shape, abundance, architecture, specialized metabolites and subsequent palatability to herbivores.

The differences in palatability of the different legumes to large mammalian herbivores may have bearings on the fate of pea aphids feeding on the same plant. Mammalian herbivores avoid unpalatable plants (Augustine & McNaughton 1998) that have chemical and physical defenses (Bryant et al. 1991). While grazing, mammalian herbivores consume large quantities of plant material, and may incidentally ingest insects that are feeding on the same plant (van Klink, van der Plas, Van Noordwijk, WallisDeVries, & Olff 2015; Gish, Ben-Ari, & Inbar 2017). This direct interaction between mammals and insects (henceforth referred to as “incidental ingestion”) has exerted substantial predation pressure on plant-dwelling

insects, as several insects have evolved the ability to detect an approaching mammalian herbivore and drop to the ground to avoid being eaten (Gish, Dafni, & Inbar 2011; Ben-Ari & Inbar 2013). Specifically, pea aphids very effectively avoid incidental ingestion by sensing the heat and humidity of the herbivore's breath and dropping to the ground (Gish, Dafni, & Inbar 2010). However, dropping to the ground has dire consequences for aphids by exposing them to ground predators (e.g. carabid beetles) and by inducing costs to re-establish on plants (Nelson 2007; Agabiti, Wassenaar, & Winder 2016). Therefore, pea aphids use several mechanisms to avoid dropping and reaching the ground unnecessarily (Gish, Dafni, & Inbar 2012; Ribak, Gish, Weihs, & Inbar 2013; Ben-Ari & Inbar 2014).

This interaction between insects and large mammals and the very distinctive dropping response it entails provide an opportunity to examine the link between host plant features and the biotype's phenotypic characteristics. In this paper we aim to explore two questions: (a) Is there variability in the behavioral response of various aphid biotypes to the threat of incidental predation? And (b) in case it exists, what factors could cause and maintain such a variability? To answer the first question, we screened the dropping response in multiple clones from the complex of pea aphid biotypes. To answer the second question, we formulated and tested several hypotheses that may account for dropping variation. These hypotheses are listed below:

1. Random, non-adaptive variability between biotypes accumulated through genetic drift.
2. Variability stems from differences in walking ability (size and leg length), which affect the clones' ability to survive on the perilous ground after dropping.
3. The variability in response to mammalian breath reflects another unknown factor in the clones' original habitat or their physiology – e.g. differences in costs of dropping to the ground or a variability of the tendency to drop in response to all threats.
4. The variability in the host plants' palatability to grazing mammalian herbivores affects a clone's level of response to the threat of incidental ingestion. Aphid biotypes specializing on a plant inedible to mammalian herbivores are less exposed to the risks of incidental ingestion, thus they will be less responsive to mammalian-born escape cues (warm and humid breath).

Materials and methods

Aphid and plant collection and rearing

We used 38 pea aphid clones belonging to 13 aphid biotypes (2–4 clones/biotype). Each clone was established from a single aphid collected from natural populations of *A. pisum* in France (Supplementary Appendix A: Table 1). Every clone used in this study has a unique genotype, as revealed by

Table 1. The legume host plants on which the aphid biotypes used in this experiment were feeding when they were collected. Palatability for mammals was determined according to the literature.

Scientific name	Common name	Palatability for mammals	Sources
<i>Medicago sativa</i>	Alfalfa, Lucerne	Edible, used for animal fodder	Hopkins and Nicholson (1999)
<i>Trifolium pratense</i>	Red clover	Edible, used for animal fodder	Collomb Bütikofer, Sieber, Jeangros, and Bosset (2002)
<i>Lotus corniculatus</i>	Bird's foot trefoil	Edible	Min et al. (1999)
<i>Vicia cracca</i>	Cow vetch, tufted vetch	Edible, used as food crop	Orhan et al. (2009)
<i>Pisum sativum</i>	Common pea	Edible, used for animal fodder	Vern et al. (2004)
<i>Medicago lupulina</i>	Hop clover, black medick	Unknown, saponins in roots	Oleszek, Price, and Fenwick (1988)
<i>Lathyrus pratensis</i>	Meadow pea	Unknown, grown in the past as fodder	http://www.luontoportti.com/suomi/en/NatureGate-plant-database
<i>Ononis spinosa</i>	Spiny restharrow	Unknown, but plant has long spines	Morisset and Boutin (1984)
<i>Melilotus officinalis</i>	Yellow sweet clover	Edible, but may be toxic when moldy	Kresge, Simoni, and Hill (2005)
<i>Securigera varia</i>	Crown vetch	Low palatability, inedible for non-ruminants	Gustine and Moyer (1990)
<i>Cytisus scoparius</i>	Common broom	Inedible	Saito, Suzuki, Yamashita, and Murakoshi (1994);
<i>Genista sagittalis</i>	Winged broom	Inedible	Harborne (1969)
<i>Genista tinctoria</i>	Dyer's broom	Inedible	Harborne (1969)

microsatellite profiles (Leclair et al. 2016). Correct assignment of these clones to their corresponding biotype has been checked using genotyping data and comparison with a reference dataset (Peccoud et al. 2009). Since their collection in 2011–2014, the clones have been maintained on *Vicia faba*, a universal host plant for all pea aphid biotypes, at 20 °C, 60% relative humidity and a 16:8 h L:D cycle. Before the experiment, adults from the various clones were placed on new *V. faba* plants and allowed to reproduce for 24 h and then removed, consequentially all adults used in the experiment were of the same age (10 days old).

Escaping in response to mammalian breath

To test for the variability in dropping response to mammalian herbivores, we exposed all clones to simulated mammalian breath. To avoid possible interference by colony members, we placed 30 single adult apterous (wingless) aphids from every clone, each on a separate small (10–15 cm high) *V. faba* plant. We used different plants for every experimental test to avoid residues of aphid feeding and pheromones. We conducted every test run on aphids from two clones, to avoid the possibility that special conditions in the experimental room affected the response of an entire clone. Plants were placed on a table, 50 cm between every two plants, and aphids were allowed one hour to acclimatize and settle. During that time, extra care was taken not to touch the experimental table or plants, or to move adjacent to the plants, which may affect their behavior (see Ben-Ari & Inbar, 2014).

After acclimatization, we exposed each aphid to two seconds of simulated mammalian breath. The mammalian breath simulator (as described in detail in Gish et al. 2010), via bubbling filtered air through hot water at a constant speed, allowed us to produce a constant airflow with heat and humidity resembling those of a mammal's breath (35.5 °C, 80–90% humidity). The airflow is of very low velocity (0.5 m/s) and is unlikely to dislodge an aphid, unless it intentionally lets go of its grip (Gish et al. 2011; Ben-Ari, Talal, & Inbar 2014). We held the muzzle of the apparatus for 2 s, 5 cm from the aphid, with the airflow reaching it from the dorsal side, further reducing the chances of unwillful dislodgement. To avoid unnecessary visual stimuli, the experimenter stood next to plants that had already been tested and approached the experimental plant only during the experiment. After two seconds, the response of the aphid was recorded (drop vs. remain on the plant).

To examine the link between dropping rates and palatability to mammals we surveyed the relevant literature on the 13 host plants as fodder or pasture for mammalian farm animals and the content of their defensive metabolites. Results of the literature survey are given in Table 1.

Escaping in response to the alarm pheromone

Aphids show variability in several behavioral responses, including the dropping response in reaction to arthropod natural enemies (Braendle & Weisser 2001). To test whether the tendency to drop in response to any cue, and not just mammalian breath, is a genetic attribute of a clone,

we applied another cue mimicking a threat of an arthropod natural enemy. We assessed the dropping response of selected biotypes to the threat of an arthropod enemy by exposing aphids to the volatile aphid alarm pheromone ((*E*)- β -farnesene—EBF) in order to examine whether there was a correlation between the response to the two cues (EBF vs mammalian breath). We tested three clones from four biotypes each (total of 12 clones) that showed the most notable contrast in dropping tendencies in the mammalian breath experiments – two biotypes with high dropping rates and two with low dropping rates.

Behavioral experiments were carried out in small tubes (3 cm diameter, 3 cm height) filled with agar-agar medium, up to a height of 3 cm, containing one leaf of *V. faba* plant. For a later application of EBF or control solution, we placed a piece of filter paper on a small toothpick, which we put into the agar next to the leaf. We then placed a single aphid of a given clone on the *V. faba* leaf and the set-up was covered with a cellophane bag to avoid aphid escape. The single experimental aphids had three to five hours to settle on the leaf before the behavioral test. The test started with the application of either 5 μ l of EBF solution (containing 100 ng EBF dissolved in *n*-hexane) or 5 μ l of the solvent (*n*-hexane) on the filter paper through a hole in the cellophane bag, using a micropipette. After five minutes of continuous observation, we recorded the response of the aphid: escaping by walking (dropping in response to EBF was very rare) or remaining on the plant. We conducted all the experiments in a laminar flow hood, in a room kept at constant 20 °C. For each aphid clone, we tested about 20 replicates per experimental treatment.

Aphid morphology

An aphid's body size and the length of its legs both reflect its ability to move on the ground after leaving the plant (Tokunaga & Suzuki 2008). We tested whether variability in dropping response was linked to body and tibia length. We preserved adult individuals of 26 of the 38 clones from the mammalian breath experiment (at least 2 clones of each biotype) in 70% ethanol. For each clone, we used 6–20 adults for morphometric analyses. These adults were not the ones used in the dropping experiment, rather they were from the same clone and sampled in the stock cultures soon after the experiment. We photographed the wingless adults using a Leica MZ12.5 binocular and an attached Olympus DP72 digital camera, and measured their body length from the base of the antennae to the cauda and tibia length using CellSense Entry software.

Statistical analyses

The data from the two behavioral experiments (i.e. escaping response to simulated breath and to EBF exposure) were analyzed separately. The dependent variable tested was the proportion of aphids dropping off the host plant, that is, a

binomial response variable. In the mammalian breath experiment, the biotype was the unique fixed independent variable. In the EBF experiment, two fixed independent variables and their interaction were analyzed: treatment (EBF or Control) and biotype. In both statistical modelling, the clone effect was included as a random factor nested within the biotype factor, and the replicate level was the single aphid. Statistical analyses were done by fitting generalized linear mixed models (GLMM) with binomial errors and a logit-link using the lme4 package (Bates & Dinkar 2007) in R 2.6.2 (R Development Core Team, 2014). Pairwise comparisons between biotypes were performed using a contrast method (DoBy package, 'esticon' function). For clones used in both behavioral experiments, we tested the correlation between the two measurements of plant leaving using the Pearson correlation index. We checked for correlation between escaping rates in the mammalian breath experiment and two morphometric criteria (body length and tibia length) using the Pearson correlation index.

Following initial results showing a marked variability in the dropping response, we tested whether dropping differences were linked to biotype genetic divergence. We used the genetic distance among these host-adapted aphid populations obtained from Peccoud et al. (2015) and tested for a correlation between the matrix of phenotypic variation (i.e. dropping rates) between all pairwise combinations of pea aphid biotypes and the genetic distance between the same pairs of biotypes.

Results

Mammalian breath experiment

Aphid biotypes showed remarkable variability in their dropping response when exposed to mammalian breath simulation (GLMM: $\chi^2 = 45.858$, $df = 12$, $p < 0.001$). While in some biotypes almost all aphids dropped immediately in response to simulated breath (93.33% dropping rates in *Medicago sativa*), aphids of other biotypes almost did not react to the mammalian presence cue (14.8% in *Genista tinctoria*) (Fig. 1). In general, within-biotype variability was low, with different clones of the same biotype displaying similar responses to the mammalian breath cue (with the exception of *Cytisus scoparius*). Standard error rates within clone were only 2–10% of the mean value, except in plant species in which the mean was very low.

Strikingly (Fig. 1), variation in dropping rates corresponded to the level of host-plant palatability as revealed in the literature survey (see Table 1 and references therein). While palatability is not a quantitative measure, plants with the highest levels of dropping rates for the corresponding aphid biotype were those described in the literature as edible for farm animals (*M. sativa*, *Trifolium pratense*, *Lotus corniculatus* and *Pisum sativum*). Conversely, aphid biotypes showing the lowest dropping rates came from plants

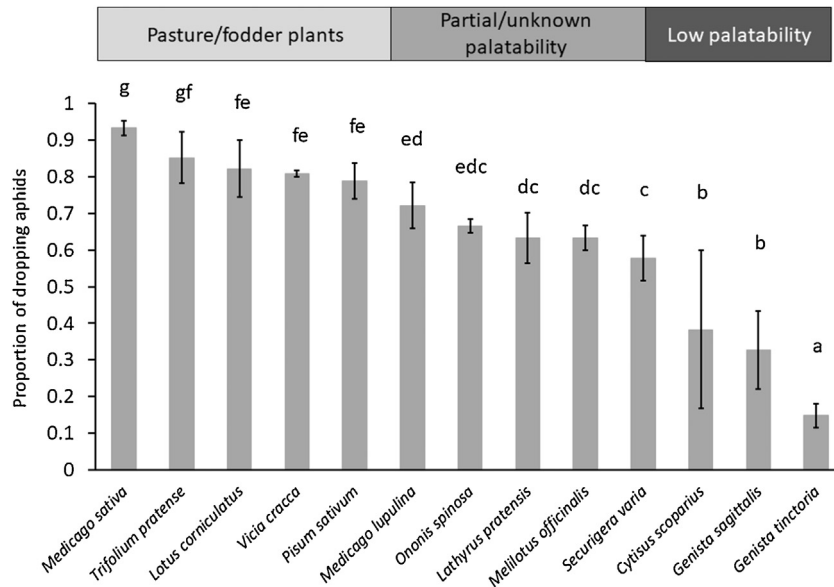


Fig. 1. The dropping rates of *Acyrtosiphon pisum* clones collected from 13 different legume species, in response to simulated mammalian breath. Bars denoted with the same letter do not differ significantly. Each value is the average of 2–4 clones tested and error bars indicate STE. Headers describe the palatability of the various host plants to mammals, as described in the literature (see sources in Table 1).

described as inedible due to toxic secondary metabolites they contain (for example, quinolizidine alkaloids in *C. scoparius* (Wink, Hartmann, Witte, & Rheinheimer 1982) and *Genista* species (Wink 1987), and flavonoids in *G. tinctoria* and *Genista sagittalis* (Harborne, 1969)). The ratio of dropping rates between biotypes did not show a significant link to genetic divergence between biotypes ($r = -0.183$, $p = 0.108$).

Alarm pheromone experiment

Aphid clones from the four tested biotypes (which had contrasting dropping rates in the mammalian breath experiment) responded significantly stronger to alarm pheromone exposure than to the control solution (Fig. 2; $\chi^2 = 62.28$, $df = 1$, $p < 0.001$). When exposed to the EBF solution, the proportion of aphids that escaped from the plant did not differ between *M. sativa*, *T. pratense*, *G. sagittalis* and *G. tinctoria* biotypes (Fig. 2; $\chi^2 = 1.48$, $df = 3$, $p = 0.685$). The *M. sativa* and *T. pratense* biotypes showed much lower escape rates when exposed to EBF than in reaction to simulated mammalian breath. Overall, for the 12 clones belonging to four studied biotypes, the responses to simulated mammalian breath and to EBF were not correlated (Fig. 3; $r = 0.30$, $p = 0.34$). There was no interaction between the biotype effect and the experimental treatment variables ($\chi^2 = 2.83$, $df = 3$, $p = 0.418$).

Aphid body morphology and dropping response under mammalian threat

The average body length of the various pea aphid clones varied considerably, between 0.97 mm (in a clone specialized

on *Ononis spinosa*) and 1.46 mm (in a clone specialized on *M. sativa*). Tibia length varied between 1.05 mm (in a different *O. spinosa* clone) and 1.48 mm (in a clone specialized on *G. tinctoria*). Nevertheless, the escape response of the various aphid clones in response to mammalian breath was correlated neither to their body length ($r = 0.19$, $p = 0.33$) nor to tibia length ($r = -0.017$, $p = 0.934$). The escape by walking rates in the pheromone control experiment were not correlated to these morphometric criteria either (body length: $r = -0.26$, $p = 0.53$; Tibia length $r = -0.04$, $p = 0.97$).

Discussion

The variety of legume plants used by the complex of pea aphid biotypes (Peccoud et al. 2009) expands the range of environmental factors aphids have to face when adapting to a new host, beyond a dichotomous change between two plants. The various hosts differ in many traits (see Table 1 and references therein) some of which may entail different kinds of selection pressures. We found that pea aphid biotypes showed remarkable variability in their dropping response to simulated mammalian breath, revealing further phenotypic divergence among biotypes. Within-biotype variability was low, further demonstrating the robustness of the differences we found. Previous studies have shown intra-species and inter-species variability in response to the alarm pheromone (Kunert et al. 2010). The unique nature of the cues we used allows us not only to highlight the extent of this variability, but also to place it in an ecological context.

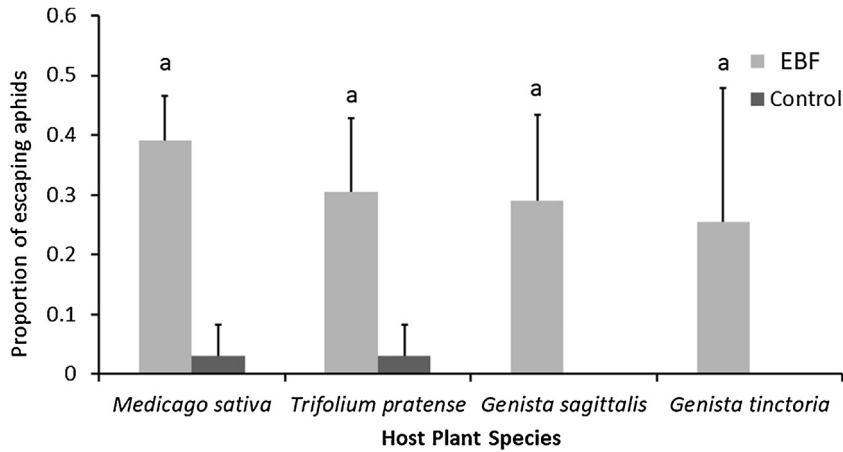


Fig. 2. The average escape rates (\pm SD) of *Acyrtosiphon pisum* clones collected from four different legume species, in response to alarm pheromone (E- β -farnesene) (treatment: control – open bars – vs. alarm pheromone (EBF) – full bars). Different letters indicate significant differences. Each value is the average of clones tested. Error bars indicate SD.

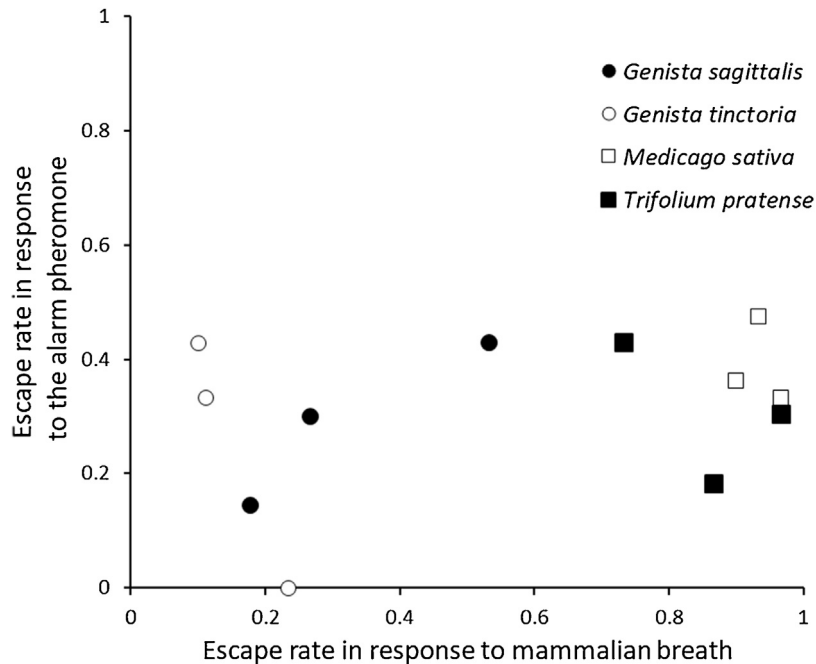


Fig. 3. Relationship between the escape rates of *Acyrtosiphon pisum* clones responding to the alarm pheromone (E- β -farnesene) and the escape rate of these clones in response to mammalian breath. One dot represents the average of one pea aphid clone. Clones were collected from four different legume species.

Reasons for variation in dropping response

In the wild, a sudden puff of hot and humid air can only be caused by an approaching mammal. Plant-dwelling insects such as aphids and ladybird beetles rarely drop when the air stream is of ambient heat and humidity (Gish et al. 2010, 2011; Ben-Ari & Inbar 2013). The extent of the present study, with the large number of clones and biotypes collected from the field, is a good indication of how variable the response to the threat of incidental ingestion by mammalian herbivores really is.

If variability were created randomly (hypothesis #1 presented in the Introduction) and relying on genetic drift, we would expect genetically distant biotypes to show greater divergence in their behavioral response. The lack of relationships between genetic distances and behavioral differences among the biotypes and the low variation in dropping rates between clones within the same biotype support the claim that the variability is indeed adaptive and not random. Hypotheses #2 and #3 (body size and general tendency to escape, respectively) seem implausible, since clones that display high dropping rates do not necessarily have longer bodies or legs,

which would have suggested improved walking abilities and increased survival on the hot ground (Ben-Ari, Gish, & Inbar 2015). Neither did they display a higher tendency to drop in response to another enemy-induced cue, which would have hinted at an overall effect of the host plant on aphid responsiveness to threats.

As suggested by hypothesis #3, different habitats and different host plants may bring about a difference in the costs aphids experience after dropping, through variation in ground temperatures (Dill, Fraser, & Roitberg 1990) and ground-dwelling predators (Losey & Denno 1998) and can affect the ability of aphids to grasp plant parts while falling (Meresman, Ben-Ari, & Inbar 2017). Such plant-mediated costs may result in different dropping tendencies as was found in different pea aphid biotypes response to the alarm pheromone (Kunert et al. 2010) and even within the same clone (Dill et al. 1990). Nevertheless, we show the variation in dropping response is cue-related. Aphid dropping response showed a markedly different pattern in response to EBF compared to mammalian threat. This latter threat induces a much more rapid response and prompts a much higher percentage of dropping aphids than the alarm pheromone response. The reaction to mammalian breath therefore reflects the special costs incidental ingestion may incur on aphids (Gish et al. 2017).

We therefore conclude that host plant palatability, and the increased threat of incidental ingestion by mammalian herbivores may be a driving factor shaping behavioral differentiation among pea aphid biotypes (hypothesis #4). This is another indication as to the importance of incidental ingestion of insects by mammals along with plant material. Recent works have shown the place of mammalian consumption in the shaping of insect behavior and physiology (Ben-Ari & Inbar 2014) and insect populations (van Noordwijk, Flierman, Remke, WallisDeVries, & Berg 2012; Gish et al. 2017).

Cascading effects of biotype formation

Attack rates of natural enemies (predators and parasitoids) are often mediated by plant morphological and chemical traits (Lill, Marquis, & Ricklefs 2002). Refuge from natural enemies may explain the host-plant shifts in herbivorous insects (Mulatu, Applebaum, & Coll 2004). Enemy-free space can drive the preference of insects for hosts on which they perform poorly (Ballabeni et al. 2001). Vosteen, Gershenson, and Kunert (2016) showed that different hosts provided enemy-free space for pea aphids against different types of predators, compared to the universal host (*V. faba*), potentially maintaining the divergence between biotypes.

The current study emphasizes the importance of host-driven pressure in the variability of biotype behavior and diversification. Research on the host plant's effects on insect biotypes tends to follow three possible paths: (a) reasons for and mechanism of the establishment of reproductive isolation

between sympatric populations (Matsubayashi, Ohshima, & Nosil 2010) (b) the different conditions experienced on the ancestral vs. new host, which provide new niches or an enemy-free space (Grosman et al. 2005) or (c) the genetic and/or phenotypic differences these biotypes exhibit (Peccoud et al. 2009; Kunert et al. 2010; Friedemann, Kunert, Gorb, Gorb, & Beutel 2015). Here, we link both (b) and (c) – the move of aphid biotypes to a new host places the aphids in a new set of selection pressures, and the phenotypic behavioral difference may be directly related to the host species and its characteristics.

Several studies have shown considerable phenotypic variation including behavioral ones between biotypes of a certain species (Via 1999; Abrahamson, Eubanks, Blair, & Whipple 2001; Prowell, McMichael, & Silvain 2004). Our study demonstrates the importance of testing as many biotypes as possible. Our ability to understand the adaptive value of a variable trait (escape response in this case) was achieved by comparing a large variety of biotypes and clones of pea aphids. The wide spectrum of reactions we discovered emphasizes the importance of looking at many different hosts (and see Wood 1993).

From patterns to mechanisms

Many works on aphids deal with the response to the alarm pheromone as a representation of response to natural enemies but such a response may manifest itself in several distinct behaviors (e.g. walking, kicking, dropping) (Dill et al. 1990; Kunert et al. 2010). The unique threat imposed by mammalian herbivores and the severity of the selection pressure it imposes on plant-dwelling insects (Gish et al. 2017) result in a very clear and rapid behavioral response (dropping). This interaction can be an invaluable tool in assessing differences in defensive behavior between biotypes and clones and in exploring the genetic architecture underlying this trait variation.

Variation in defense responses could be associated with the aphid microbiota. Both facultative and obligate symbionts (Dion, Polin, Simon, & Outreman 2011) induce phenotypic changes in their aphid host, some of which confer protection against natural enemies, such as parasitoid wasps (Oliver et al. 2003; Oliver, Degnan, Burke, & Moran 2010; Leclair et al. 2016) and pathogens (Scarborough, Ferrari, & Godfray 2005). Pea aphids from different biotypes have disparate symbiont compositions (Simon et al. 2003; Tsuchida, Koga, & Fukatsu 2004), and differences in symbiont assortment cause differences in defensive abilities against natural enemies (Dion et al. 2011; Polin, Simon, & Outreman 2014). It would be interesting to explore whether symbiont composition (as affected by biotype and clone) affects the tendency of aphids to drop in response to mammalian presence. Initial reports on aphids from several species have yielded negative results (Lavy, Sher, Malik, & Chiel 2015) but examining these differences in our system, with its range of different biotypes

(Peccoud et al. 2009) and our ability to control symbiont composition (e.g. Leclair et al. 2016) may provide further insights.

In conclusion, the gradient of responses to a single predation cue over a large variety of hosts demonstrates the extent to which biotypes can accumulate phenotypic differences. The pattern of diversity suggests that changes are not merely the results of drift, but are rather host-driven and related to host plant characteristics and host plant interactions with other unrelated organisms. The various biotic and abiotic conditions an organism acquires on its new host contribute to further ecological differentiation, and in the end may bring about diversification.

Acknowledgements

The authors would like to thank Jean Peccoud for early discussion on this project and fruitful feedbacks on dropping variation in pea aphid biotypes, and Rami Reshef from the university of Haifa for the use of his lab's microscope. This work was supported by the Agence Nationale de la Recherche (grant ANR-11-BSV7-007 to JCS) and by The Israel Science Foundation (grant No. 248/17 to MI).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.baae.2018.10.005>.

References

- Abrahamson, W. G., Eubanks, M. D., Blair, C. P., & Whipple, A. V. (2001). Gall flies, inquilines, and goldenrods: A model for host-race formation and sympatric speciation. *American Zoologist*, *41*, 928–938. <http://dx.doi.org/10.1668/0003-1569>
- Agabiti, B., Wassenaar, R. J., & Winder, L. (2016). Dropping behaviour of pea aphid nymphs increases their development time and reduces their reproductive capacity as adults. *PeerJ*, *4*, e2236 <http://dx.doi.org/10.7717/peerj.2236>
- Augustine, D. J., & McNaughton, S. J. (1998). Ungulate effects on the functional species composition of plant communities: Herbivore selectivity and plant tolerance. *Journal of Wildlife Management*, *62*, 1165–1183. <http://dx.doi.org/10.2307/3801981>
- Ballabeni, P., Włodarczyk, M., & Rahier, M. (2001). Does enemy-free space for eggs contribute to a leaf beetle's oviposition preference for a nutritionally inferior host plant? *Functional Ecology*, *15*, 318–324. <http://dx.doi.org/10.1046/j.1365-2435.2001.00529.x>
- Ben-Ari, M., Gish, M., & Inbar, M. (2015). Walking aphids can partake in within-field dispersal to distant plants. *Basic and Applied Ecology*, *16*, 162–171. <http://dx.doi.org/10.1016/j.baae.2014.11.007>
- Ben-Ari, M., Talal, S., & Inbar, M. (2014). Anticipatory and reactive crouching of pea aphids in response to environmental perturbations. *Environmental Entomology*, *43*, 1319–1326. <http://dx.doi.org/10.1603/EN14046>
- Ben-Ari, M., & Inbar, M. (2014). Aphids link different sensory modalities to accurately interpret ambiguous cues. *Behavioral Ecology*, *25*, 627–632. <http://dx.doi.org/10.1093/beheco/aru033>
- Ben-Ari, M., & Inbar, M. (2013). When herbivores eat predators: Predatory insects effectively avoid incidental ingestion by mammalian herbivores. *PLoS One*, *8*, e56748 <http://dx.doi.org/10.1371/journal.pone.0056748>
- Bryant, J. P., Provenza, F. D., Pastor, J., Reichardt, P. B., Clausen, T. P., & du Toit, J. T. (1991). Interactions between woody plants and browsing mammals mediated by secondary metabolites. *Annual Review of Ecology and Systematics*, *22*, 431–446. <http://dx.doi.org/10.1146/annurev.es.22.110191.002243>
- Collomb, M., Bütikofer, U., Sieber, R., Jeangros, B., & Bosset, J. O. (2002). Correlation between fatty acids in cows' milk fat produced in the lowlands, mountains and highlands of Switzerland and botanical composition of the fodder. *International Dairy Journal*, *12*, 661–666. [http://dx.doi.org/10.1016/S0958-6946\(02\)00062-6](http://dx.doi.org/10.1016/S0958-6946(02)00062-6)
- Dill, L. M., Fraser, A. H., & Roitberg, B. D. (1990). The economics of escape behaviour in the pea aphid, *Acyrtosiphon pisum*. *Oecologia*, *83*, 473–478. <http://dx.doi.org/10.1007/BF00317197>
- Dion, E., Polin, S. E., Simon, J. C., & Outreman, Y. (2011). Symbiont infection affects aphid defensive behaviours. *Biology Letters*, *7*, 743–746. <http://dx.doi.org/10.1098/rsbl.2011.0249>
- Feder, J. L. (1995). The effects of parasitoids on sympatric host races of *Rhagoletis pomonella* (Diptera: Tephritidae). *Ecology*, *76*, 801–813. <http://dx.doi.org/10.2307/1939346>
- Ferrari, J., West, J. A., Via, S., & Godfray, H. C. J. (2012). Population genetic structure and secondary symbionts in host-associated populations of the pea aphid complex. *Evolution*, *66*, 375–390. <http://dx.doi.org/10.1111/j.1558-5646.2011.01436.x>
- Friedemann, K., Kunert, G., Gorb, E., Gorb, S. N., & Beutel, R. G. (2015). Attachment forces of pea aphids (*Acyrtosiphon pisum*) on different legume species. *Ecological Entomology*, *40*, 732–740. <http://dx.doi.org/10.1111/een.12249>
- Gish, M., Ben-Ari, M., & Inbar, M. (2017). Direct consumptive interactions between mammalian herbivores and plant-dwelling invertebrates: Prevalence, significance, and prospectus. *Oecologia*, *183*, 347–352. <http://dx.doi.org/10.1007/s00442-016-3775-2>
- Gish, M., Dafni, A., & Inbar, M. (2012). Young aphids avoid erroneous dropping when evading mammalian herbivores by combining input from two sensory modalities. *PLoS One*, *7*, e32706 <http://dx.doi.org/10.1371/journal.pone.0032706>
- Gish, M., Dafni, A., & Inbar, M. (2011). Avoiding incidental predation by mammalian herbivores: Accurate detection and efficient response in aphids. *Naturwissenschaften*, *98*, 731. <http://dx.doi.org/10.1007/s00114-011-0819-7>
- Gish, M., Dafni, A., & Inbar, M. (2010). Mammalian herbivore breath alerts aphids to flee host plant. *Current Biology*, *20*, R628–R629.
- Grosman, A. H., Van Breemen, M., Holtz, A., Pallini, A., Rugama, A. M., Pengel, H., et al. (2005). Searching behaviour of an omnivorous predator for novel and native host plants of its herbivores: A study on arthropod colonization of eucalyptus in Brazil. *Entomologia Experimentalis et Applicata*, *116*, 135–142. <http://dx.doi.org/10.1111/j.1570-7458.2005.00307.x>

- Gross, P. (1993). Insect behavioral and morphological defenses against parasitoids. *Annual Review of Entomology*, *38*, 251–273. <http://dx.doi.org/10.1146/annurev.en.38.010193.001343>
- Gustine, D. L., & Moyer, B. G. (1990). Legumes and oilseed crops I. In Y. P. S. Bajaj (Ed.), *Biotechnology in agriculture and forestry* (pp. 341–354). Heidelberg, Berlin: Springer.
- Harborne, J. B. (1969). Chemosystematics of the Leguminosae. Flavonoid and isoflavonoid patterns in the tribe Genisteae. *Phytochemistry*, *8*, 1449–1456. [http://dx.doi.org/10.1016/S0031-9422\(00\)85914-2](http://dx.doi.org/10.1016/S0031-9422(00)85914-2)
- Hopkins, D. L., & Nicholson, A. (1999). Meat quality of wether lambs grazed on either saltbush (*Atriplex nummularia*) plus supplements or lucerne (*Medicago sativa*). *Meat Science*, *51*, 91–95. [http://dx.doi.org/10.1016/S0309-1740\(98\)00105-3](http://dx.doi.org/10.1016/S0309-1740(98)00105-3)
- Hufbauer, R. A., & Via, S. (1999). Evolution of an aphid–parasitoid interaction: Variation in resistance to parasitism among aphid populations specialized on different plants. *Evolution*, *53*, 1435–1445. <http://dx.doi.org/10.1111/j.1558-5646.1999.tb05408.x>
- Kunert, G., Belz, E., Simon, J. C., Weisser, W. W., & Outreman, Y. (2010). Differences in defensive behaviour between host-adapted races of the pea aphid. *Ecological Entomology*, *35*, 147–154. <http://dx.doi.org/10.1111/j.1365-2311.2009.01146.x>
- Kresge, N., Simoni, R. D., & Hill, R. L. (2005). Hemorrhagic sweet clover disease, dicumarol, and warfarin: The work of Karl Paul link. *Journal of Biological Chemistry*, *280*, e5–e6.
- Lavy, O., Sher, N., Malik, A., & Chiel, E. (2015). Do bacterial symbionts govern aphid's dropping behavior? *Environmental Entomology*, *44*, 588–592. <http://dx.doi.org/10.1093/ee/nvv044>
- Leclair, M., Pons, I., Mahéo, F., Morlière, S., Simon, J. C., & Outreman, Y. (2016). Diversity in symbiont consortia in the pea aphid complex is associated with large phenotypic variation in the insect host. *Evolutionary Ecology*, *30*, 925–941.
- Lill, J. T., Marquis, R. J., & Ricklefs, R. E. (2002). Host plants influence parasitism of forest caterpillars. *Nature*, *417*, 170–173. <http://dx.doi.org/10.1038/417170a>
- Losey, J. E., & Denno, R. F. (1998). Interspecific variation in the escape responses of aphids: Effect on risk of predation from foliar-foraging and ground-foraging predators. *Oecologia*, *115*, 245–252. <http://dx.doi.org/10.1007/s004420050513>
- Matsubayashi, K. W., Ohshima, I., & Nosil, P. (2010). Ecological speciation in phytophagous insects. *Entomologia Experimentalis et Applicata*, *134*, 1–27. <http://dx.doi.org/10.1111/j.1570-7458.2009.00916.x>
- Meresman, Y., Ben-Ari, M., & Inbar, M. (2017). Turning in mid-air allows aphids that flee the plant to avoid reaching the risky ground. *Integrative Zoology*, *12*, 409–420. <http://dx.doi.org/10.1111/1749-4877.12263>
- Min, B. R., McNabb, W. C., Barry, T. N., Kemp, P. D., Waghorn, G. C., & McDonald, M. F. (1999). The effect of condensed tannins in *Lotus corniculatus* upon reproductive efficiency and wool production in sheep during late summer and autumn. *The Journal of Agricultural Science*, *132*, 323–334.
- Morisset, P., & Boutin, C. (1984). The biosystematic importance of phenotypic plasticity. In W. F. Grant (Ed.), *Plant biosystematics* (pp. 293–306). Ontario, Canada: Academic Press.
- Mulatu, B., Applebaum, S. W., & Coll, M. (2004). A recently acquired host plant provides an oligophagous insect herbivore with enemy-free space. *Oikos*, *107*, 231–238. <http://dx.doi.org/10.1111/j.0030-1299.2004.13157.x>
- Nelson, E. H. (2007). Predator avoidance behavior in the pea aphid: Costs, frequency, and population consequences. *Oecologia*, *151*, 22–32. <http://dx.doi.org/10.1007/s00442-006-0573-2>
- Oleszek, W., Price, K. R., & Fenwick, G. R. (1988). Triterpene saponins from the roots of *Medicago lupulina* L. (black medick trefoil). *Journal of the Science of Food and Agriculture*, *43*, 289–297. <http://dx.doi.org/10.1002/jsfa.2740430402>
- Oliver, K. M., Russell, J. A., Moran, N. A., & Hunter, M. S. (2003). Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences*, *100*, 1803–1807. <http://dx.doi.org/10.1073/pnas.0335320100>
- Oliver, K. M., Degnan, P. H., Burke, G. R., & Moran, N. A. (2010). Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Annual Review of Entomology*, *55*, 247–266. <http://dx.doi.org/10.1146/annurev-ento-112408-085305>
- Orhan, I., Kartal, M., Abu-Asaker, M., Şenol, F. S., Yilmaz, G., & Şener, B. (2009). Free radical scavenging properties and phenolic characterization of some edible plants. *Food Chemistry*, *114*, 276–281. <http://dx.doi.org/10.1016/j.foodchem.2008.09.005>
- Peccoud, J., Mahéo, F., Huerta, M., Laurence, C., & Simon, J. C. (2015). Genetic characterisation of new host-specialised biotypes and novel associations with bacterial symbionts in the pea aphid complex. *Insect Conservation and Diversity*, *8*, 484–492. <http://dx.doi.org/10.1111/icad.12131>
- Peccoud, J., Ollivier, A., Plantegenest, M., & Simon, J. C. (2009). A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. *Proceedings of the National Academy of Sciences*, *106*, 7495–7500. <http://dx.doi.org/10.1073/pnas.0811117106>
- Peccoud, J., & Simon, J. C. (2010). The pea aphid complex as a model of ecological speciation. *Ecological Entomology*, *35*, 119–130. <http://dx.doi.org/10.1111/j.1365-2311.2009.01147.x>
- Polin, S., Simon, J. C., & Outreman, Y. (2014). An ecological cost associated with protective symbionts of aphids. *Ecology and Evolution*, *4*, 836–840. <http://dx.doi.org/10.1002/ece3.991>
- Prowell, D. P., McMichael, M., & Silvain, J. F. (2004). Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*, *97*, 1034–1044. <http://dx.doi.org/10.1603/0013-8746>
- Ribak, G., Gish, M., Weihs, D., & Inbar, M. (2013). Adaptive aerial righting during the escape dropping of wingless pea aphids. *Current Biology*, *23*, R102–R103. <http://dx.doi.org/10.1016/j.cub.2012.12.010>
- Saito, K., Suzuki, H., Yamashita, Y., & Murakoshi, I. (1994). Isolation and enzymatic synthesis of an ester alkaloid, (–)-3-(hydroxy-13 α -tigloyloxylupanine, from *Cytisus scoparius*. *Phytochemistry*, *36*, 309–311. [http://dx.doi.org/10.1016/S0031-9422\(00\)97066-3](http://dx.doi.org/10.1016/S0031-9422(00)97066-3)
- Scarborough, C. L., Ferrari, J., & Godfray, H. C. J. (2005). Aphid protected from pathogen by endosymbiont. *Science*, *310*, 1781. <http://dx.doi.org/10.1126/science.1120180>
- Simon, J. C., Carré, S., Boutin, M., Prunier-Leterme, N., Sabater-Muñoz, B., Latorre, A., et al. (2003). Host-based divergence in populations of the pea aphid: Insights from nuclear markers and the prevalence of facultative symbionts. *Proceedings of the Royal Society of London B: Biological Sciences*, *270*, 1703–1712. <http://dx.doi.org/10.1098/rspb.2003.2430>
- Tokunaga, E., & Suzuki, N. (2008). Colony growth and dispersal in the ant-tended aphid, *Aphis craccivora* Koch, and the

- non-ant-tended aphid, *Acyrtosiphon pisum* Harris, under the absence of predators and ants. *Population Ecology*, 50, 45–52. <http://dx.doi.org/10.1007/s10144-007-0065-1>
- Tsuchida, T., Koga, R., & Fukatsu, T. (2004). Host plant specialization governed by facultative symbiont. *Science*, 303, 1989. <http://dx.doi.org/10.1126/science.1094611>
- Van Emden, H. F., & Harrington, R. (2007). *Aphids as crop pests*. Oxfordshire: Cabi International.
- van Klink, R., van der Plas, F., Van Noordwijk, C. G. E., WallisDeVries, M. F., & Olff, H. (2015). Effects of large herbivores on grassland arthropod diversity. *Biological Reviews*, 90, 347–366. <http://dx.doi.org/10.1111/brv.12113>
- van Noordwijk, C. G. E., Flierman, D. E., Remke, E., WallisDeVries, M. F., & Berg, M. P. (2012). Impact of grazing management on hibernating caterpillars of the butterfly *Melitaea cinxia* in calcareous grasslands. *Journal of Insect Conservation*, 16, 909–920. <http://dx.doi.org/10.1007/s10841-012-9478-z>
- Vern, S., Baron, A. A., Clayton, G. W., Campbell Dick, A., & McCartney, D. H. (2004). Swath grazing potential of spring cereals, field pea and mixtures with other species. *Canadian Journal of Plant Science*, 84, 1051–1058. <http://dx.doi.org/10.4141/P03-143>
- Via, S. (1999). Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. *Evolution*, 53(5), 1446–1457. <http://dx.doi.org/10.1111/j.1558-5646.1999.tb05409.x>
- Vosteen, I., Gershenzon, J., & Kunert, G. (2016). Hoverfly preference for high honeydew amounts creates enemy-free space for aphids colonizing novel host plants. *Journal of Animal Ecology*, 85, 1286–1297.
- Wink, M. (1987). Quinolizidine alkaloids: Biochemistry, metabolism, and function in plants and cell suspension cultures. *Planta Medica*, 53, 509–514.
- Wink, M., Hartmann, T., Witte, L., & Rheinheimer, J. (1982). Interrelationship between quinolizidine alkaloid producing legumes and infesting insects: Exploitation of the alkaloid-containing phloem sap of *Cytisus scoparius* by the broom aphid *Aphis cytisorum*. *Zeitschrift für Naturforschung C*, 37, 1081–1086. <http://dx.doi.org/10.1515/znc-1982-11-1206>
- Wood, T. K. (1993). Speciation of the *Enchenopa binotata* complex (Insecta: Homoptera: Membracidae). In D. R. Lees, & D. Edwards (Eds.), *Evolutionary patterns and processes* (pp. 299–317). New York: Academic Press.

Available online at www.sciencedirect.com

ScienceDirect