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ORIGINAL ARTICLE

The effect of host plants on genotype variability in fitness and honeydew composition of *Aphis fabae*

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Abstract

Aphid species can be polyphagous, feeding on multiple host plants across genera. As host plant species can have large variation in their phloem composition, this can affect aphid fitness and honeydew composition. Previous research showed significant intraspecific genotype variation in the composition of the honeydew carbohydrates of the black bean aphid *Aphis fabae*, with the ant attractant trisaccharide melezitose showing especially large variation across different genotypes. In the present study, we test if variation in melezitose and carbohydrate composition of aphid honeydew could be linked to the adaptation of specific aphid genotypes to particular host plants. To this end, four high and five low melezitose secreting genotypes of the black bean aphid *Aphis fabae* were reared on four common host plants: broad bean, goosefoot, beet and poppy. The carbohydrate composition, and in particular melezitose secretion, showed important aphid genotype and host plant interactions, with some genotypes being high melezitose secreting on one host plant but not on another. However, the interaction effects were not paralleled in the fitness measurements, even though there were significant differences in the average fitness across the different host plants. On the whole, this study demonstrates that aphid honeydew composition is influenced by complex herbivore-plant interactions. We discuss the relevance of these findings in the context of ant-aphid mutualisms and adaptive specialization in aphids.

Introduction

Ecological specialization is an ongoing process whereby every species exhibits patterns of resource and habitat use (Futuyma & Moreno, 1988). Insects, especially plant-feeding insects, have a high species richness which is likely spurred by specialization on host plants (Ehrlich & Raven, 1964; Janz *et al.*, 2006). There is a competition driven directional evolution toward increased specialization (Nosil, 2002; Nurmi *et al.*, 2008), resulting in resource polymorphism with coexistence or the evolution to new species (Pfennig & Pfennig, 2012). The mechanisms behind specialization are a complex mix of biotic and abiotic interactions, behavior and genetic factors (Jaenike, 1990; Nurmi *et al.*, 2008; Forister *et al.*, 2012).

Many aphid species are highly specialized and occur only on one specific host plant, however, another strategy is to be polyphagous and have the ability to feed on multiple host plants. Polyphagous aphids can sometimes be mistaken for cryptic species with often still a shared host plant for their sexual reproduction, nevertheless there are still truly polyphagous aphid species (Müller, 1982; Raymond *et al.*, 2001; Carletto *et al.*, 2009; Derocles *et al.*, 2015). Even though polyphagous aphids have a wide range of host plant species there are fitness variations between host plants and even within a specific host plant species (Edwards, 2001; Awmack & Leather, 2002; von Burg *et al.*, 2008; Vorburger *et al.*, 2008). Those variations can result in trade-offs between different host plants where specific genotypes perform better on a certain host plant which could lead to speciation (Fry, 1996; Mackenzie, 1996; Berlocher & Feder, 2002).

The black bean aphid (*Aphis fabae* Scopoli) is a polyphagous species, common in the northern hemisphere. It has more than 100 different secondary herbaceous host plants ranging from cultivated to wild plant species (Stroyan, 1984). The primary host plants, mainly the

European spindle tree (*Euonymus europaeus*), are used in autumn, winter and spring for survival of eggs deposited after sexual reproduction. In spring the eggs hatch and viviparous, parthenogenetic females start reproducing, after which offspring migrates to the secondary host plants where they reproduce asexually for the remainder of the spring and summer. Based on those secondary host plants it is possible to distinguish four different subspecies of *A. fabae*. Besides an overlapping range of host plants, each subspecies also has the ability to use a specific host plant that cannot be used by any of the other subspecies (Stroyan, 1984). Despite this clear specialization, even with modern technologies it is still difficult to have a clear phylogenetic distinction between the subspecies (Coeur d'Acier *et al.*, 2004; Béji *et al.*, 2015).

Aphids feed on the phloem sap of plants and as a result excrete honeydew, a carbohydrate-rich waste product that also contains small amounts of amino acids and vitamins (Mittler, 1958; Auclair, 1963). Aphid honeydew plays an important role in the ant-aphid mutualism, whereby ants collect the honeydew as food and in return protect the aphids against natural enemies (Nixon, 1951; Banks, 1962; Buckley, 1987). The composition of the honeydew is highly dependent on the phloem sap composition which varies within and between host plant species. Within species, phloem sap composition can alter due to diurnal shifts (Taylor *et al.*, 2012) and seasonal variation (Douglas, 1993; Wool *et al.*, 2006), which was shown to also affect honeydew composition. Feeding on different host plants has also been shown to affect the amount of the trisaccharide melezitose secreted in the honeydew of *Chaitophorus populialbae* (Fischer & Shingleton, 2001) as well as the total carbohydrate concentration of *A. fabae* (Fischer *et al.*, 2005). Pringle *et al.* (2014) also found differences in the concentration of honeydew carbohydrates glucose, sucrose and xylose of *Aphis nerii* when feeding on two different host plants. Recently, intraspecific variation in honeydew composition was detected between genotypes, especially in the concentration of melezitose,

in *A. fabae* (Vantaux *et al.*, 2011b) and *Aphis craccivora* (Katayama *et al.*, 2013) with melezitose being either almost absent or representing up to 50% of the total carbohydrate concentration. The trisaccharide melezitose has been shown to play an especially important role in the ant-aphid symbiosis (Kiss, 1981; Volkl *et al.*, 1999; Woodring *et al.*, 2004; Detrain *et al.*, 2010). In particular, higher melezitose concentrations have been shown to increase ant attendance (Volkl *et al.*, 1999; Woodring *et al.*, 2004) and ant feeding efficiencies (Detrain & Prieur, 2014).

The purpose of this study was to test if specific genotypes of *A. fabae* showed adaptions to particular host plants and if this resulted in intraspecific variation in melezitose production and carbohydrate composition of the honeydew. It is discussed how this could lead to changes in honeydew quality which could affect ant-aphid mutualism and speciation.

Materials and methods

Study organism and host plants

The studied aphids are *A.fabae fabae*, which has broad bean (*Vicia faba*) as the main secondary host plant. For our study, we collected nine different genotypes from broad bean host plants in Belgium. All genetic lineages could be distinguished by microsatellites following the protocol in Vantaux *et al.* (2011a) (Table S1). Based on the composition of honeydew produced while feeding on broad bean, we then decided to focus on 4 genotypes which were categorized as high melezitose genotypes with melezitose levels > 20% of the total carbohydrates found in the honeydew and 5 genotypes which were categorized as low melezitose genotypes with melezitose levels < 1%.

Besides broad bean, three other herbaceous plants were used that are commonly found to be infested with *A. fabae*, namely goosefoot (*Chenopodium album*), beet (*Beta vulgaris*) and poppy (*Papaver dubium*). All were grown from seeds at 21 ± 1°C with a 16 : 8 h L : D photoperiod and 60% ± 5% humidity and experiments were carried out under the same conditions. Pre-flowering plants with at least 4 leafs were inoculated with aphids to either test fitness performance or to collect honeydew.

Previous to testing aphid fitness and honeydew excretion on host plants other than broad bean, new lineages were created by transferring adult aphids of one genotype to another host plant. After 24 h of reproduction the adults were removed and with daily monitoring the 1st generation adults were removed when the first offspring was produced. This was repeated to get 3rd generation adults for the experiments.

Honeydew collection

Ten aphids were placed on the top leaf of a young, previously uninfested plant and were enclosed in a plastic box. To minimize variation in honeydew excretion due to different settling times on the plant, honeydew collection was started 24 hours after the transfer. To this end, the aphids were enclosed in a new box where they were left undisturbed for another 24 hours. The droplets were gathered with 400 μL azide water and boiled during 5 minutes to stop enzymatic and bacterial activity. This was repeated 5 to 9 times for each genotype on each plant.

High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD, Dionex ICS 3000, USA) was used to analyse the carbohydrates present in all honeydew samples (Vantaux *et al.*, 2011b) The molar concentrations of the different

carbohydrates in each analysed sample were estimated by comparing the area under the chromatogram peaks with standards using the software package Chromeleon (Dionex, USA). Using this method we quantified 8 carbohydrates, namely monosaccharides fructose and glucose, disaccharides maltose, sucrose and trehalose and trisaccharides erlose, maltotriose and melezitose.

Fitness measurements

Fitness was measured by using two common used fitness indices: the mean relative growth rate (MRGR) and the intrinsic rate of increase (r_m) (Leather & Dixon, 1984; Kindlmann *et al.*, 1992; Vantaux *et al.*, 2015). MRGR is calculated based on the weight gain from larvae to adult, MRGR = (ln W2 – ln W1)/ (DT), with W1 and W2 as the averaged dry masses of the larvae and the adults, respectively, and DT the development time from birth to the start of first reproduction (Fisher, 1921). The intrinsic rate of natural increase relates the fecundity of an aphid to its development time. It is calculated using the formula $r_m = 0.74 \times (\ln FD/ DT)$, in which FD is the number of larvae produced over the development time DT and in which 0.74 is a correction factor for the reproductive time calculated by Wyatt and White (1977).

Adult aphid apterae were placed on a previously uninfested plant and left to reproduce for 24h. The development time of the larvae from birth up to first reproduction was monitored daily. When reaching the reproductive stage 1 to 3 adults were left to reproduce for a period based on the development time and offspring was counted and removed daily. Up to three 1st stage larvae and adults were collected and killed by putting them at -20°C for 20 minutes, then dried by placing them in an oven for 48 hours at 37°C and kept in a box with calcium chloride before weighing to measure the average larval weight (W1) and adult weight (W2). The genotypes were tested against each host plant 5 to 9 times.

Statistical analysis

The data were analysed with linear mixed models with Gaussian distribution (Bates *et al.*, 2014) on log transformed data for all compounds found in the honeydew and the untransformed data from the fitness measurement r_m and MRGR. The full model consisted of a random intercept model with aphid genotype, plant species and their interaction as fixed factors, the number of aphids coded as a covariate and replicate as a three-level nested random factor in both aphid genotype and melezitose level. We used backward stepwise model simplification based on AIC to arrive at a minimum adequate model considering the structure of the experimental setup. This resulted in omitting the interaction term for both fitness measurements and all honeydew compounds except for melezitose. All results are displayed as least square means ± CI (95%). Significant differences were corrected for multiple comparison using Tukey post hoc tests and can be found as supplemental material. All statistical analyses were performed in R 3.2.0 (R Core Team, 2015).

Results

Honeydew composition

The difference in melezitose concentration between low and high melezitose aphid genotypes found in broad bean was also observed in goosefoot and poppy, even if melezitose concentrations were slightly reduced (Fig. 1). On beet however, two aphid genotypes characterized as high melezitose genotypes on broad bean (melezitose concentration (μmol/L), H3: 13.59 ± (6.99 – 11.41) and H4: 8.28 ± (3.70 – 6.68)) showed a significant reduction in melezitose concentration (1.81 ± (0.80 – 0.45) and 1.20 ± (0.59 – 0.30) for H3

and H4 respectively; $P < 0.001$) and would even be characterized as low melezitose genotypes (< 1% of total carbohydrate concentration) (Table S2–S3).

All other carbohydrates detected in the honeydew showed host plant induced differences. Aphids feeding on broad bean showed a general trend of higher values compared to aphids feeding on the other host plants (Fig. 2).

Fitness measurements

MRGR and r_m, differed between the host plants and had similar high values on broad bean and poppy. The aphids had a decreased performance on goosefoot and beet, with even lower MRGR measurements on beet (Fig. 3A and B). There was genotypic variability in fitness, however, both measures showed differences between different genotypes (Fig. 3C and D). For rm the best performing genotypes were H1 and L5 and H4, L1, L2 and L4 had the lowest performance. For MRGR the best performing genotype was H1 and the lowest performance was measured in H4. Overall, there was a positive correlation between MRGR and rm with genotype variation ranging from moderate (R> 0.5) for genotypes H2, H3, H4 and L5 to a strong (R> 0.7) correlation for the other genotypes (Table S4).

Discussion

The carbohydrate composition, and in particular melezitose secretion, showed host plant species dependent variation. Two high melezitose genotypes, as characterized on broad bean, showed strong reduced melezitose production on beet. However, the genotypes showed no decrease in the total amount of carbohydrates in their honeydew. Additionally, the interaction effects in melezitose production were not paralleled by aphid genotype and host plant

interaction effects on fitness. Thus, the reduced melezitose production is unlikely to be a genotype specific maladaptation to the host plant. Previous research neither detected fitness differences between high and low melezitose genotypes when reared on broad bean (Vantaux *et al.*, 2015) nor changed total amounts of carbohydrates related to the melezitose levels found in the honeydew of *A. fabae* (Vantaux *et al.*, 2011b). The detected variation in melezitose levels seems to be a host plant induced nonadaptive plasticity (Fitzpatrick, 2012). Nonetheless, fitness trade-offs are not essential in evolutionary pathways leading to specialization (reviewed in Forister *et al*. 2012), hence the detected plasticity could indicate an ongoing specialization process most likely fueled by competition (Bono *et al.*, 2015). Although not tested, the plasticity in melezitose production might be linked to a preference for specific host plant species. Especially interactions with natural enemies could influence this preference as aphids can choose for nutritionally inferior host plants to avoid predation (Wilson & Leather, 2012).

Melezitose is the most susceptible carbohydrate in the honeydew, probably because of its special function to regulate osmoregulation and the additional function of acting as an ant-attractant and reducing the suitability of honeydew for parasitoids and predators (Wackers, 2000). Melezitose is an important carbohydrate in the ant-aphid mutualism as it acts as an ant attractant (Kiss, 1981; Volkl *et al.*, 1999; Woodring *et al.*, 2004; Detrain *et al.*, 2010) and ant-tended aphid species have in general higher amounts of melezitose in their honeydew (Woodring *et al.*, 2004). However, the presence of *L. niger* ants could be one factor impacting the ant-aphid mutualism with *A. fabae* by preferential tending specific genotypes and/or selectiveness in the level of protection provided depending on the amount of melezitose found in the honeydew. Alternatively, the plasticity in melezitose production could also indicate a host plant dependent strategy to deal with predators and a changed dependency on the ant mutualism for defense as most non-tended aphids have very little

melezitose in their honeydew (Woodring *et al.*, 2004). In addition, ants can also be a limited factor (Cushman & Addicott, 1989), thus resulting in interspecies and possibly also intraspecific competition. Further research is necessary to gain more insight in the eco-evolutionary dynamics of plant–herbivore communities and the effects on predator-prey systems regarding the ant-aphid mutualism (Ohgushi, 2016).

Aphids reared on broad bean produced honeydew with higher total carbohydrate concentrations compared to beet, goosefoot and poppy. However, the fitness measurements, MRGR and rm, indicated diet specialization with higher fitness for aphids reared on broad bean and poppy and lower fitness when reared on beet and goosefoot. The results can probably be explained by the quality of the phloem sap and mainly the carbohydrate : amino acid ratio of the phloem sap of the different host plants. Broad bean has a 1.1 ratio and this ratio is at least 3 times higher for goosefoot and beet and only 0.4 for poppy, resulting in nutritionally different phloem sap compositions (Lohaus *et al.*, 1994; Wilkinson *et al.*, 2001). Since phloem sap is a diet generally lacking sufficient amounts of essential amino acids (Douglas, 1993), the increased levels of essential amino acids in the phloem sap of poppy plants could reduce the needed ingestion of phloem sap. In addition, honeydew is mostly a waste product to deal with osmotic stress, hence a lower ingestion of phloem sap could lower the production of honeydew and melezitose without having an impact on fitness (Karley *et al.*, 2002). It remains an interesting research question how melezitose low genotypes, either on the studied host plants or other host plants, deal with the osmotic stress that is supposed to be similar to the high melezitose genotypes since there are no indications of a reduced phloem sap intake as there were no general differences in total amount of carbohydrates found in the honeydew nor in fitness parameters between the high and low melezitose genotypes.

In conclusion, this study found a host plant induced plasticity in melezitose production of *A.*

fabae which could ultimately result in specialization depending on resource competition and possibly change the level of dependency on the ant mutualism.

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Legend figures

Fig. 1 Mean concentration (+ 95% CI) of the trisaccharide melezitose found in the honeydew of *Aphis fabae* genotypes fed on four different host plants. Genotypes are high (H) or low (L) melezitose producing genotypes as characterized on broad bean. Significant differences are displayed in more detail in Table S2–S3.

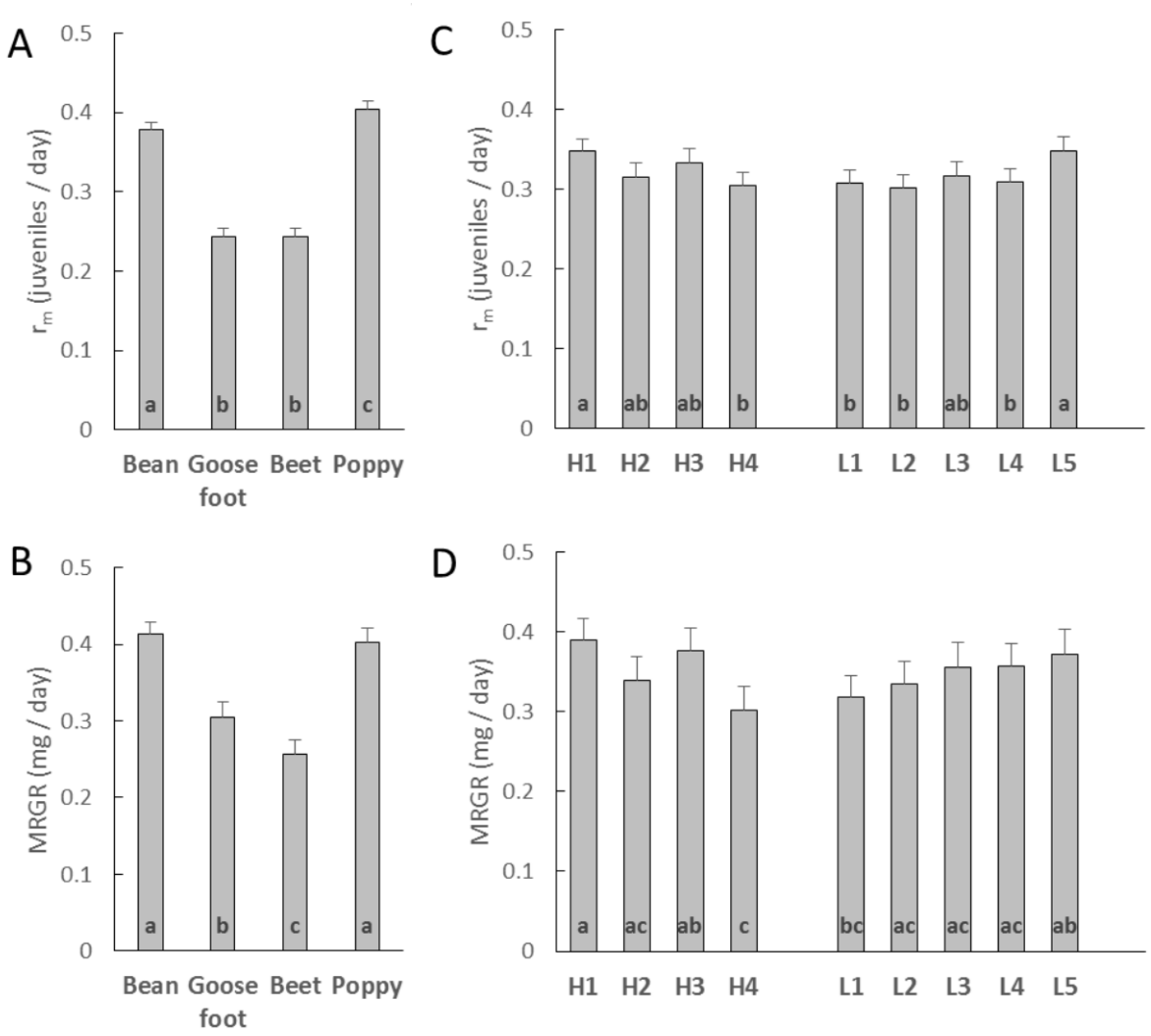

Fig. 2 Mean concentration (+ 95% CI) of the seven other carbohydrates found in the honeydew of *Aphis fabae* genotypes fed on four different host plants. The total concentration is calculated on all eight carbohydrates found in the honeydew. Significant differences after post hoc correction within a carbohydrates between plants are indicated by distinct letters.

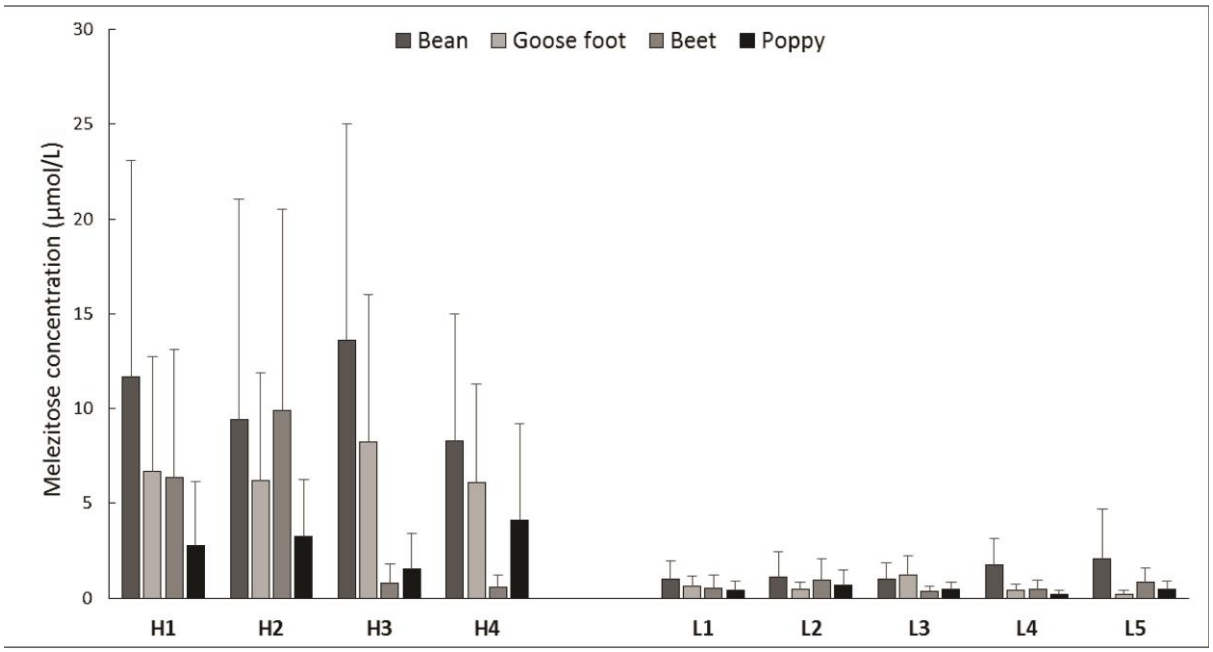

Fig. 3 Mean intrinsic growth rate (r_m) (+ 95% CI) of *Aphis fabae* (A) fed on four different host plants and (C) between the high (H) and low (L) melezitose producing genotypes. Mean relative growth rate (MRGR) (+ 95% CI) of *Aphis fabae* (B) fed on four different host plants and (D) between the high (H) and low (L) melezitose producing genotypes. Significant differences after post hoc correction are indicated by distinct letters.