

# Mechanisms of species-sorting: effect of habitat occupancy on aphids' host plant selection

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**Abstract.** 1. Interspecific interactions such as competition are important factors affecting insect herbivore fitness. Host choice in herbivorous insects including aphids has largely been studied with respect to host plant condition, while the role of competition is also very important.

2. Tansy (*Tanacetum vulgare* L.) hosts three specialised aphids, *Macrosiphoniella tanacetaria* (Kaltenbach), *Metopeurum fuscoviride* Stroyan and *Uroleucon tanacetii* (L.) (Hemiptera: Aphididae). In this study, a set of greenhouse and field experiments were carried out to investigate whether aphid host plant choice was affected by the presence of the other aphid species.

3. When winged individuals were given the choice between unoccupied plants and plants occupied by the same or another aphid species, choices generally reflected the outcome of competitive interactions among aphids.

4. Colonisation of plants by winged individuals was influenced not only by the presence of other aphids on the host plant but also by previous infestation. The host choice of winged individuals basically reflected competitive hierarchies, i.e. aphids in most cases selected plants where future competition was less likely. By contrast, unwinged aphids did not show any host plant preference. For *M. tanacetaria* previously infested plants promoted the production of winged offspring.

5. Our results show that competitive interactions could affect host selection behaviour by aphids. In a metacommunity context, such preferences in colonising different habitats leads to species-sorting through habitat heterogeneity.

**Key words.** Aphid, choice, colonisation, competition, performance, preference, species interactions, winged morph.

## Introduction

Herbivorous insects that share a common host plant can affect each other directly or indirectly. In addition to direct competition for nutrients, feeding of these insects may induce host plant responses (Karban & Baldwin, 1997), which can promote interactions between species even when they

occur on different parts of the host plant or occupy the plant at different times (Denno *et al.*, 1995, 2000). In the case of induced defences, herbivore attack may also prime plants for a more efficient activation of defence responses in the case of subsequent attack by the same or another herbivore (Bruinsma & Dicke, 2008). Host plant-mediated competitive interactions are considered to be important for the structuring of herbivorous insect communities (Kaplan & Denno, 2007). According to the preference–performance hypothesis (PPH), parental insect herbivores should select plants where competitive interactions are minimised, i.e. where performance of the offspring is maximal, and there are several examples that host plant choice is affected by interspecific competition (e.g. Clark *et al.*, 2011).

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Aphids (Hemiptera: Aphididae) are phloem feeders, and interactions between them are likely to be frequent because of their reliance on the plant vascular system (Denno *et al.*, 1995, 2000; Karban & Baldwin, 1997; Petersen & Sandstrom, 2001). For example, Messina *et al.* (2002) showed that previous infestation by conspecifics considerably reduced the performance of *Rhopalosiphum padi* (L.). Winged individuals of *R. padi* and *Diuraphis noxia* (Mordvilko) also preferentially settled on uninfested plants than on plants previously infested by *R. padi* (Messina *et al.*, 2002). In this case, dispersing aphids chose plants with a lower chance of future competitive interactions.

Tansy (*Tanacetum vulgare* L. (Asteraceae)) is a perennial herbaceous plant that hosts three specialist aphids: *Macrosiphoniella tanacetaria* (Kaltenbach), *Metopeurum fuscoviride* Stroyan and *Uroleucon tanaceti* (L.) (Aphididae). These three species are monoeconomic, remaining on the same host throughout their annual life cycle (Blackman & Eastop, 2006; Mehrparvar *et al.*, 2013). *Macrosiphoniella tanacetaria* is not ant-attended (Stadler, 2004; Mehrparvar *et al.*, 2013) while *M. fuscoviride* is an obligatory myrmecophilous aphid commonly attended by ants such as the black garden ant, *Lasius niger* (L.) (Flatt & Weisser, 2000; Loxdale *et al.*, 2011; Mehrparvar *et al.*, 2013). Both species feed on the apex of ramets, but mixed colonies have not been observed. The third species, *U. tanaceti*, feeds on the underside of lower leaves of its host plant and is also a non-ant-attended species (Loxdale *et al.*, 2011). All species have a classical metapopulation structure, and together they form a metacommunity (Weisser, 2000; Massonnet *et al.*, 2002; Loxdale *et al.*, 2011; Mehrparvar *et al.*, 2013).

In a separate study, we showed that there are strong interspecific competitive interactions among the three aphid species that are partly mediated by ants (Table 1) (M. Mehrparvar *et al.*, unpublished). In that study, for all combinations of one, two or three species, five nymphs of each species were placed on the plants initially and the survival and growth of the colonies were observed until the end of the experiment. While this setup only allows limited conclusions about a situation when one of the species colonises in low numbers in a large colony of another species, it will reveal the presence of competitive interactions between species and resembles the situation in the field in the early season when winged individuals might colonise plants where small colonies of another species are present. In the presence of ants, the ant-tended *M. fuscoviride* is competitively dominant, due to preferential predation by ants on the other aphid species and improvement of *M. fuscoviride* survival, reproduction and developmental rates, even in the absence of predators (Flatt & Weisser, 2000; Fischer *et al.*, 2001). The non-tended *M. tanacetaria* is competitively superior in the absence of ants. For the non-tended aphid species, *U. tanaceti*, the situation is slightly more complicated (Table 1): the net effect of ant presence is positive only in the presence of *M. fuscoviride* and predators, because ant tending reduces the risk of predation so that they do not let natural enemies remain on the plant, eliminating them or chasing them away. The competitive interactions may result in competitive exclusion of one or more species from a

**Table 1.** Outcome of competitive interactions between tansy aphids in the greenhouse experiment (lasting 20 days, no predators) and the field experiment (lasting 21 days, in presence of natural enemies) (M. Mehrparvar *et al.*, unpublished). The results are based on colony persistence and the cumulative number of aphids at the end of experiments. A species was declared to be competitively superior (winner) if it showed longer colony persistence and a greater cumulative number of individuals on the plant.

Species combination	Winner	Remark
MA + ME	With ants: ME	Ants tend ME and attack and kill MA
	Without ants: MA	ME became extinct due to little reproduction (c.f. Flatt & Weisser, 2000) and/or predation (field experiment)
MA + UR	With ants: UR	Ants attack MA but not UR
	Without ants: MA/UR	In the absence of ants in the field (in the presence of predators), MA was the superior competitor (based on cumulative number), but in the greenhouse (absence of predators) MA = UR. However if populations grow, interspecific competition is likely
ME + UR	With ants: ME	Ants tend ME and increase its population growth
	Without ants: UR	ME extinct because of competition or/and predation
MA + ME + UR	With ants: ME	Ants tend ME and increase its population growth while attacking MA
	Without ants: MA/UR	In the absence of ants in the field (in the presence of predators), MA was the superior competitor (based on cumulative number only) but in the greenhouse MA = UR. However if populations grow, interspecific competition is likely

MA, *Macrosiphoniella tanacetaria*; ME, *Metopeurum fuscoviride*; UR, *Uroleucon tanaceti*.

plant (Table 1), and thus it would be advantageous for winged mothers to choose the birthplace of their offspring such that they avoid such competitive interactions.

In this study, we conducted several greenhouse and field experiments in order to understand: (i) whether host choice in tansy aphids is affected by the presence of conspecific or heterospecific aphids on the host plant; (ii) whether previous infestation of the host plant by conspecific or heterospecific individuals affects aphid host choice; (iii) whether there is a difference in settling behaviour between winged and unwinged

individuals; and (iv) whether previous infestation by aphids results in a measurable effect on fitness for aphids subsequently feeding on the plant.

## Materials and methods

### *Experimental plants and aphids*

Tansy plant is native to Europe and Asia (Mitich, 1992). It can be found growing as isolated patches or stands of single plants on riverbanks and wastelands. Tansy hosts more than 23 aphid species globally (Blackman & Eastop, 2006; Holman, 2009).

At the end of March 2011, tansy plants were grown individually from seeds in 1-litre pots (11 cm diameter). The plants were kept in a greenhouse at a temperature of about 25 °C during the day, 20 °C at night, and with an LD 16:8 h regime using artificial lighting. Plants were used for experiments when their height reached about 20 cm.

We used both winged and unwinged aphids for the experiments. At the beginning of May 2011, adult unwinged aphids of each of the three species, *M. tanacetaria*, *M. fuscoviride*, and *U. tanacetii*, were collected from the field and then separately reared in the greenhouse on potted tansy plants. Winged aphids used in choice experiments were collected 12 h before starting the experiments from the field and kept in 300-ml capacity empty plastic jars until required.

### *Colonisation experiments*

All experiments were performed in June 2011, at a time when the number of winged individuals in the field is large (Mehrparvar *et al.*, 2013). Plexiglas cages (35 cm long × 35 cm wide × 90 cm high) were used in the greenhouse experiments in order to prevent the escape of aphids (Figure S1). To facilitate air ventilation, the front of the cages consisted of a fine mesh.

*Choice Experiment I – choice of occupied plants by winged aphids in the greenhouse.* For this experiment, four potted tansy plants were placed inside each cage in the corners of an imaginary square of about 25 cm (Figures S2 and S3). Three plants were infested, each with five third instars of one of the aphid species (*M. tanacetaria*, *M. fuscoviride* or *U. tanacetii*), whilst one plant was left uninfested as an unoccupied plant. Nymphs were allowed to settle down for 2 h and then their number was checked again; if the number had decreased, new individuals were placed on the plant to maintain a starting density of five nymphs. The position of each treatment within the cage was chosen randomly for each replicate. By using non-reproductive nymphs, all offspring found on the plants during the experiment were the outcome of immigrant reproduction.

At the start of the experiment, a small plastic vial (Eppendorf, 1.5 ml) containing a winged individual was hung by a thin wire in a central position from the ceiling of the cage above the plants and was then opened to allow the aphid

to emerge from the vial. Thereafter, the winged aphid was allowed to choose a host plant. Thus, aphids had to fly to reach a host plant. In some cases, the winged aphids climbed up the wire to walk to the bottom of the cage or fell down. In this case, they were picked up carefully by a paintbrush and put back on the plastic vial. All plants were checked hourly for 6 h after the start of the experiment for the position of the winged aphid and production of nymphs. The first plant on which the aphid landed was noted. All aphids were observed to choose a host plant within 6 h after release. The inspection was repeated after 24 h. In total, 50 replicates using 50 different winged adults were run for each aphid species.

*Choice Experiment II – choice of occupied plants by winged aphids in the field.* This experiment was essentially the same as Experiment I except that plants were placed in the field and were colonised by winged individuals that had emigrated from other plants in the region. Four plants were placed at the corner of a 25 cm square. In total, 30 groups of four plants were placed in a field in the botanical garden of Jena, Germany, with a distance of 80 cm between groups. The experiment was carried out on 17–19 June 2011 [3-day average: temperature 15 °C (range 10.3–21.3 °C); relative humidity 66.3% (range 38.3–97.8%)]. Plants were placed in the field at 07.00 hours and checked daily for 3 days for the presence of winged immigrants. The winged immigrants were identified to species and removed from the plant together with any offspring that were produced. If the number of third-instar nymphs on the plants was lower than five, nymphs from the stem culture were added to the plants.

*Choice Experiment III – choice of previously infested plants by winged aphids in the greenhouse.* At the start of this experiment, 200 potted tansy plants were randomly divided into four subsets of 50. Three subsets were used for infestation by the three aphid species and one subset was kept uninfested. In order to infest a plant, it was placed in a Plexiglas cage (cf. Choice Experiment I) and 15 newly moulted unwinged adults were placed on the plant and allowed to feed and reproduce for 15 days. Thereafter, all aphids and any visible traces of their presence (e.g. exuviae) were removed. We also cleaned the honeydew away (if any) using a wet paintbrush. Control plants were also placed in Plexiglas cages for 15 days. For the choice experiment, three plants previously infested by *M. tanacetaria*, *M. fuscoviride* or *U. tanacetii* and one uninfested plant were placed inside a single cage as in Choice Experiment I. One winged aphid was released in the cage as described previously. Fifty replicates using 50 different winged adults were carried out for each of the three aphid species.

*Choice Experiment IV – choice of previously infested plants by unwinged aphids in the greenhouse.* This experiment was exactly the same as Choice Experiment III, except that unwinged aphids were used instead of winged individuals and these were released in the centre of the cage floor between the plants using a fine paintbrush. The unwinged aphids were

collected 12 h before starting the experiment from the field and kept in 300-ml capacity empty plastic jars until required. In total, there were 50 replicates using 50 different unwinged adults for each aphid species.

#### *Performance experiment of M. tanacetaria on previously infested plants*

The results of the colonisation experiments prompted us to perform a performance experiment with *M. tanacetaria* on previously infested plants. This experiment assessed whether previous infestation influences: (i) offspring survival and the allocation of mothers into winged and unwinged offspring; and (ii) the number of offspring born to second-generation unwinged mothers on the plants.

The experiment was conducted in the greenhouse using two treatments: plants previously infested by *M. tanacetaria* and control plants never exposed to infestation by aphids. Ten tansy plants grown from seeds as described earlier were placed in Plexiglas cages and each was infested with 10 adult individuals of *M. tanacetaria* which were allowed to reproduce for 10 days. Ten other plants were used as controls and were kept free of infestation and also placed in cages. After 10 days, aphids (including any offspring produced and exuviae) were carefully removed from the infested plants using a paintbrush. We also cleaned the honeydew away using a wet paintbrush, but some honeydew may have remained on the plants. There was no discernible damage to the infested plants. After a further 3 days (days 11–13), three unwinged fourth-instar nymphs of *M. tanacetaria* were placed on each of the 20 plants and allowed to develop into adults and to reproduce for 3 days. The mothers and all nymphs, except for 10 of them, were then removed from the plant. The remaining nymphs were allowed to develop into adults. After adult moult, the number of survivors (of 10) was noted and their phenotype (winged/unwinged) was assessed. Five unwinged adults were allowed to remain on the plants; all others (including any offspring) were removed. Adults were allowed to reproduce for another 10 days when the total number of offspring was assessed.

#### *Statistical analysis*

Choice experiments were analysed using  $\chi^2$  tests implemented in the SPSS 16 software (SPSS Inc. 2007, procedures NPAR TEST, CHISQUARE).

For the performance experiment, we used a generalised linear model (procedure GLM) implemented in the statistical package R (R version 2.14.0, 2011) to compare the proportions of winged offspring between treatment and controls. As our count data were overdispersed, the cbind function and a quasibinomial distribution with logit link function were used. Offspring survival from birth to adult moult was analysed using a *t*-test (procedure T-TEST) and the total number of aphids in the end of experiment was analysed using a general linear model (procedure UNIANOVA) with the number of mothers as a covariate using the SPSS 16 software (SPSS Inc. 2007).

## Results

### *Colonisation experiments*

All aphids were observed to choose a host plant within 6 h after release. In all cases, aphids produced offspring on the plants where they were observed first. In the following, 'aphid choice' therefore refers to both the first settling of an individual on a plant and subsequent production of offspring. As we only observed the aphids during first 6 h and after 24 h, we have no data on the timing of reproduction, but all reproduction occurred after the first 6-h observation interval.

*Choice Experiment I – choice of occupied plants by winged aphids in the greenhouse.* Most *M. tanacetaria* winged morphs chose plants occupied by *U. tanacetii* followed by plants occupied by conspecifics ( $\chi^2 = 25.2$ , d.f. = 3,  $P < 0.001$ ,  $N = 50$ ; Fig. 1a). Plants occupied by *M. fuscoviride* were very rarely chosen.

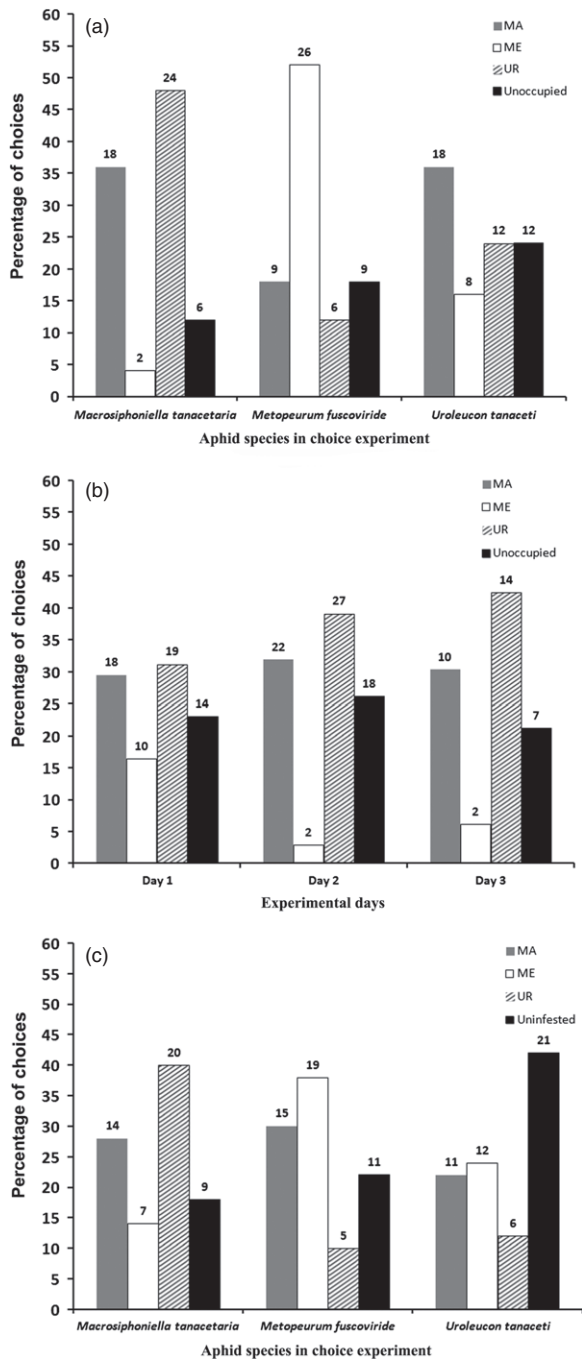
Winged individuals of *M. fuscoviride* preferred plants occupied by conspecific individuals, followed by plants occupied by *M. tanacetaria* and unoccupied plants, and plants occupied by *U. tanacetii* ( $\chi^2 = 19.92$ , d.f. = 3,  $P < 0.001$ ,  $N = 50$ ; Fig. 1a).

Winged individuals of *U. tanacetii* did not show a clear preference ( $\chi^2 = 4.08$ , d.f. = 3,  $P = 0.25$ ,  $N = 50$ ), even though a majority of individuals (36%) landed on plants occupied by *M. tanacetaria* (Fig. 1a).

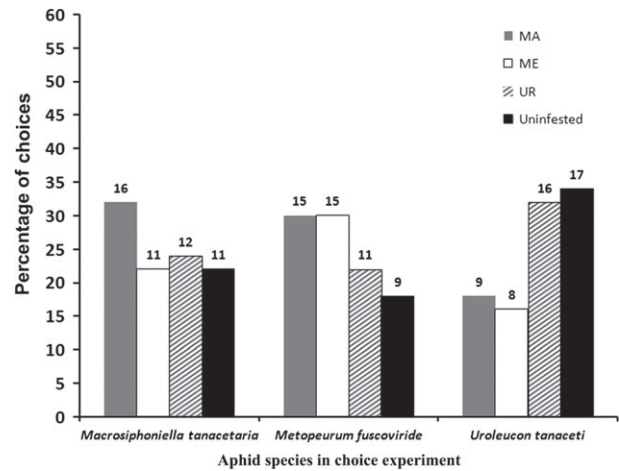
*Choice Experiment II – choice of occupied plants by winged aphids in the field.* In 163 out of 168 cases, the winged individuals that landed on the experimental plants were *U. tanacetii*. Thus, the results described here are only based on the 163 *U. tanacetii* individuals. The distribution of winged individuals over plants was sometimes more than one individual on the same plant.

The distribution of *U. tanacetii* winged individuals among the different plants on the first day of the experiment was not different from a random distribution ( $\chi^2 = 3.33$ , d.f. = 3,  $P = 0.34$ ,  $N = 61$  individuals; Fig. 1b). On the second ( $\chi^2 = 20.33$ , d.f. = 3,  $P < 0.001$ ,  $N = 69$  individuals) and third days ( $\chi^2 = 9.3$ , d.f. = 3,  $P < 0.05$ ,  $N = 33$  individuals), the distribution of new immigrant winged individuals among the plant treatments was non-random so that winged individuals were rarely found on plants occupied by *M. fuscoviride* (Fig. 1b). When plants with *M. fuscoviride* were excluded from the analysis, there was no longer a significant difference among the treatments (day 2:  $\chi^2 = 1.82$ , d.f. = 2,  $P = 0.4$ ,  $N = 67$  individuals; day 3:  $\chi^2 = 2.39$ , d.f. = 2,  $P = 0.3$ ,  $N = 31$  individuals).

*Choice Experiment III – choice of previously infested plants by winged aphids in the greenhouse.* For *M. tanacetaria*, a pattern similar to that of the Choice Experiment I was observed. Winged *M. tanacetaria* preferred plants that had previously been infested by *U. tanacetii* followed by plants



**Fig. 1.** Host choice by winged morphs of tansy aphids. The graph shows the percentage of aphid individuals choosing a plant infested by conspecifics, individuals of another species or uninfested plants. The total number choices for each species was  $N = 50$  individuals. (a) Greenhouse experiment with infested plants plus control plant without aphids; (b) field experiment with infested plants plus control plant without aphids for host plant selection by *Uroleucon tanacetii*; (c) greenhouse experiment with previously infested plants plus control plant that was never infested with aphids. The number of individuals choosing a particular option is shown at the top of each bar. MA, *Macrosiphoniella tanacetaria*; ME, *Metopeurum fuscoviride*; UR, *Uroleucon tanacetii*.



**Fig. 2.** Host choice by unwinged morphs of tansy aphids in the greenhouse. The graph shows the percentage of aphid individuals choosing a plant infested by conspecifics, individuals of another species or uninfested plants. Total no. choices for each species was  $N = 50$  individuals. The number of individuals choosing a particular option is shown at the top of each bar. MA, *Macrosiphoniella tanacetaria*; ME, *Metopeurum fuscoviride*; UR, *Uroleucon tanacetii*.

previously infested by *M. tanacetaria* ( $\chi^2 = 8.08$ , d.f. = 3,  $P < 0.05$ ,  $N = 50$ ; Fig. 1c).

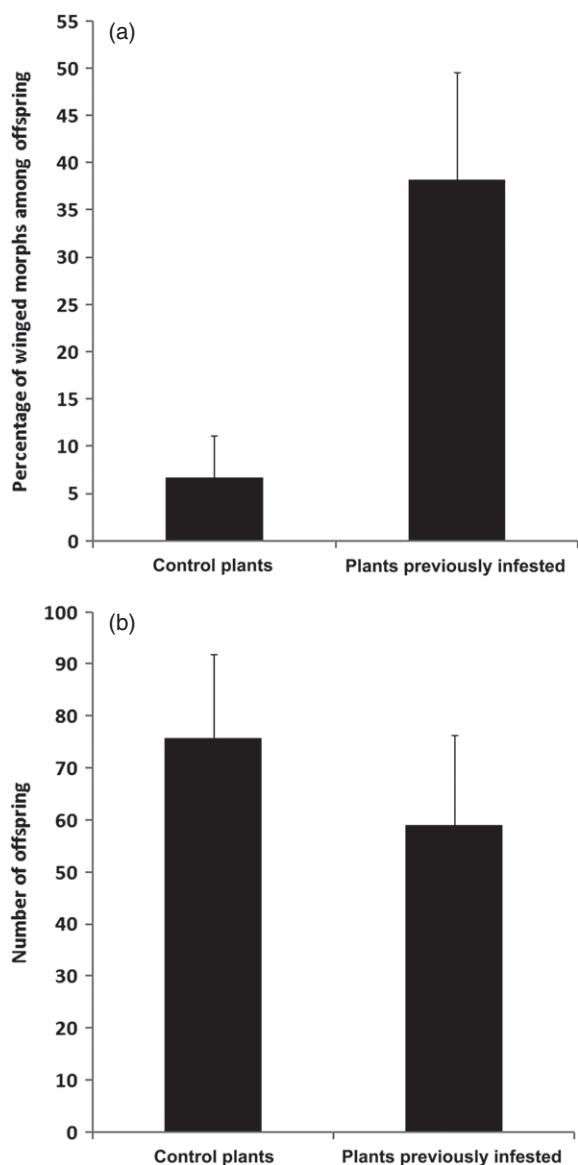
*Metopeurum fuscoviride* mostly chose plants (38% of choices) that had previously been infested by conspecific individuals. The second most common choice comprised plants previously infested by *M. tanacetaria* and then uninfested plants. The plants previously infested by *U. tanacetii* were rarely chosen ( $\chi^2 = 8.56$ , d.f. = 3,  $P < 0.05$ ,  $N = 50$ ; Fig. 1c).

Winged individuals of *U. tanacetii* preferred uninfested plants (42% of choices) over plants that were previously infested ( $\chi^2 = 9.36$ , d.f. = 3,  $P < 0.05$ ,  $N = 50$ ; Fig. 1c). When the uninfested plants were excluded from the analysis, there was no significant preference for certain plants ( $\chi^2 = 2.14$ , d.f. = 2,  $P = 0.34$ ,  $N = 29$ ).

*Choice Experiment IV – choice of previously infested plants by unwinged aphids in the greenhouse.* The host choice of unwinged individuals of *M. tanacetaria* and *M. fuscoviride* was not different from random (*M. tanacetaria*,  $\chi^2 = 1.36$ , d.f. = 3,  $P = 0.72$ ,  $N = 50$ ; *M. fuscoviride*,  $\chi^2 = 2.16$ , d.f. = 3,  $P = 0.54$ ,  $N = 50$ ; Fig. 2). For *U. tanacetii*, there was a tendency for aphids to avoid plants previously infested by *M. tanacetaria* or *M. fuscoviride*, but this was not statistically significant ( $\chi^2 = 5.2$ , d.f. = 3,  $P = 0.16$ ,  $N = 50$ ; Fig. 2).

#### *Performance experiment of M. tanacetaria on previously infested plants*

Previous infestation had no effect on offspring survival, i.e. the number of first instars out of 10 developing successfully into adults (mean =  $7.39 \pm 0.77$ ,  $t = -0.07$ , d.f. = 16,  $P = 0.945$ ). The percentage of winged morphs among these



**Fig. 3.** Greenhouse experiment on the performance of *Macrosiphoniella tanacetaria* on previously infested (treatment) and uninfested (control) plants. (a) Percentage of winged morphs among first-generation offspring; (b) number of offspring produced by unwinged first-generation adults (mean  $\pm$  SE).

adults was significantly higher on previously infested plants than on uninfested plants ( $t = 2.527$ , d.f. = 16,  $P < 0.05$ ; Fig. 3a).

Whenever there were fewer than five unwinged adults on the plants (min = 3,  $N = 8$ ), all remaining unwinged adults were used in the reproduction trial. The number of offspring produced at the end of experiment was independent of the treatment ( $F = 0.491$ , d.f. = 1,  $P = 0.495$ ; Fig. 3b). The initial number of *M. tanacetaria* mothers had no effect on the number of *M. tanacetaria* offspring produced at the end of experiment ( $F = 2.773$ , d.f. = 1,  $P = 0.118$ ).

## Discussion

In this study, we tested how occupancy of plants affected host plant choice of winged and unwinged tansy aphids. We hypothesised that aphids select plants where the risk of future competition, for some species mediated by the presence of mutualistic ants, is reduced (cf. Table 1). Our results show that colonisation of plants by dispersing aphid individuals was, in fact, influenced by the presence of other aphids on the host plant. Importantly, we found that not only present occupation of aphids, but also previous occupation, affected the colonisation of plants. Thus, aphids apparently detected that plants were occupied beforehand and this affected host choice. In contrast to winged individuals, unwinged females exhibited little host preference. However, we should state that these findings may not apply to all other aphid species.

Avoiding competitive interactions is a strategy that may increase insect herbivore fitness. For example, the bark beetle *Pityogenes chalcographus* (L.) does not select the trees that are already infested by another bark beetle infesting Norway spruce, *Ips typographus* (L.), avoiding interspecific competition (Byers, 1993). An effect of previous infestation on host choice by insect herbivores has also been shown in a number of studies. For example, females of *Leptinotarsa juncta* (Germar) preferred to oviposit on undamaged plants than on plants that had been infested by their competitor, *Epitrix fuscula* (Crotch) (Wise & Weinberg, 2002). Similarly, Agrawal (1999) showed that colonisation of wild radish plants by leaf-mining flies, *Liriomyza* sp., was lower on plants that had received previous damage by a lepidopteran larva, *Pieris rapae* (L.), than on controls.

We found for the tansy–aphid system that winged aphid individuals in most cases avoided host plants where there was a high chance of competitive interactions. The avoidance of *M. fuscoviride*-infested plants by winged individuals of *M. tanacetaria* and vice versa reflects such a host choice, as *M. fuscoviride* is competitively dominant on plants with ants, whereas *M. tanacetaria* is competitively dominant on plants without ants (Table 1). Because our aphid colonies were not ant-tended, our results suggest that winged individuals of *M. tanacetaria* base their choice on the presence of *M. fuscoviride* aphids, rather than on the presence of ants. In nature, it is in fact very rare to find both species together on the same host plant (e.g. Weisser & Harri, 2005).

In addition to these avoidance reactions, the preferences exhibited by winged individuals of *M. fuscoviride* and *M. tanacetaria* were also interesting. *Metopeurum fuscoviride* preferred plants that were occupied or previously infested with conspecifics. Because *M. fuscoviride* is an obligate myrmecophilous species (Fischer *et al.*, 2001) and needs mutualistic ants for survival and reproduction (Flatt & Weisser, 2000), aggregation may be beneficial as large colonies of aphids are often more likely to be attended or attended more intensively (Addicott, 1979; Itioka & Inoue, 1996; Fischer *et al.*, 2001). Benefits of aggregation for aphids have also been shown by Sauge *et al.* (2002, 2006) for peach plants where colonisation by *Myzus persicae* (Sulzer) led to easier feeding and settlement for conspecific individuals. However,

it should be mentioned that *M. fuscoviride* is most likely not competitively dominant *per se*, but rather that other aphid species suffer from predation by ants. This does not, however, explain the preference of *M. fuscoviride* for plants previously infested by *M. fuscoviride*. Possibly plants where *M. fuscoviride* occurred have a higher chance of being attended by ants, i.e. aphids choosing those plants that will be more likely to be tended, but this remains to be tested. In addition, previous infestation may indicate a suitable host plant which again needs to be tested.

Winged individuals of *M. tanacetaria* often chose plants occupied by *U. tanacetii*. While *M. tanacetaria* and *U. tanacetii* do compete, this probably only occurs when colonies have reached a large size (Table 1), so infestation by *U. tanacetii* may indicate host plant suitability, as has been shown before. Brunissen *et al.* (2009) presented evidence that potato plants previously infested by *M. persicae* were more attractive for winged individuals of *Macrosiphum euphorbiae* Thomas than uninfested plants or previously infested by conspecifics. Such effects have also been reported in other plant–aphid systems (Prado & Tjallingii, 1997; Gonzales *et al.*, 2002). In our study system, at low densities, this behaviour may also reflect facilitation, as *U. tanacetii* causes occupied leaves to turn yellow, which may lead to a higher nutritional value of the plant for *M. tanacetaria*. In the field, we frequently observed that tansy plants were colonised by both *M. tanacetaria* and *U. tanacetii*, such that sometimes individuals of *M. tanacetaria* settled within *U. tanacetii* colonies on the underside of the lower leaves of the host plant.

The host plant selection scenario was different and more complicated for winged individuals of *U. tanacetii*. In the greenhouse, the presence of other aphid species on the plant had no observable effect on selection behaviour (Choice Experiment I), except in the case of previous plant infestation (Choice Experiment III). Here, winged individuals of *U. tanacetii* mostly chose plants that had previously been uninfested. Interestingly, in the field (Choice Experiment II), initial plant choice was as in greenhouse Choice Experiment I, i.e. for immigrating winged *U. tanacetii* the choice appeared to be random, and a preference developed until the second and third day. Over the next 2 days, the patterns indicated an increased avoidance of plants infested by *M. fuscoviride*. If plants with *M. fuscoviride* were excluded from the analysis, aphid choice was random in all 3 days. Importantly, ants may have played a crucial role in the choice of winged *U. tanacetii*. On the first day of the experiment, plants were placed in the field, and at this point, plants were still ant-free. Thus, on day 1, the conditions were similar to the greenhouse Choice Experiment I, and the results were essentially the same. From the second day onwards, ants explored the colonies of *M. fuscoviride* on the plants and starting to attend them. One possibility for the distribution of winged *U. tanacetii* on days 2 and 3 is that while winged *U. tanacetii* arrived randomly on the different plants, they were subsequently attacked by ants that arrived to attend *M. fuscoviride*.

Incidentally, in the field experiment, a considerable number of winged individuals of *U. tanacetii* (~62%) landed on the plants occupied by conspecifics and settled down into an

existing *U. tanacetii* colony. This may be indicative of a preference for settling in a colony with conspecifics.

Phytophagous insects, including aphids, use a combination of different pre- and post-feeding stimuli, such as chemical and visual cues, to detect a suitable host plant (Chapman *et al.*, 1981; Nottingham & Hardie, 1993; Bernays & Chapman, 1994; Powell *et al.*, 2006; Robert *et al.*, 2012). In the case of our experiments, winged aphids always remained on the plant where they alighted first; thus there was no preliminary probing, which has been shown to be important for host choice in some species (Powell *et al.*, 2006; Pettersson *et al.*, 2007). There is growing evidence to indicate that herbivore infection induces emission of plant volatiles, which can be detected by other insects including aphids (e.g. Pickett *et al.*, 1992; Li *et al.*, 2002). Aphids have complex interactions with plants (Völkel & Baldwin, 2004) and feeding has been shown to induce volatile production in plants (e.g. Du *et al.*, 1998). Even though we do not know the exact cue used by the winged aphids in our experiments, it is likely that volatile rather than visual cues affected host selection, as, in the case of previously infested plants, no trace of aphids should have been left on the plants. In fact, for *M. tanacetaria* and *M. fuscoviride*, the observed patterns of host plant selection by winged individuals were the same when the plants were occupied or previously infested (see Fig. 1a,c). If this is true then the volatiles released by tansy plants must differ depending on the aphids that feed or previously fed on them. The unwinged individuals of all three aphid species did not exhibit preferences for particular plants. The absence of host choice in the unwinged individuals suggests that these morphs are not specialised for host plant selection.

In the performance experiment with *M. tanacetaria*, there were no fitness differences between aphids reared on uninfested and previously infested plants, yet interestingly the percentage of winged morphs among the offspring was higher on previously infested plants than on uninfested plants. In aphids, the production of winged morphs is a general response to adverse environmental conditions (Dixon, 1998; Weisser *et al.*, 1999; Braendle *et al.*, 2006; Balog *et al.*, 2013; Mehrparvar *et al.*, 2013). In the present study, prior plant infestation was a stimulus for wing induction in offspring and hence reflects the decision to leave the plant in the next generation. However, this was not consistent with the decision of winged individuals to choose plants occupied by *M. tanacetaria* (Choice Experiment I). It is important to point out that while the proportion of winged morphs was increased, not all offspring were destined to become winged morphs and hence it is conceivable that previous infestation prompts aphids to ‘spread the risk’ by colony growth on the current plant and colonisation of a few other plants.

The host selection behaviour observed in this study may lead to different colony compositions in the field. Tansy aphids show a classical metapopulation structure (Weisser, 2000; Massonnet *et al.*, 2002; Loxdale *et al.*, 2011) where the extinction rates are high and, as a result, a large proportion of local habitats (plants) are always unoccupied. Based on the results obtained in this study, a number of assembly rules can be derived. In the beginning of the season when most

tansy plants are not occupied (except for those where aphids overwinter as eggs) (Weisser, 2000), there is the same chance for each of the three specialised tansy aphids to occupy the plants. However, one of the most important factors affecting the distribution of aphids at this time is the presence of mutualistic ants in habitat patches, which increases the chance of successful occupation for *M. fuscoviride* (Flatt & Weisser, 2000). After the establishment of an aphid colony on a plant, the subsequent occupation and colonisation will be affected by the aphid species already present on the plant, resulting in particular assembly rules. In the field observations, joint occupation of *M. tanacetaria* and *M. fuscoviride* of the same plant is rarely observed (cf. Weisser & Harri, 2005); however, a combination of *M. tanacetaria* and *U. tanacetii* or *M. fuscoviride* and *U. tanacetii* can be observed more frequently (Weisser & Harri, 2005).

In conclusion, we have shown that the tansy aphid metacommunity shows strong elements of species-sorting, i.e. different aphid species preferentially sort into different plants (=habitats), creating separate spatial niches through habitat heterogeneity. Thus the interactions among aphids and with ants are structuring forces of this metacommunity via competition effects (sensu Stewart, 1996), and have ultimately also affected the evolution of the different species.

### Acknowledgements

We would like to thank Hugh Loxdale for his valuable comments on an early draft of this paper. M.M. and S.M.M. were supported financially by the Iranian Ministry of Science, Research and Technology. W.W.W. gratefully acknowledges support from the German Science Foundation (WE 3081/2-3).

### Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12096

**Figure S1** The Plexiglas cages (35 cm long × 35 cm wide × 90 cm high) that was used for the experiments in the greenhouse in order to prevent the escape of aphids. To facilitate air ventilation, the front of the cages was covered by a fine mesh.

**Figure S2** Tansy pots inside the Plexiglas cages. For the greenhouse experiments, four potted tansy plants were placed inside each cage in the corners of an imaginary square of about 25 cm at the same distance from each other.

**Figure S3** Final design of the inside of the cages for the experiments.

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Accepted 5 December 2013

First published online 7 March 2014