Effects of Aggregation Size and Host Plant on the Survival of an Ant-Tended Membracid (Hemiptera: Membracidae): Potential Roles in Selecting for Generalized Host Plant Use

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ABSTRACT Insect herbivores that are tended by ants exhibit a range in host plant use from specialists to extreme generalists. Potential factors that may influence relative suitability of different host plants include the presence or absence of ants and the size of aggregations formed by nymphs feeding on a host. We used a membracid–ant mutualism to test whether presence of ants or aggregation size (from 20 to 320 nymphs per plant) resulted in variable selection for host use by the generalist membracid *Publilia modesta* (Uhler) (Hemiptera: Membracidae) that feeds on host species *Chrysothamnus viscidiflorus* (Hook.) Nutt. and *Wyethia* spp., among others. Survival of nymphs as mid-instars and the percentage of nymphs developing into adults on *C. viscidiflorus* were greater for nymphs in small than in large aggregations. Survival of nymphs as mid-instars on *Wyethia* spp. was also greater in smaller aggregations, but the percentage of nymphs developing into adults was independent of aggregation size. The presence of the tending ant, *Formica obscuripes* Forel, had no effect on the percentage of nymphs developing into adults during this experiment. The survival of membracids on *C. viscidiflorus* was always greater than survival of membracids on *Wyethia* spp., regardless of aggregation size or presence of ants; the favored host did not vary in a way that would explain the observed generalized host plant use. *C. viscidiflorus* was a superior host plant, because it senesced after nymphs became adults, whereas *Wyethia* spp. senesced early enough that most nymphs died before reaching adulthood.

KEY WORDS density dependence, host range, mutualism, *Publilia modesta*, spatial variation

Mutualistic interactions can exhibit substantial spatial and temporal variation (for review, see Bronstein 1994). That variation may lead to spatially or temporally varying selection, which in turn may have profound effects on the process of coevolution between the mutualistic partners (Thompson 1994). Some examples of variation in the strength of interaction are provided by studies of ant-tended members of the Membracidae (Cushman and Whitham 1989, DelClaro and Oliveira 2000, Billick and Tonkel 2003) and other Hemiptera (Addicott 1979, Offenberg 2001). In these associations, the hemipterans feed on phloem of the host plant and excrete honeydew that is collected and eaten by the ants. The ants provide benefits that can include protection from predators (Fritz 1982, Cushman and Whitham 1989, Morales 2000a), facilitation of feeding (Banks and Nixon 1958, Way 1963, Bristow 1984, Morales 2000a), control of sooty mold growth on the honeydew excretions (McEvoy 1979), and maintenance of host plant quality (Messina 1981).

Membracids usually form aggregations of nymphs on single plants, which attract ants (McEvoy 1979,

Wood 1993). Aggregations are likely made up of both siblings and unrelated individuals, because multiple females oviposit on a single plant. Unlike aphids, which reproduce clonally, membracids only reproduce sexually, and, as a result, aggregation size is determined by the number of eggs females lay on a plant. In principle, the survival of membracids can depend not only on host plant and whether or not ants are present but also on the size of the aggregation.

The purpose of this study is to examine how the effects of aggregation size and ant tending lead to variation in survival of membracids on different host plants, and influence selection for host plant use. Our intent is to investigate how aggregation size influences selection for host use, rather than to examine selection on clutch size. Because the size of aggregations often shows natural variation (Morales 2000a,b), if the carrying capacity for membracids differs between host plant species, one host plant could be superior for small aggregations and another for larger aggregations. The result would be spatial variation in selection for host use. Alternatively, spatially varying selection for host plant use could be generated if the relative fitness of membracids on different host species depends on

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the abundance of ants. This trade-off would occur when one plant species is superior in the presence of ants and a second species is superior in the absence of ants. This effect could occur either when ants are better protectors or when predators are more common on one host species. Such spatial variation in selection can, in principle, favor generalists over specialists (Levins 1968), although additional conditions are usually required for a generalist to evolve (MacArthur 1972, Slatkin 1973, Real 1980, Lynch and Gabriel 1987, Futuyma and Moreno 1988, VanTienderen 1991, Rausher 1993). Despite the potential evolutionary importance of spatially variable selection for host use, it has only rarely been documented for any kind of insect herbivore (Singer and Thomas 1996). Here, we explore whether there is variation in survival of membracids, generated either by variation in aggregation size or by variation in the benefit of ant tending across host plants, that in principle could promote generalized host plant use.

Comparative studies of membracids point to associations between the degree of host plant generalization and certain ecological factors (Wood 1984). Membracids in the tropics are usually more generalized in their use of hosts than membracids in the temperate zone; tropical species on average feed on several host genera, whereas temperate zone membracids tend to specialize on a single genus (Wood 1984). In addition, specialist membracids rarely associate with ant mutualists and live solitary lives, whereas tropical generalist membracids are more likely to associate with ant mutualists and form aggregations (Wood 1984). These data suggest that ant tending and formation of aggregations may cause some membracids to be host plant generalists. Similar observations have been made for host use by lycaenid butterflies; ant-tended lycaenids tend to have larger host ranges than untended lycaenids (Pierce and Elgar 1985).

These comparative data are complemented by empirical studies examining relationships between aggregation size, ant tending, and survival of membracids. These studies have described contrasting patterns in which the benefit of ant tending increases with aggregation size (Cushman and Whitham 1989), decreases with aggregation size (Morales 2000a), or decreases with membracid density (Cushman and Whitham 1991). Variation in the benefits of ant tending has been ascribed to changes in per capita tending rates (Morales 2000b), persistence of ant attendance throughout the day (McEvoy 1979), and competition among the membracid nymphs (Cushman and Whitham 1991). Furthermore, ants occasionally abandon membracid aggregations, which reduces membracid survival (Morales 2000b). These examples suggest that ant tended membracids experience considerable variation in their environment. However, it is difficult to ascertain whether these effects are due to aggregation size rather than another correlated variable such as proximity to an ant colony (McEvoy 1979, Wood 1982, Wimp and Whitham 2001, Morales 2002, Reithel 2003), because aggre-

gation size on single plants was not manipulated in these studies as in other Hemiptera (e.g., aphids, Breton and Addicott 1992a, and scales, Itioka and Inoue 1996). A manipulative experiment would provide a more definitive test of the influence of aggregation size on membracid survival, and it could clear up the discrepancy between the extant observational studies.

To test for variation across host plants in the effect of aggregation size or the benefit of ant tending, it is necessary to manipulate aggregation size and the presence of ants across more than one plant species. Most studies of ant-tended Hemiptera have been confined to a single plant species (but see Addicott 1978). We recently demonstrated that membracid survival depends on both the species of host plant and the presence of tending ants. However, the effect of host plant species was confounded with aggregation size (Reithel and Billick 2006). In the current study, we manipulated aggregation sizes of membracid nymphs on two host species to separate these two effects. In addition, we crossed aggregation size with the presence versus absence of ants to test whether the benefit of ant tending interacted with aggregation size and host plant species to influence membracid survival. Manipulations were performed on two common host plant species of the generalist species *Publilia modesta* (Uhler) (Hemiptera: Membracidae), an ant-tended membracid found throughout the western United States (Kopp and Yonke 1973). The two host plant species vary markedly in phenology, with *Wyethia* spp. sometimes senescing before membracid nymphs become adults and *Chrysothamnus viscidiflorus* (Hook.) Nutt. senescing long after nymphs become adults. To incorporate this aspect of host quality into our estimates of survival, we also manipulated plant senescence and followed survival of nymphs as they attempted to move to new host plants.

This study addressed three questions: 1) How does aggregation size influence nymph survival? 2) Do aggregation size, ant tending, or their interaction affect the relative survival of a generalist membracid on the two host plant species? and 3) Are these differences in survival such that the superior host varies in space, potentially favoring generalized host use?

Materials and Methods

Study Site and Species. Study sites were located in southwest facing meadows in the Almont Triangle (elevation 2,720 m) in Gunnison Co., CO. Experiments were conducted on land managed by the Colorado Department of Wildlife. Cattle grazing is not permitted, though grazing by deer and elk is common. Dominant vegetation includes *Artemisia tridentata* Nutt. (sagebrush), *Chrysothamnus* spp. (rabbitbrush), grasses, and herbaceous perennials.

Publilia modesta (Membracidae) is a phytophagous ant-tended hemipteran. At Almont Triangle, *P. modesta* is most commonly tended by *Formica obscuripes* Forel. The ants protect membracids from predators including spiders, lacewing larvae, and coccinellids

(Fritz 1982; Cushman and Whitham 1989; Morales 2000a; J. R., unpublished data). *P. modesta* produces one generation per year at this site. In May and June, females lay eggs in stems of herbaceous and woody plants. At Almont Triangle, females oviposit on at least six genera: *Wyethia* spp., *Chrysothamnus, Erigeron, Balsamorhiza, Helianthella,* and *Lupinus.* Nymphs eclose continuously over the course of ≈3 wk and feed on the host plant from which they emerged. Aggregation sizes on individual plants peak in early to midsummer after all the eggs have hatched. Nymphs do not move from their host plant unless it begins to senesce, in which case they move onto nearby plants. This species is known to feed on at least 16 species of plants from four families: Asteraceae, Fabaceae, Polygonaceae, and Scrophulariaceae. Host plants at Almont Triangle include *Wyethia* spp., *C. viscidiflorus, Chrysothamnus parryi* (Gray) Greene, *Balsamorhiza sagittata* (Pursh) Nuttall, *Artemisia tridentata, Achillea lanulosa* Nuttall, *Solidago* sp., *Tragopogon* sp., *Erigeron* sp., *Helianthella quinquenervis* (Hooker) Gray, *Lupinus* sp., *Lathyrus leucanthus* Greene, *Vicia americana* Muhlenberg, *Oxytropis* sp., *Eriogonum subalpinum* Greene, and *Castilleja* sp. Nymphs become adults in August and September. Both male and female adults overwinter at the roots of plants and mate in the spring.

We focused on the two most common host plants at Almont Triangle, the perennials *Wyethia* spp. and *C. viscidiflorus. Wyethia* spp., which includes *Wyethia arizonica* Gray and hybrids of *Wyethia arizonica* and *Wyethia amplexicaulis* (Nuttall) Nuttall (Weber and Wittmann 1996), are sunflowers with broad leaves. Pure *W. arizonica* is difficult to differentiate from hybrids, because the degree of pubescence on leaves and stems is the distinguishing characteristic. It is possible that the entire population is made up of hybrids originating from initial contact between the two parent species during the Pleistocene (Weber and Wittmann 1996). There is relatively little variation in phenology of *Wyethia* spp. at Almont Triangle compared with variation among sites within the region. *Wyethia* spp. bloom in late June–early July, and most individuals have completely senesced by late August, roughly the same time that membracid nymphs become adults. *C. viscidiflorus,* a small shrub, flowers in late July–early August and senesces in mid- to late-September at the earliest.

Natural Nymph Aggregation Sizes. We recorded natural aggregation sizes on a random sample of *C. viscidiflorus* and *Wyethia* spp. to ensure that the experimentally manipulated aggregations were representative of natural aggregation sizes. From the edge of each of 12 *Formica* ant colonies at Almont triangle, we ran a 7–12-m-long transect. On all *C. viscidiflorus* and *Wyethia* spp. within 5 cm of the transect, we recorded the number of nymphs every 2 wk from 10 May to 14 September 2000. The number of nymphs per plant peaked between 6 July and 2 August, depending on the plant. We report the average number of nymphs per plant on 2 August, omitting plants without nymphs.
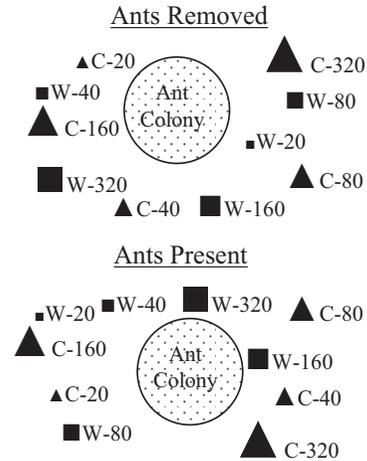

Fig. 1. Schematic representation of one of 12 blocks in the split-plot experimental design. Each block contained a pair of whole plots, each with an ant colony. For one of the plots, ants were removed from around the 10 experimental plants. Each plot included 10 plants (subplots), each represented with a symbol and labeled with the treatments, plant species (C, *C. viscidiflorus*; W, *Wyethia* spp.) and aggregation size (20, 40, 80, 160, or 320 nymphs per plant). The size of the symbol roughly corresponds to the size of the aggregation on the plant, either *C. viscidiflorus* (▲) or *Wyethia* spp. (■).

Aggregation Size and Ant Manipulation. In 1999, we tested the effects of nymph aggregation size and the presence of ants on the relative suitability of host plants. We used a split-plot design where each ant treatment was split into observations on two plant species and five nymph aggregation sizes (Fig. 1). Twelve pairs of *F. obscuripes* colonies were located; the two colonies of each pair were closer to each other (<20 m apart) than to other pairs. Each of the 12 pairs of colonies constituted a block, within which there were two "whole-plot" treatments; ants were removed from plants at one of the colonies (total of 12 colonies), and ants remained present for plants at the second colony (total of 12 colonies). Each of those ant colonies was split into 10 treatments ("subplot" level), which themselves constituted a two-way factorial of two plant species (*Wyethia* spp. vs. *C. viscidiflorus*) crossed with five aggregation sizes, each on a single plant within 5 m of the colony. Although plant species is not a factor that can be deliberately assigned to individual plants at random, for all colonies the five plants from each species were heavily interspersed (Fig. 1). The aggregation sizes were 20, 40, 80, 160, or 320 nymphs per plant. The total number of subplots in this design was 240 (two plant species × 5 aggregation sizes × 24 colonies). To manipulate the presence of ants, aluminum cylinders (≈10 cm in height and 30 cm in diameter) were assembled around all plants, and nearby vegetation was trimmed to prevent ants from using vegetation to climb over cylinders. Cylinders used to exclude ants were complete cylinders with Tanglefoot (Grand Rapids, MI) applied to the outside of cylinders, whereas cylinders around plants with

ants had openings through which ants could travel. The Tanglefoot treatment was highly effective at manipulating ant tending. During the first survey on 30 July, plants in the "ant removal" treatment had fewer ants than controls ($F_{1,131} = 31.3$; $P < 0.0001$). Additionally, ant tending did not differ between host species, nor was there an ant treatment x plant species interaction (both $F_{1,131} < 0.2$; NS). Tending was reduced from 3.1 ± 0.5 ants per plant for controls to 0.3 ± 0.8 ants per plant on ant removal plants.

Before manipulating nymph aggregation size, we removed all adults and nymphs from experimental plants. Plants with >20 naturally occurring nymphs were not used in the experiment, because it was difficult to remove large numbers of nymphs. We manipulated aggregation size by moving nymphs from plants outside the experiment to experimental plants. Following Breton and Addicott (1992a), we cut plant stems with nymphs and tied them to experimental plants. As plant stems senesced, nymphs moved onto experimental plants. We adjusted aggregation sizes by adding or removing a few nymphs with a camel's-hair paintbrush. Initial aggregation sizes were recorded on 24 July 1999. Initial aggregation sizes had to fall within a certain range around the target aggregation size to be included in the experiment; for example, plants with a target aggregation of 20 nymphs actually had 10–30 nymphs. Likewise, the treatment 40 nymphs per plant had a range of 31–60, 80 nymphs per plant had 61–120, 160 nymphs per plant had 121–240, and 320 nymphs per plant had 241–480. The assigned aggregation size was achieved on 135 plants, and the remaining plants were excluded from the experiment. Average aggregation sizes ± 1 SE and sample sizes for plants in the five aggregation treatments were 21.2 ± 0.96 ($N = 34$), 44.5 ± 1.44 ($N = 32$), 86.7 ± 2.53 ($N = 29$), 162.6 ± 3.88 ($N = 26$), and 343.0 ± 18.51 ($N = 14$). Nymphs were approximately in their third instar at the start of the experiment. Once a week for the next 7 wk, we surveyed the number of nymphs remaining on each plant and the number of new adults on each plant. We differentiated adults emerging in different weeks by marking new adults with nontoxic water-based Painters Speedball (Statesville, NC) paint markers.

We used split-plot analysis of variance (ANOVA) to test whether the percentage of nymphs developing into adults differed among the random effect of blocks (=pair of colonies), ant treatments (whole-plot fixed factor), host plants, aggregation treatments, or interactions. The percentage of nymphs developing into adults is a cumulative measure of nymph survival over the entire experiment and is therefore the most complete measure of survival. This split-plot design has the most power for detecting the fixed effects of host plant, aggregation size, and interactions between these factors and the presence of ants. We chose the design to maximize power for determining whether aggregation size affected membracid survival (question 1) and whether the effects of ants, aggregation size, and host plant interacted in such a way that the superior host plant changed spatially (questions 2 and

3). An interaction of aggregation size with host plant would indicate that the superiority of a particular host varied with aggregation size, whereas its interaction with ants would indicate that the benefit of ant tending changed with aggregation size. An interaction between host plant and ants could indicate that the superior host varied with presence of ants. Finally, a three-way interaction could indicate that the effect of aggregation size on the benefit of ant tending varied with host plant species. In addition to this analysis of final survival, we used repeated measures, split-plot ANOVA to examine any changes over time in the effects of treatments. We tested whether the percentage of mid-instar nymphs surviving 1, 2, 3, and 4 wk (30 July, 6 August, 13 August, and 20 August) depended on the block, the presence of ants, host plant, aggregation size, or their interactions with time. This analysis was limited to the first four weeks of the experiment because in subsequent weeks, many nymphs had developed into adults, leaving some plants with no nymphs and generating many empty cells in the analysis. All analyses were performed with PROC GLM in SAS version 9.1 (SAS Institute, Cary, NC) following procedures for analysis of split-plot designs and repeated measures designs described in Littell et al. (2002). Univariate and multivariate methods of analysis for the repeated measures design yielded the same conclusions regarding statistical significance, so for simplicity we report only the univariate results.

Senescence Manipulation. One aim of the previous experiment was to determine whether the superior host plant depended on nymph aggregation size and/or the presence of ants. However, the relative survival of membracids on the two host plants was difficult to assess because some portion of the nymphs on *Wyethia* spp. moved to new plants when *Wyethia* spp. senesced. The above-mentioned analyses assumed that these nymphs died as a result of plant senescence. To obtain an estimate of survival during these movements, and thus evaluate the role of senescence, we experimentally paired *Wyethia* spp. plants with another *Wyethia* or *C. viscidiflorus* plant and we followed survival of nymphs to adulthood as they moved from senescing *Wyethia* spp. to another plant. Because experimental pairs were within 0.5 m of each other, this experiment should provide an upper bound on survival during that final stage.

In 2000, 88 pairs of plants were located and assigned one of the following four treatments: 1) an unmanipulated *Wyethia* sp. paired with another *Wyethia* sp., 2) an unmanipulated *Wyethia* paired with a *C. viscidiflorus*, 3) a *Wyethia* sp. plant with midribs cut to simulate senescence paired with *C. viscidiflorus*, and 4) a *C. viscidiflorus* paired with *C. viscidiflorus.* The last treatment served as a control to see whether survival of nymphs always differed between the initial host plant species regardless of *Wyethia* treatment. We assembled aluminum cylinders around each pair of plants and placed Tanglefoot around most of the inside rim of the cylinders to prevent membracids from leaving cylinders, while still allowing the ants access. Within each pair of conspecific plants, one plant was

Table 1. Split-plot ANOVA for the percentage of nymphs developing into adults on individual plants

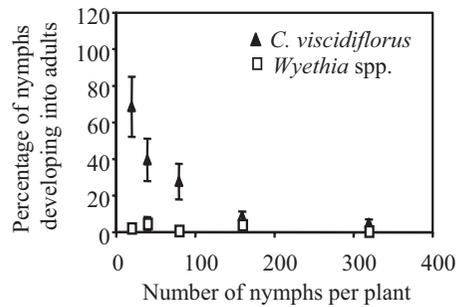| Source	df	MS	F	P
Main plot				
Block	11	0.0887	1.02	0.4844
Ants	1	0.0701	0.81	0.3875
Block × ants	11	0.0866	1.13	0.3448
Subplot				
Plant	1	1.6910	22.13	0.0001
Aggregation size	4	0.4800	6.28	0.0002
Plant × ants	1	0.0270	0.35	0.5539
Aggregation × ants	4	0.0808	1.06	0.3821
Plant × aggregation	4	0.4589	6.01	0.0002
Plant × aggregation × ants	4	0.1060	1.39	0.2445
Subplot error	93	0.0764		

Whole-plot factors are the random effect of block and fixed effect of ants (presence vs. absence). Whole-plot factors are tested over block × ants (Littell et al. 2002). Subplot factors are the fixed effects of plant species (*C. viscidiflorus* and *Wyethia* spp.) and aggregation size (20, 40, 80, 160, and 320 nymphs per plant).

Fig. 2. Mean percentage of nymphs developing into adults on *C. viscidiflorus* and on *Wyethia* spp. for each of five manipulated aggregation categories (20, 40, 80, 160, and 320 nymphs per plant) (±1 SE).

chosen at random to be the "source" plant, and the other plant was the "recipient" plant. For the mixed species pairs, *Wyethia* sp. was the source plant. We did not manipulate the number of naturally occurring nymphs on source plants. However, all nymphs and adults were removed from recipient plants before the start of the experiment. Because most eggs had hatched by this time, nymphs on recipient plants in subsequent weeks must have walked there from source plants. On 13 July, we counted the number of nymphs on each pair of plants. Also, on 13 July, *Wyethia* spp. were naturally starting to senesce, so we chose this week to manipulate senescence in one of the mixed species pairs by cutting the midribs of all the *Wyethia* spp. leaves. Each week for the next 4 wk, we counted the number of new adults on each pair of plants. Using the number of nymphs on 13 July as a baseline, we calculated the percentage nymphs developing into adults. Surveys were discontinued after 4 wk because remaining nymphs began to move in and out of cylinders. As a result, estimates of the percentage of nymphs developing into adults were underestimates, because not all nymphs had fully developed. We used ANOVA to determine whether the percentage of nymphs developing into adults differed among the four types of species pairs. A posteriori pairwise comparisons among the four treatments were made with Tukey's studentized range test.

Results

Natural aggregation sizes of nymphs per plant averaged 82.3 ± 19.2 on *C. viscidiflorus* and 92.4 ± 17.7 on *Wyethia* spp. Ninety-eight percent of *C. viscidiflorus* and 94% of *Wyethia* spp. had 360 or fewer nymphs per plant, making the aggregation size categories in the experimental manipulation (20–320) comparable with natural aggregation sizes.

The percentage of nymphs developing into adults on a host plant is a cumulative estimate of nymph survival (assuming survival during movement to new plants is low; see below). This measure of nymph survival depended on the size of nymph aggregations, the host plant, and their interaction (Table 1, plant × aggregation). The percentage of nymphs developing into adults on *C. viscidiflorus* was greater for small than large aggregations of nymphs, whereas the percentage of nymphs developing into adults on *Wyethia* spp. was independent of aggregation size (Fig. 2). Even though the effect of aggregation size differed for membracids on the two hosts, *C. viscidiflorus* was the superior host for all aggregation sizes. Thus, although the strength of selection favoring use of *C. viscidiflorus* changed with aggregation size, there was no evidence for a reversal in the favored host. Moreover, we detected neither an effect of the presence of ants nor an interaction of ants × host plant (Table 1), indicating that the favored host did not depend on the presence of ants.

The repeated measures analysis also showed that survival of mid-instar nymphs was greater on *C. viscidiflorus* than on *Wyethia* spp. (Table 2; Fig. 3). In addition, nymphal survival was greater for small aggregations than large aggregations on both plants (Fig. 3). Through time, however, nymph survival on

Table 2. Summary of the split plot ANOVA with repeated measures analyzing the percentage of mid-instar nymphs surviving 1, 2, 3, and 4 wk after the experiment began

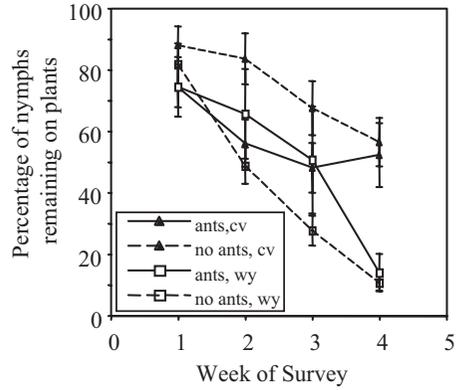| Source of variation	P	P for interaction with time
Main plot		
Block	0.1388	0.2254
Ants	0.6940	0.4778
Block × ants	0.0393	0.6158
Subplot		
Plant	0.0255	0.0067
Aggregation size	0.0026	0.4978
Plant × ants	0.1674	0.0593
Aggregation × ants	0.1714	0.5573
Plant × aggregation	0.5035	0.2690
Plant × aggregation × ants	0.8358	0.9480

Whole-plot factors are block and ants (presence vs. absence). Whole-plot factors are tested over block x ants (or, for the interaction with time, over block × ants × time). Subplot factors are plant species (*C. viscidiflorus* and *Wyethia* spp.) and aggregation size (20, 40, 80, 160, and 320 nymphs per plant). Because the assumption of sphericity was violated, Greenhouse–Geisser corrected *P* values are reported for all of the interactions with time.
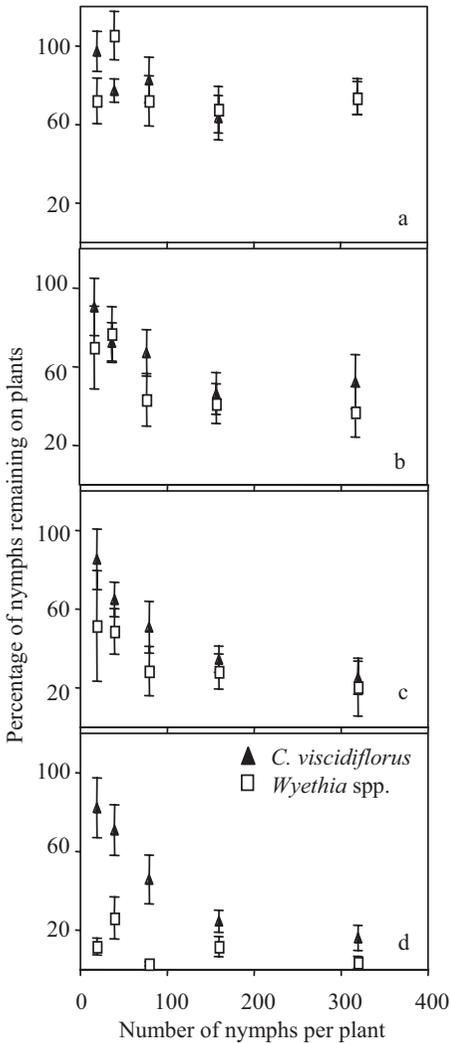
Fig. 3. Mean survival of nymphs on two host plants and from each of five aggregation categories (20, 40, 80, 160, and 320 nymphs per plant) (a) 1 wk after treatment setup, (b) 2 wk after treatment setup, (c) 3 wk after treatment setup, and (d) 4 wk after treatment setup (±1 SE).

Fig. 4. Mean survival of nymphs over time on two host plants (cv, *C. viscidiflorus*; wy, *Wyethia* spp.) and in the presence and absence of ants (±1 SE).

new plants, and developed into adults to provide a more precise comparison of survival of membracids originating on the two host plants. Of the three treatments where nymphs originated on *Wyethia* spp., survival of nymphs to adulthood was at best 8%, whereas survival to adulthood for nymphs on *C. viscidiflorus* was 43% over the same period of time (Fig. 5); survival on the four species pair combinations differed significantly (ANOVA: $F = 25.96$; df = 3, 81; $P = 0.0001$). Survival of nymphs originating on *Wyethia* did not depend on whether senescence of that plant was manipulated or natural (Tukey's a posteriori test). Nor did the species of the recipient plant, either *Wyethia* spp. or *C. viscidiflorus,* significantly influence survival of nymphs moving from *Wyethia* spp.
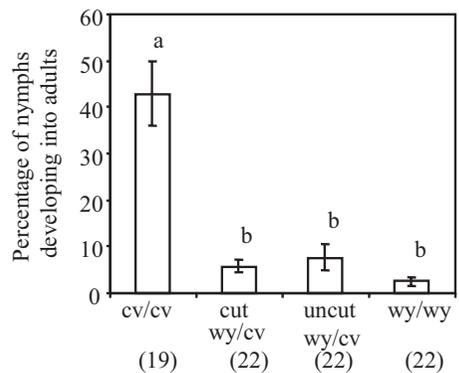
Wyethia spp. decreased more drastically than nymph survival on *C. viscidiflorus* (plant interaction with time in Table 2 and Fig. 4). Survival was likely lower on *Wyethia* spp. because these plants were senescing. The ant × plant × time interaction was marginally significant (Table 2; $P = 0.0593$). Ants tended to positively affect nymph survival on *Wyethia* spp. and negatively affect nymph survival on *C. viscidiflorus,* although by week 4 ants were not beneficial to membracids on either species (Fig. 4).

Our original analysis of final survival could have underestimated survival on *Wyethia* spp. because it assumed that all nymphs moving from senescent *Wyethia* spp. died. In a second experiment, we followed moving nymphs to estimate the percentage of nymphs that originated on *Wyethia* spp., moved to

Fig. 5. Mean percentage of nymphs developing into adults for each pair of plants (cv/cv, two *C. viscidiflorus*; cut wy/cv, one *C. viscidiflorus* and one *Wyethia* spp. with senescence manipulated; uncut wy/cv, one *C. viscidiflorus* and one naturally senescing *Wyethia* spp.; and wy/wy, two *Wyethia* spp.) (±1 SE). Different letters above bars indicate significant differences among treatments (Tukey's studentized range test, significance at the $P = 0.05$ level). Sample sizes are in parentheses.

Discussion

Aggregation Size. Individuals of the membracid species *P. modesta* develop as nymphs in aggregations on several host plants. Here, we found that the effect of aggregation size on survival of mid-instar nymphs during the early weeks of the experiment was similar on both host plants; mid-instar nymphs in smaller aggregations had greater survival. However, the cumulative survival of nymphs over the entire summer, measured as the percentage of nymphs developing into adults, differed between host species. The percentage of nymphs developing into adults on *C. viscidiflorus* decreased as aggregation size increased, whereas the percentage of nymphs developing into adults on *Wyethia* spp. seemed independent of aggregation size (Fig. 2).

Selection for generalized host use was not observed; *C. viscidiflorus* was always a superior host plant than *Wyethia* spp. Although there was an aggregation × plant interaction in cumulative survival (question 2) (Table 1), the favored host plant did not reverse with aggregation size (question 3). Nor was there a three-way interaction of aggregation × plant × ant tending that could have led to spatial variation in the selected host. Instead, *C. viscidiflorus* was the superior host for all aggregation sizes in the presence and absence of ants. In addition, nymphs originating on *Wyethia* spp. and moving to *C. viscidiflorus* after *Wyethia* spp. senesced had much lower survival to adulthood than nymphs spending their entire lives on *C. viscidiflorus* (Fig. 5).

The host plant effect is clearly explained by differences in host plant senescence. *Wyethia* spp. senesced before all nymphs could develop into adults. Many nymphs either died on senescing *Wyethia* spp. or died as they attempted to move to new host plants. Even with another plant very nearby, fewer than 8% of nymphs could survive the transition. In contrast, *C. viscidiflorus* did not senesce before September, allowing nymphs time to develop into adults.

Although the mechanism behind the host plant effect is clear, there are several ways in which aggregation size can affect survival. Negative density-dependent survival (higher growth rates or survival at smaller aggregation sizes than at larger aggregation sizes) (e.g., Breton and Addicott 1992a, Billick and Tonkel 2003, Agrawal et al. 2004, this study) can be explained by decreased quality of host plants at higher densities (Hughes 1963, Denno 1985, Itioka and Inoue 1996), possibly increased predation or parasitism of large aggregations, or, for ant-tended insects, lower per capita number of ants tending insects in large aggregations (Breton and Addicott 1992a, Morales 2000b). However, some ant-tended insects may be better off in large than small aggregations. Positive density-dependent survival (increasing survival as aggregation size increases) could occur if large aggregations attract larger ant guards (Fritz 1982, Wood 1982, Itioka and Inoue 1996) and receive greater benefits from ant tending (Cushman and Whitham 1989). In the current study, membracid nymphs experienced

negative density-dependent survival, at least on *C. viscidiflorus* (question 1). We found no interaction between the presence of ants and aggregation size (question 2), suggesting that the effect of aggregation size in this case was driven by competition for plant resources, rather than a reduction in per capita ant tending, decreased protection from predators for large aggregations, or both. Our results also illustrate how the effect of aggregation size can depend on the host plant, because the effect was weak or absent on the alternative host *Wyethia.* Nymph mortality on *Wyethia* was almost entirely due to plant senescence, which was independent of aggregation size.

Despite that membracids experience strong negative density dependence, large aggregations numbering in the hundreds are often observed. One explanation is that egg hatch success (Zink 2003a) and nymph survival (Billick et al. 2001) depend on the presence of adults, but *Publilia* mothers, which can lay can close to 100 eggs (McEvoy 1979), can only live on one plant during nymph development. Most males die shortly after mating in the spring, so it is left to the females to provide protection to hatching eggs and developing nymphs. In an effort to ensure protection for their offspring, *P. modesta* mothers likely lay and abandon eggs on plants with unrelated adults, just as do females of the closely related species, *Publilia concava* Say (Zink 2003b). The result is larger than optimal aggregations of nymphs.

Ant Mutualism. The abundance of ants did not alter the relative suitability of the two host plants (Table 1, no ant × plant interaction; question 2). *C. viscidiflorus* was always the superior host regardless of the presence of ants. In fact, the presence of ants may have affected survival of mid-instar nymphs (Table 2; Fig. 4), but ants apparently had no effect at all on the cumulative survival of nymphs to adulthood at this site (Table 1). There are at least two explanations for the lack of an ant effect. First, the ant effect may have been lower than average in the year of study (Billick and Tonkel 2003), and the use of a split-plot design had limited power to detect a small main effect of ants. Second, ants may only be beneficial to membracids during certain periods in their life cycle. In this study, treatments were applied when nymphs had already reached their third instars. In contrast, similar experiments conducted at nearby sites (Billick and Tonkel 2003, Reithel and Billick 2006) detected an ant effect when following the survival of nymphs from their earliest instars when they are likely to be more vulnerable to predation (Wood 1982).

Although ants had no effect on cumulative nymph survival to adulthood, during the early weeks of the experiment ants positively affected survival of mid-instar nymphs on *Wyethia* and negatively affected survival of mid-instar nymphs on *C. viscidiflorus* (Table 2, ant × plant × time; $P = 0.0593$). This anomalous result remains unexplained, because our other studies in the same location show that ant-tending positively benefited survival of mid-instar nymphs on both plant species in 2000 and 2001 (Reithel and Billick 2006). Additionally, other researchers report ants as having

no effect or a positive effect on *P. modesta*'s survival, but never having a negative effect (Cushman and Whitham 1989, Billick et al. 2001, Billick and Tonkel 2003).

Host Plant Generalism. Results to date suggest that membracids specializing on *C. viscidiflorus* are favored over membracids capable of generalizing across hosts, regardless of aggregation size or ants (question 3). There are at least two plausible explanations for why *P. modesta* remains a host generalist. The first explanation is that the superior host plant may change over the life cycle of the membracids. Host generalism could be favored if *Wyethia* spp., or one of the many other host plants, is more suitable than *C. viscidiflorus* for overwintering adults or during the egg stage, whereas *C. viscidiflorus* is superior during nymph development. Whether this type of variable selection favors host switching or a generalist strategy depends on whether membracids can discriminate among host species at several points during their life cycle. Indeed, whereas host switching by aphids is relatively common (Moran 1992), there are no confirmed cases of membracids specializing on different host species for specific periods during their life cycle.

A second explanation for generalism by *P. modesta* could be the possible use of plant phenology as an oviposition cue. *P. modesta* may preferentially oviposit on host plants at the correct phenological stage, such as bud break in the spring when plants have a flush of nutrients and water in their stems to support new growth. Similarly, the membracid *Platycotis vittata* (F.) can be induced to break winter diapause early and oviposit on host trees with experimentally accelerated phenology; newly flushing trees have increased water content and nitrogen levels in their sap (Keese and Wood 1991). Because *P. modesta*'s life history is not synchronized with a single host species, spatial or temporal variation in plant phenology could result in spatial or temporal variation in the preferred host plant for oviposition. Indeed, other studies show that the preferred host plant for *P. modesta* differs markedly between two sites separated by 20 km and having similar host plant communities (Reithel 2003). Suitability of hosts for survival might vary spatially as well.

Several ecological factors associated with ant mutualisms are known to vary over space or time. These include the species of ant (Addicott 1979), ant tending rate (Cushman and Addicott 1989, Morales 2000b), aggregation size (Addicott 1979, Wood 1982, Cushman and Whitham 1989, Morales 2000a, this study), host quality (Breton and Addicott 1992b, Reithel 2003), and the abundance of predators (Cushman and Whitham 1989, Del-Claro and Oliveira 2000). However, relatively little is known about the implications of this variation for evolution of host plant use or coevolution in ant–hemipteran mutualisms (Thompson 1999). Here, we addressed the potential for selection favoring generalized host plant use in membracids, by performing manipulations of ant tending and aggregation size on multiple plant species, and examining the effects on membracid survival. Although our study did not find evidence for this type of selection, a broad understanding of the evolutionary significance of spatial and temporal variation will require many more studies that go beyond simple documentation of variation. In particular, future work on ant-tended hemipterans should investigate the underlying causes of spatial and temporal variation in fitness.

Acknowledgments

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