Coexisting congeners: demography, competition, and interactions with cardenolides for two milkweed-feeding aphids

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Explaining the coexistence of closely related species sharing a single resource has been a long-standing challenge in ecology. Here we report on studies comparing the aphids *Aphis nerii* and *A. asclepiadis* that feed sympatrically on the milkweed *Asclepias syriaca* in northeastern North America. We sought to identify tradeoffs among species' attributes that might promote coexistence, but in most instances *A. nerii* was superior to *A. asclepiadis*. *Aphis nerii* was 84% more fecund, fed upon 880% more phloem sap, and was affected 70% less by intraspecific competition as compared to *A. asclepiadis*. In interspecific competition, *A. nerii* reduced *A. asclepiadis* abundance by 77%, whereas *A. asclepiadis* did not affect *A. nerii*. In dispersal trials, 10% of winged *A. nerii* but only 1% of *A. asclepiadis* successfully moved from non-host plants to *A. syriaca*. We also investigated whether there were differences in aphid interactions with milkweed cardenolides. Jasmonic acid increased milkweed cardenolides by 33%, a realistic amount similar to that induced by several leaf-chewing herbivores. Nevertheless, jasmonate-induced cardenolides failed to affect aphid performance and aphid feeding had no effect on milkweed cardenolide concentration. Yet cardenolides were important for aphid resistance to predators; *A. nerii* sequestered 25% more cardenolides and was preyed upon 50% less than *A. asclepiadis*. Interactions with cardenolides thus again favored *A. nerii* over *A. asclepiadis*. Given that *A. nerii* and *A. asclepiadis* are decidedly not equivalent in their demography, competitive ability, defense and dispersal, our results strongly refute the notion that neutral processes can explain coexistence of these aphids. Based on field observations, we propose two tradeoffs – timing of milkweed colonization and relationships with ants – as putative mechanisms for the coexistence of these congeners.

For 70 years, understanding the mechanisms that promote the coexistence of species has been a central problem in community ecology. Gause was perhaps the first to experimentally examine the mechanisms by which species coexist in his classic experiments using congeneric paramecia (Gause 1934). Based on his findings, Gause proposed the principle of competitive exclusion, an idea that is still debated and studied. Coexistence is especially difficult to explain in species that appear to utilize a similar set of resources in a similar way (Hutchinson 1961).

Most theory predicts that tradeoffs in fitness-enhancing traits are necessary for species to coexist, e.g. the ability to exploit a particular resource, predator avoidance, or tolerance of abiotic stress (MacArthur 1972, Tilman 1982, McPeek 1996, but see Hubbell 2001). The bewildering diversity of insects on plants has been attributed to the fact that most herbivores have relatively narrow diet breadths, thus allowing a large number of species to occupy unique niches within a community. These narrow diet breadths are probably driven by tradeoffs in fitness on alternative hosts (Futuyma and Moreno 1988). In this way, two species that could not coexist on a single resource due to competitive exclusion persist by resource partitioning, i.e.

maintaining competitive dominance on separate resources (Chase and Leibold 2003). Still, in the contemporary context, every plant species is attacked by at least several herbivorous insect species, often with several in the same feeding guild. The factors organizing the community and promoting coexistence of multiple herbivore species have been hotly debated, although rarely systematically unraveled (Rathcke 1976, Lawton and Strong 1981, Karban 1989, Denno et al. 1995, Denno and Kaplan 2007).

Herbivore species sharing a host plant can influence each other not only by exploitative competition – i.e. drawing down the quantity of a shared resource – but also by altering the quality of those resources. In the case of competing herbivores, one species might affect another indirectly by inducing changes in the secondary chemistry of a shared host plant (Denno et al. 1995, Karban and Baldwin 1997, Van Zandt and Agrawal 2004a, 2004b). Induced changes in plant secondary chemistry may directly affect herbivore performance and competitive ability based on their tolerance of those compounds (Karban and Baldwin 1997). Plant secondary chemistry also has implications for interactions of herbivores with predators, especially in instances where herbivores sequester defensive

compounds (Brower and Moffitt 1974, Nishida 2002). Herbivore tolerance, induction, and sequestration of plant secondary compounds may thus play an important role in determining the modes of competitive interactions and coexistence.

Pairs of congeneric species, especially those that are sympatric, present unique opportunities for the study of coexistence. Because of their recent common ancestry, sympatric congeners are likely to be similar in many phenotypic and ecological traits, with the exceptions likely being those aspects of their biology that have diverged as a function of competition. Examples of character displacement between congeners are widespread (Schluter 2000) and have provided important observational evidence for the types of tradeoffs that mediate coexistence.

The aphids (Hemiptera: Aphididae) *Aphis asclepiadis* and *Aphis nerii* coexist with each other, and with other herbivore species, on the common milkweed *Asclepias syriaca* in northeastern North America. We conducted a series of laboratory experiments with this system to address the following questions. First, how do the two aphid species compare with respect to their demography and competitive ability? Second, are there differences between the traits of the two species suggesting a tradeoff that might mediate their coexistence? Specifically, we compared these species with respect to interactions with milkweed cardenolides (tolerance, induction, and sequestration), defense against predators, dispersal ability, and temporal (seasonal) partitioning of resource use. Finally, we consider our results and the natural history of these congeners to formulate a testable hypothesis for the means of their coexistence.

Methods

Natural history

Aphis nerii and *A. asclepiadis* are members of a guild of 11 species, including the aphid *Myzocallis asclepiadis*, that regularly feeds on milkweeds (*Asclepias*: Apocynaceae) (Mooney and Agrawal in press, Smith et al. in press). *Aphis nerii* feeds broadly within the Apocynaceae while *A. asclepiadis* is restricted to relatively few *Asclepias* species (Blackman and Eastop 2006). In eastern North America, *Aphis nerii* and *A. asclepiadis* feed upon the common milkweed *Asclepias syriaca* (hereafter referred to as milkweed), a native perennial that grows in old fields throughout the region. Both aphids feed in dense aggregations on apical leaves, but they also feed on the underside of lower leaves when apical leaves are crowded and thus unavailable. *Aphis nerii* is bright yellow in coloration and is rarely tended by ants. *Aphis asclepiadis* is cryptically colored, ranging from brown to green, and is nearly always ant-tended (Mooney and Agrawal unpubl.). Both aphids reproduce by parthenogenesis (i.e. asexually) during summer months and can produce winged morphs under dense conditions (Groeters 1989, Mooney and Agrawal unpubl.). *Aphis nerii* is believed to be obligately parthenogenetic and cannot tolerate freezing temperatures (Groeters 1989, Blackman and Eastop 2006; but see Takada and Miyazaki 1992). It is unknown whether *A. asclepiadis* is obligately parthenogenetic like *A. nerii*, or whether it is cyclically parthenogenetic, i.e. with a sexual

generation in the fall and an overwintering egg stage. The latter is suggested by the fact that *A. asclepiadis* overwinters in central New York where an egg stage may be necessary to tolerate temperatures well below freezing (Knight et al. 1986, Clough et al. 1990, Strathdee et al. 1995).

Milkweed's well-known toxicity comes from cardiac glycosides (cardenolides), bitter-tasting steroids that have toxic effects on most animals by disrupting the sodium and potassium flux in cells (Malcolm 1991). Cardenolides are present constitutively in milkweed and their production is also known to be induced following damage by several species of foliage-chewing herbivores (Malcolm and Zalucki 1996, Agrawal unpubl.). Past work suggests *A. nerii* can sequester cardenolides (Rothschild et al. 1970, Malcolm 1986) but it is unknown whether this is the case for *A. asclepiadis*.

Experimental plants and aphid colonies

We germinated seeds collected in Tompkins County, NY, USA (42°50′04″N, 76°43′57″W) and planted them individually in 500-ml pots with potting soil. We then grew plants in growth chambers (25°C: 22°C on a 14L: 10D cycle) where we watered them as needed and fertilized them weekly. At the time of the experiments, the plants were approximately one month old, with 8–10 leaves. Thus all plants were seedlings; as the goal here was to compare aphid performance under controlled conditions, the logistical ease of working with smaller plants justified their use. We conducted all of our experiments under these same experimental conditions. We founded our aphid colonies from single individuals of both aphid species collected from *Asclepias syriaca* in Tompkins County in August 2004, and maintained small populations by serial transfer on potted *A. syriaca* in a growth chamber under the conditions described above.

Feeding and reproduction

We compared *A. nerii* and *A. asclepiadis* with respect to their feeding rate and fecundity. To measure the aphids' feeding rates, we collected honeydew from beneath individual aphids on pre-weighed, dry filter paper disks. We used eight plants for this experiment, where each plant had one aphid of each species and six plants had control disks beneath a leaf without an aphid. We removed the nymphs produced by each aphid on a daily basis, and after four days, we re-weighed the dried disks. We analyzed these data by mixed-model ANOVA using the MIXED procedure in SAS ver. 9.1, testing for differences in feeding rate with plant included in the model as a random effect.

To measure fecundity and lifespan, we placed adult aphids of each species singly on milkweeds and after 24 h we removed these adults and all but one nymph. We then monitored six focal aphids of each species daily through their development to the adult stage and then through their reproductive life span. Each day we counted and removed all nymphs produced by the focal aphids and thus determined both daily and lifetime fecundities of each aphid. We analyzed these data by ANOVA using the SAS procedure GLM to compare the species with respect to age

at first reproduction, reproductive life spans, and lifetime fecundity.

Intraspecific competition and milkweed cardenolides

The goals of this experiment were threefold: first, to measure and compare the population growth-rate and the strength of density dependence (i.e. intraspecific competition) for the two aphids; second, to manipulate cardenolide levels in milkweed and thus determine the effects of these compounds on aphid growth and density dependence; and third, to determine whether aphid feeding itself induces changes in milkweed cardenolides.

We stocked individual milkweed plants with nymphs of a single species at densities ranging from 1 to 15 nymphs per plant. To manipulate milkweed cardenolide levels, we applied 0.5 mM jasmonic acid (hereafter JA) 48 h prior to aphid inoculation according to the methods of Thaler et al. (1996). When applying JA, we sprayed plants to coat the leaf surfaces, while spraying a similar amount of acetone in water on control plants. After 13 days (two to three aphid generations) we counted all aphids. We conducted three iterations of this experiment, with sample sizes of 120, 80 and 60 plants, each of which included a complete range of aphid starting densities (i.e. 1 to 15) for each aphid species and at each level of the JA treatment.

We calculated aphid per capita daily growth rate dN/Ndt (hereafter "growth rate") as $(\ln N_2 - \ln N_1)/(T_2 - T_1)$ where N_1 and N_2 are the population size at times T_1 and T_2 respectively. We regressed growth rate against initial aphid density (N_1), thereby testing whether growth rate was affected by aphid density for each species. We performed these mixed-model ANOVAs with the SAS procedure MIXED, modeling growth rate as a function of aphid species, initial density and JA treatment and including experimental iteration as a random effect.

In the first iteration of the experiment, we measured milkweed cardenolide levels from leaves of all 120 plants collected at the conclusion of the experiment. We also measured cardenolides from 10 aphid-free plants at the beginning and end of the experiment that were divided evenly between JA and control treatments. We measured cardenolide concentration spectrophotometrically following the methods of Agrawal (2004b, 2005). Briefly, powdered tissue was extracted in 1.9 ml of 95% ethanol, sonicated in a water bath (65°C) for 10 min, and then centrifuged at 5000 rpm for five min. Two aliquots of each extracted sample were added to a 96-well plate (active sample and blank), and 0.15% 2,204,40-tetranitrodiphenyl (TNDP) in EtOH was added only to the active sample. The colorimetric reaction was catalyzed with 0.1 M aqueous NaOH and the plate was read after 18 min at 620 nm using a microplate reader. Differences between sample and blank were calibrated with digitoxin, which was used as standard. We analyzed these data by ANOVA using the SAS procedure GLM to test for the effects of JA on cardenolide levels at the beginning and end of the experiment on aphid-free plants. To test for the effects of aphid feeding on cardenolides, we regressed cardenolide concentration on aphid counts also using GLM.

In a separate experiment, we compared cardenolide induction by other (non-aphid) herbivores with those induced by aphids to assess how these species might affect aphid performance, and to compare the cardenolide induction from JA with that from actual feeding damage. We exposed plants to JA, mechanical damage to mimic herbivory, or true herbivory by *Tetraopes tetrophthalmus* (Cerambycidae), *Labidomera clivicollis* (Chrysomelidae), *Danaus plexippus* and the aphid *A. nerii*. We allowed the chewing herbivores (*T. tetrophthalmus*, *L. clivicollis*, *D. plexippus*) to feed until 10-15% of each plant's leaf area was removed. Herbivory by chewing herbivores occurred over three days and we applied JA in the middle of the second day. For the aphid treatment, we inoculated plants with five adult *A. nerii* each and allowed them to feed and reproduce for five days. For the mechanical damage treatment, we removed 10% of leaf area spread over the leaves with a hole puncher. Six days after treatments were initiated, we collected leaf tissue for cardenolide analysis from treated and untreated control plants. Sample size for each treatment was between nine and 11 plants. We tested for differences in cardenolide levels among these seven treatments by ANOVA using the SAS procedure GLM, and tested all pairwise comparisons among treatment levels with Duncan's post-hoc tests (Zar 1999).

Interspecific competition

To determine the relative competitive abilities of the two species, we placed aphids on plants either alone at a density of three aphids per plant (n = 9 plants per aphid species) or in combination (n = 9 plants) at a density of three aphids per species (six aphids total). After 10 days, we compared aphid counts by ANOVA using the SAS procedure GLM where the two factors were competition (present, absent) and aphid species. In this analysis, a difference in the effects of competition is indicated by a competition-by-aphid interaction. Because *A. asclepiadis* was much less fecund than *A. nerii* (Results), competition effects in terms of reduced fecundity would be expected to be less for *A. asclepiadis* simply because there is less reproductive potential upon which competition might act. Consequently, we used a multiplicative null model by analyzing log-transformed data (Sih et al. 1998). This approach is analogous to testing for the effect of competition proportionally to the abundance of each aphid species.

Cardenolide sequestration and predator resistance

We measured aphid cardenolide content by collecting samples of 25 mg wet mass (several hundred aphids) on three separate occasions (n = 26 and 30 for *A. asclepiadis* and *A. nerii,* respectively) from our laboratory colonies. Across the three sampling dates, aphids were collected from over 30 different plants. We allowed aphids to live for 24 h to clear their guts before freezing them. We measured cardenolide content spectrophotometrically as before and tested for the effect of aphid species by mixed-model ANOVA using the SAS procedure MIXED, including experimental iteration as a random effect.

To compare the palatability of the aphids to predators, we conducted both choice and no-choice predator bioassays using the seven-spotted lady beetle, *Coccinella septempunctata*, from a laboratory colony maintained on the pea aphid *Acyrthosiphon pisum*. In the no-choice test, we placed 35 aphids with a single ladybug in a 9.0 cm diameter petri dish arena and counted the number of aphids remaining after 16, 24, 40 and 48 h (n = 9 per aphid species). In the choice test we followed the same protocol but placed in the arena 15 aphids of each species for a total of 30 aphids (n = 10). In the no-choice experiment we tested for differences in the number of aphids remaining at the last census (48 h) by ANOVA using the SAS procedure GLM. In the choice experiment we analyzed data by contingency table analysis.

Resource partitioning and dispersal ability

To investigate whether there was seasonal partitioning of milkweed use by the aphids, we documented the dates of first and last detection of each aphid species at local field sites for two consecutive summers (2005, 2006). During each summer, we worked extensively with several milkweed populations near Ithaca, NY (USA) and kept records of the milkweed herbivore fauna encountered. Because we visited these field sites on a weekly to bi-weekly basis, these times of first detection are only approximate.

We also investigated whether the two species differ in dispersal ability. In 15 separate trials we placed aphid-free "target" milkweeds in growth chambers 20 cm away from non-host (Crassulaceae spp.) plants on which we placed 10 winged aphids of each species. After 24 h, we recorded the number of aphids that had dispersed to the target plants. Because the data were highly non-normal, we tested for differences in dispersal rate using the non-parametric Wilcoxon signed-rank test (Zar 1999).

Results

Feeding and reproduction

After four days, the honeydew accumulated on filter paper differed among experimental treatments ($F_{2,20} = 109.96$, $p < 0.0001$), with *A. nerii* producing 0.44 ± 0.04 mg honeydew day^{-1} (mean $\pm$ 1SE), nine times more than the 0.05 ± 0.01 mg honeydew day^{-1} produced by *A. asclepiadis*. Although the mean mass of filter paper beneath *A. asclepiadis* was 14 times greater than that on control disks (0.004 ± 0.009 mg honeydew day^{-1}), this difference was not significant in Duncan's post-hoc pairwise comparisons. The two aphids did not differ in age of first reproduction (about 6 days, $F_{1,11} = 0.05$, $p = 0.83$). *Aphis asclepiadis* had a 16% longer reproductive lifespan than *A. nerii* ($F_{1,6} = 8.33$, $p = 0.0278$), but *A. nerii* had nearly twice the lifetime fecundity ($F_{1,8} = 9.63$, $p = 0.0146$) of *A. asclepiadis* (Fig. 1).

Intraspecific competition and milkweed cardenolides

The application of jasmonic acid (JA) to aphid-free milkweeds increased cardenolide concentration by 33%
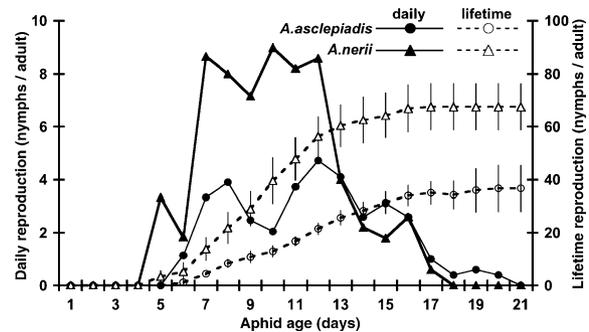

Fig. 1. Mean daily and lifetime fecundity ($\pm$1SE) for *A. asclepiadis* (n = 6) and *A. nerii* (n = 7). SE are omitted from daily fecundity data for clarity. *Aphis nerii* has a significantly longer reproductive life span and higher lifetime fecundity than *A. asclepiadis*, but the two species do not differ for the age of first reproduction.

from 0.15 ± 0.009 (mean percent dry mass $\pm$ SE) in control plants to 0.20 ± 0.009 in treated plants ($F_{1,10} = 8.10$, $p = 0.0174$). Cardenolide levels did not change over the course of the 13 day experiment (JA $\times$ time: $F_{1,10} = 0.45$, $p = 0.52$). Nevertheless, there was no main effect of induced cardenolides on aphid growth rate ($F_{1,243} = 0.01$, $p = 0.92$), nor an interaction between induction and aphid species ($F_{1,243} = 0.03$, $p = 0.87$) or between induction and aphid density ($F_{1,243} = 0.78$, $p = 0.38$).

Growth rate for the aphids varied as a function of aphid species ($F_{1,243} = 121.39$, $p < 0.0001$), initial aphid density ($F_{1,243} = 11.44$, $p = 0.0008$), and an interaction between the two (density $\times$ aphid species interaction: $F_{1,243} = 12.12$, $p = 0.0006$). Separate regressions of growth rate on starting density for the two species revealed negative density dependence for *A. nerii* ($F_{1,123} = 32.29$, $p < 0.0001$, $R^2 = 0.53$, slope $= -0.007$) but not for *A. asclepiadis* ($F_{1,124} = 0.06$, $p = 0.81$).

Final aphid populations differed more than three-fold between the two species ($F_{1,235} = 195.92$, $p < 0.0001$), with *A. nerii* and *A. asclepiadis* having 746 ± 28 and 210 ± 28 aphids per plant, respectively. Because of these large differences in aphid abundance, we performed a second analysis to compare density dependence between the aphid species over an identical range of aphid counts. We limited our dataset to observations where final aphid counts were greater than 70 aphids but less than 550. This range generated the most balanced sample size for the two aphid species, and it constituted the 8th to 50th percentiles for *A. nerii* (n = 54 of 127 total plants) and the 50th to 96th percentiles for *A. asclepiadis* (n = 57 of 126 total plants). With this restricted analysis, density dependence again differed between the two aphid species (density $\times$ aphid species interaction: $F_{1,101} = 17.87$, $p < 0.0001$) (Fig. 2). Here, separate regressions for each species showed density dependence being stronger for *A. asclepiadis* than *A. nerii*, with the slope of the later being 70% less than that of the former (statistics provided in Fig. 2). We tested for effects of JA-mediated induction on this restricted dataset, but as before we saw no main effects of, or interactions with induction ($p > 0.23$ for all tests). *Aphis asclepiadis* thus showed negative density dependence in the upper – but not lower-half of its density range, suggesting that there may be
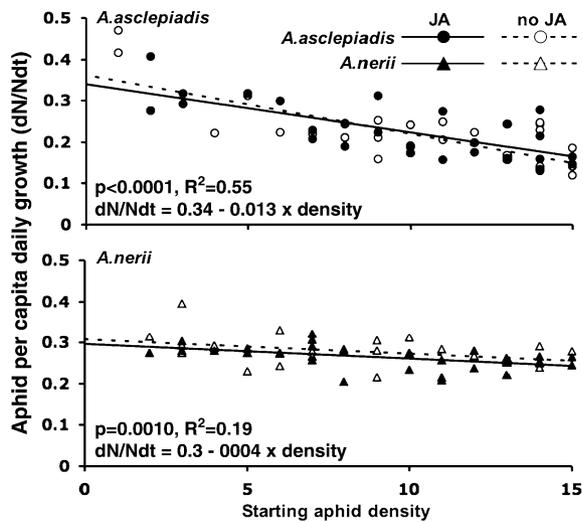
Fig. 2. Effect of aphid starting density and jasmonic acid (JA) application on aphid growth rate for each aphid species. The analyses shown here are restricted to plants with aphid counts of 70 to 550 aphids for *A. nerii* (8th to 50th percentiles, n = 54 plants) and *A. asclepiadis* (50th to 96th percentiles, n = 57 plants). Filled symbols are plants treated with JA and open circles are controls. There was negative density dependence for both species, with the effect being significantly stronger for *A. asclepiadis* than *A. nerii*. There were no detectable (p > 0.05) effects of JA and the statistics and regression equations in each panel are pooled across the JA treatment.

a lower threshold of aphid abundance (i.e. approximately 70 aphids per plant) where intraspecific competition is minimal. Whether or not this same threshold holds for *A. nerii* cannot be determined from our data as few plants (8%) had fewer than 70 *A. nerii*.

Milkweed cardenolide levels were unaffected by aphids; there were no detectable effects of aphid species ($F_{1,104} = 0.10$, $p = 0.76$), aphid abundance ($F_{1,104} = 0.01$, $p = 0.97$), or their interaction ($F_{1,104} = 0.01$, $p = 0.95$). As we saw with the aphid-free plants, the application of JA on plants with aphids increased cardenolide levels by 19% from 0.16 ± 0.008 to 0.19 ± 0.01 percent cardenolides ($F_{1,104} = 9.82$, $p = 0.0022$).

When we compared the chemistry of plants attacked by different herbivores, cardenolide levels differed among plants based on herbivore damage and our manipulations ($F_{1,63} = 3.75$, $p = 0.0030$, Fig. 3). Post-hoc Duncan's tests revealed that application of JA and damage by *D. plexippus* resulted in cardenolide levels that were 48% higher compared to controls, while plants with mechanical damage or plants fed upon by *A. nerii* and *T. tetrophthalmus* did not differ. Plants damaged by *L. clivicollis* were intermediate between these groups and did not differ significantly from any other treatment.

Interspecific competition

There was a significant interaction between aphid species and competition ($F_{1,32} = 4.68$, $p = 0.0380$, Fig. 4). Separate post-hoc tests for each aphid species showed that competition reduced *A. asclepiadis* abundance by 77% ($F_{1,16} = 6.76$, $p = 0.0193$), while *Aphis nerii* abundance
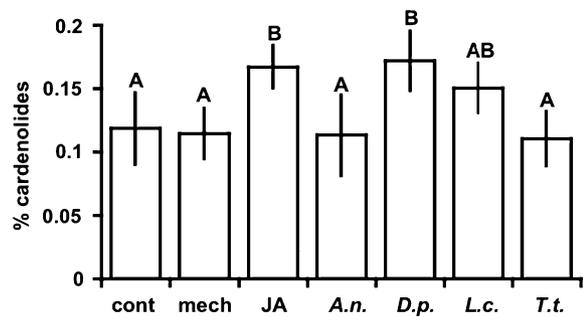

Fig. 3. Mean (± 1SE, n ranges from 9 to 11) leaf cardenolide levels for milkweeds in the following treatments: Control ("cont") mechanical damage ("mech"), jasmonic acid application ("JA") and herbivory by *Aphis nerii* ("*A.n.*"), *Danaus plexippus* ("*D.p.*"), *Labidomera clivicollis* ("*L.c.*") and *Tetraopes tetraophthalmus* ("*T.t.*"). Means not sharing letters differed in post-hoc tests (p < 0.05).

was reduced by 7% which was not statistically significant ($F_{1,16} = 0.46$, $p = 0.51$).

Cardenolide sequestration and palatability

The cardenolide concentration of *A. nerii* (4.5 ± 0.9 percent cardenolides wet mass), was 22% higher than that in *A. asclepiadis* (3.7 ± 0.9) ($F_{1,52} = 4.47$, $p = 0.0393$). After 48 h, there were 146% and 210% more *A. asclepiadis* consumed by lady beetles compared to *A. nerii* in the choice ($\chi^2_{(DF = 3)} = 17.3$, $p = 0.0006$) and no-choice ($F_{1,16} = 5.32$, $p = 0.0348$) experiments respectively (Fig. 5).

Resource partitioning and dispersal ability

In both 2005 and 2006 we first observed *A. asclepiadis* at our field sites in early- to mid-June, while we did not observe *A. nerii* until August. In nine of the 15 dispersal
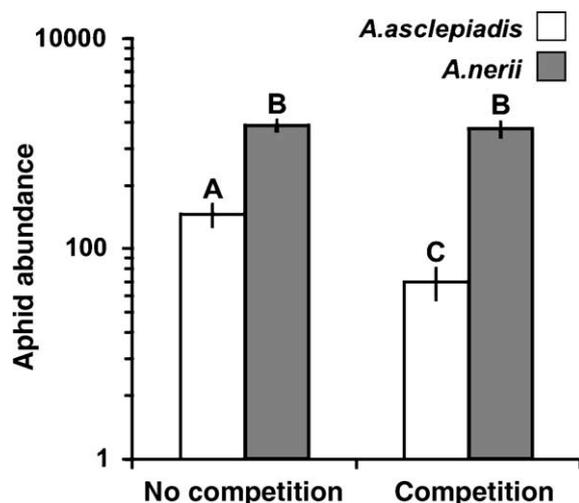

Fig. 4. Effects of intraspecific competition on *A. asclepiadis* and *A. nerii*. Competition reduced mean aphid abundance for *A. asclepiadis* by 77%, but had no detectable effects on *A. nerii*. Analyses were performed on log-transformed data. Means not sharing letters differed in post-hoc tests (p < 0.05).
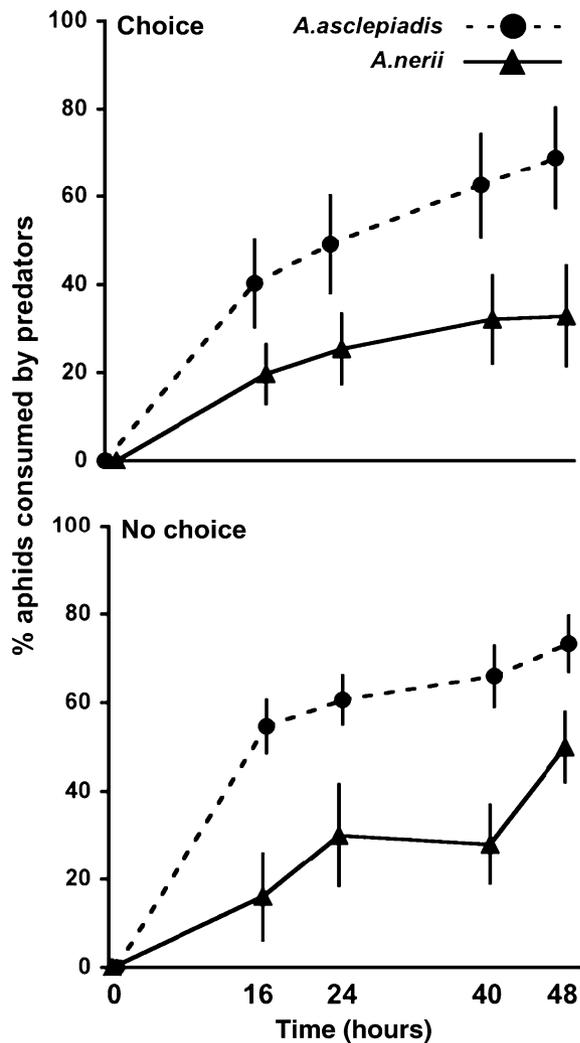
Fig. 5. Predation of *A. asclepiadis* and *A. nerii* by sevenspotted lady beetle *Coccinella septempunctata* in choice and no-choice experiments. Mean ($\pm$1SE, n=9) aphids consumed at four time periods. *Aphis asclepiadis* was consumed more than *A. nerii* (p <0.05) in both cases.

trials at least one aphid moved to *A. syriaca*, and there were eight trials where the rate of dispersal was not a tie between the two aphid species, which is a requirement of the Wilcoxon signed-rank test. Statistical tests for these eight non-tied trials showed *A. nerii* to have superior dispersal ability compared to *A. asclepiadis* (W =36, n =8, p = 0.01); for *A. nerii* 10 ± 3% of aphids located the host plant (range 0–30%) while for *A. asclepiadis* only 1 ± 1% (a single aphid) did so.

Discussion

Traits and coexistence

Aphis nerii was superior to *A. asclepiadis* in every trait and ecological attribute measured in our studies. As compared to *A. asclepiadis*, *A. nerii* fed at a greater rate, reproduced faster (Fig. 1) and better tolerated both intraspecific (Fig. 2) and interspecific (Fig. 4) competition. Both aphid species

sequestered significant concentrations of milkweed cardenolides, although other work using our same methods has shown that *A. nerii* sequestered only 57% the cardenolide concentration of adult monarch butterflies when they were compared directly (Agrawal unpubl.). Nevertheless, *A. nerii* was better defended with cardenolides than *A. asclepiadis*, and this difference was reflected in a lower palatability to predators (Fig. 5). Although both aphid species were represented by single genotypes, these stark differences almost certainly are qualitatively similar among other, untested genotypes. The apparently uncompromising superiority of *A. nerii* raises the question of how *A. asclepiadis* manages to persist (Chase and Leibold 2003)?

Resource partitioning is considered one of the principal mechanisms promoting coexistence (Hutchinson 1961, MacArthur 1972). We compared these aphids on only one of several host plant species, and it could be that *A. asclepiadis* may persist in the field by virtue of outperforming *A. nerii* on some alternative host species. This appears unlikely based on our observations; *Asclepias syriaca* is the most common, and often the only host plant for *A. asclepiadis*, while *A. nerii* can be found in large numbers on a few locally occurring host species (Mooney and Agrawal unpubl.). Blackman and Eastop (2006) report that *A. nerii* feeds broadly across the Apocynaceae while *A. asclepiadis* is limited to a few species in the genus *Asclepias* and one species of *Apocynum*. They also provide aphid host plant records for 25 *Asclepias* species; 48% are fed upon by *A. nerii* alone, 8% by *A. asclepiadis* alone, and 20% by both aphids (with 24% being fed upon by neither aphid), demonstrating the much larger diet breadth of *A. nerii*. Resource partitioning among herbivores might also occur within a single host plant species via specialization on specific plant parts. For example, the coexistence of three aphids on birch *Betual pendula* and a guild of seven sapfeeding insects on saltmeadow cordgrass *Spartina patens* may be promoted by feeding on different plant parts (Denno 1980, Hajek and Dahlsten 1986). Yet in the present case, both *A. nerii* and *A. asclepiadis* preferentially select apical leaves as a feeding location (Mooney and Agrawal unpubl.). Consequently, resource partitioning, either within or among host plant species, does not likely play an important role in providing a refuge to *A. asclepiadis* from competition with *A. nerii*. *Aphis nerii* thus appears to be an exception to the adage that a "jack of all trades is master of none".

Colonization-competition tradeoffs have also been proposed as a mechanism for promoting the coexistence of species where resources are patchily distributed (Holmes and Wilson 1998). In this scenario, competitively inferior species coexist with superior ones by having a higher dispersal rate. In our dispersal experiment we measured one aspect of dispersal ability, that of winged individuals to successfully locate a suitable host plant. In this regard, *A. nerii* was again superior to *A. asclepiadis*.

A second aspect of dispersal ability in aphids is the propensity with which winged offspring are formed. Aphids produce winged young in response to a decline in resource quality or a perceived risk of predation (Dixon 1998). This is most readily seen where winged aphids are formed in response to host plant overcrowding. While we did not compare *A. asclepiadis* and *A. nerii* with respect to this

aspect of their biology, it is our impression that they produce winged offspring in response to over-crowding at a similar rate. Given the clear superiority of winged *A. nerii* to locate host plants, it is unlikely that a tradeoff between dispersal ability and competitive ability is the principal explanation for their coexistence.

In contrast to the apparent lack of a colonization-competition tradeoff with respect to dispersal ability, there was an apparent tradeoff between timing of milkweed colonization, i.e. phenology, and competitive ability. *Aphis asclepiadis* was present throughout the growing season while *A. nerii* only arrived in late summer, probably after moving north from warmer climates. This observed difference in phenology is likely due to differences in over-wintering ability associated with the aphid's life histories. *Aphis nerii* is obligately parthenogenetic through most of its range (Groeters 1989, Blackman and Eastop 2006, but see Takada and Miyazaki 1992) and thus lacks a sexual generation and egg stage. Aphid eggs are cold hardy (Strathdee et al. 1995) compared to nymphs (Knight et al. 1986, Clough et al. 1990). While it is undetermined whether *A. asclepiadis* is cyclically parthenogenetic, this is likely the case given its early occurrence at our field site (late May). *Aphis asclepiadis* may thus escape a competitively superior *A. nerii* for much of the summer by virtue of over-wintering locally in the egg stage.

Different relations with ants may also be important to the coexistence of *A. nerii* and *A. asclepiadis*. Where we consistently find *A. asclepiadis* to be tended by ants, *A. nerii* is most often untended. Although invasive argentine ants *Linepithema humile* have been shown to benefit *A. nerii* in California (Bristow 1991), at our study sites ants benefit *A. asclepiadis* (Mooney and Agrawal unpubl., Smith et al. in press) but reduce *A. nerii* abundance (Smith et al. in press). The traits of these species are consistent with the hypothesis that mutualism with ants (or not) is an important axis separating the two. *Aphis nerii* is warningly colored and sequesters relatively high levels of cardenolides compared to *A. asclepiadis*. Our bioassay showed *A. nerii* to be less palatable than *A. asclepiadis* (Fig. 5), and similar work comparing a taxonomically diverse group of aphid species has consistently found *A. nerii* to be the least consumed by a variety of predators (Malcolm 1986, Omkar and James 2004). In contrast, *A. asclepiadis* is relatively undefended, is cryptic in coloration, and appears to rely substantially on ants for protection from predators (Mooney and Agrawal unpubl., Smith et al. in press). Because mutualism with ants can carry substantial fitness costs (Stadler and Dixon 2005), this difference between the two aphids may present a tradeoff mediating their coexistence. There are several other examples of ant-tended and untended pairs of congeneric aphids sharing the same host plant species (Shingleton and Foster 2001, Mondor et al. 2002, Mooney 2006, 2007), suggesting that such dynamics may frequently play a role in mediating the coexistence of otherwise ecologically similar species.

There are some models that seek to explain coexistence in the absence of trait differences and tradeoffs among species. For instance, neutral theory has recently provided explanations for coexistence among demographically equivalent species (Hubbell 2001). A neutral-based explanation for the coexistence of *A. nerii* and *A. asclepiadis*

seems unlikely; *Aphis nerii* and *A. asclepiadis* are decidedly not equivalent in their demography (Fig. 1), competitive ability (Fig. 4), nor in other traits important to persistence. So called "aggregation models" predict that intraspecific aggregations of individuals across patches reduce the importance of interspecific relative to intraspecific competition, and thus reduce the probability of competitive exclusion (Atkinson and Shorrocks 1981). While aphid distributions are aggregated, these models suggest coexistence is limited to species using ephemeral resource patches (Reader et al. 2006). Long-lived milkweeds are not ephemeral, making it unlikely that the aggregation models are relevant to aphid coexistence on milkweed.

Cardenolide-mediated interactions among herbivores

We did not detect any effects of cardenolides on aphid performance (Fig. 2), nor did we see an influence of aphids on milkweed cardenolide content (Fig. 3). The fact that cardenolides were present in sizable quantities in aphid tissues documents that sap-feeding herbivores do, in fact, encounter and sequester these compounds. Cardenolides have been shown to affect the performance of a diversity of chewing herbivore species on milkweeds (Cohen 1983, Zalucki et al. 2001). In our studies, we increased cardenolide levels by one-third with the application of JA, representing levels of induction comparable to those from herbivory by milkweed's foliage-chewing herbivores (Fig. 3). Other studies have looked at the effects of interspecific variation in milkweed cardenolides on *A. nerii* performance. For example, Martel and Malcolm (2004) found *A. nerii* was insensitive to large differences in levels of cardenolides between *Asclepias incarnata* and *A. curassavica*. In contrast, Agrawal (2004a) found a negative relationship between *A. nerii* fecundity and a 10-fold range in cardenolide content among 18 *Asclepias* species. In addition, where we failed to find induction of cardenolides in *A. syriaca* by even high densities of *A. nerii* and *A. asclepiadis*, Martel and Malcolm (2004) found induction from *A. nerii* on *A. curassavica* but not *A. incarnata*. Aphid performance thus appears to be responsive to the level of variation in cardenolides found among milkweed species. However, milkweed species vary in their inducibility by aphids.

While it is clear that herbivores can interact indirectly via induced plant defenses (Karban and Baldwin 1997, Denno and Kaplan 2007), our results from the milkweed system demonstrate that such dynamics can differ between sap-feeding and tissue-feeding guilds; foliage-chewing herbivores apparently both induce (Fig. 3) and are negatively affected by cardenolides (Cohen 1983, Zalucki et al. 2001), but such dynamics are apparently weaker or absent for aphids. A field study supports this conclusion; Van Zandt and Agrawal (2004a) showed that herbivory by both monarchs *D. plexippus* and leaf beetles *L. clivicollis* did not influence aphid abundance, but had a marginally significant negative effect on a stem-feeding weevil (Curculonidae: *Rhyssomatus lineaticollis*). Further supporting our results, Zehnder and Hunter (in press) found *A. nerii* did not induce cardenolide production in four milkweed species including *A. syriaca*. At least with respect to milkweed

cardenolides, sap-feeders thus appear to be functionally isolated from indirect interactions with other herbivores. This pattern for aphids is in stark contrast to a wealth of data, from both the milkweed system and elsewhere, showing the importance of induced plant defenses in mediating inter- and intraspecific interactions among tissue-feeding herbivores (Van Zandt and Agrawal 2004a, 2004b).

Conclusions

Using the comparative method to study species coexistence is hypothesis-generating, not hypothesis-testing in nature; unmeasured traits and tradeoffs might mediate species coexistence and identifying differences among coexisting species is only a first step. We showed that *A. nerii* is competitively, demographically, and defensively superior to *A. asclepiadis*. If coexistence requires tradeoffs, we suggest that coexistence in these two aphids is based upon tradeoffs in phenology and/or mutualism with ants. ith respect to phenology, our hypothesis makes the prediction that *A. asclepiadis* should be excluded from habitats where *A. nerii* overwinters and precludes the pre-emptive demographic response of *A. asclepiadis* seen at our field sites. With respect to ants, our hypothesis makes the prediction that *A. asclepiadis*, the ant mutualist, should be competitively superior to *A. nerii* in the presence but not absence of ants. The importance of these two factors can be tested either by observing natural patterns of co-variance of the two aphids, or by manipulating these factors via a combination of ant exclusion and early-season introduction of *A. nerii*. It may also be that these two factors work in a complementary fashion to allow *A. asclepiadis* to persist in the face of *A. nerii*'s competitive dominance. Regardless of the precise mechanism promoting their coexistence, our results demonstrate that congeners of radically different competitive ability can coexist on a single resource and that ecological factors other than resource partitioning play an important role in promoting niche partitioning and biodiversity.

References

Agrawal, A. A. 2004a. Plant defense and density dependence in the population growth of herbivores. – Am. Nat. 164: 113–120.

Agrawal, A. A. 2004b. Resistance and susceptibility of milkweed to herbivore attack: consequences of competition, root herbivory, and plant genetic variation. – Ecology 85: 2118–2133.

Agrawal, A. A. 2005. Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. – Evol. Ecol. Res. 7: 651–667.

Atkinson, W. D. and Shorrocks, B. 1981. Competition on a divided and ephemeral resource: a simulation-model. – J. Anim. Ecol. 50: 461–471.

Blackman, R. L. and Eastop, V. F. 2006. Aphids on the world's herbaceous plants and shrubs. – Wiley.

Bristow, C. M. 1991. Are ant-aphid associations a tritrophic interaction: oleander aphids and Argentine ants. – Oecologia 87: 514–521.

Brower, L. P. and Moffitt, C. M. 1974. Palatability dynamics of cardenolides in monarch butterfly. – Nature 249: 280–283.

Chase, J. M. and Leibold, M. A. 2003. Ecological niches: linking classical and contemporary approaches. – Univ. of Chicago Press.

Clough, M. S. et al. 1990. Differential cold hardiness in adults and nymphs of the peach-potato aphid *Myzus persicae*. – Ann. Appl. Biol. 116: 1–9.

Cohen, J. A. 1983. Chemical interactions among milkweed plants (Asclepiadaceae) and lepidopteran herbivores. Thesis, Dept of Biology. – Univ. of Florida.

Denno, R. F. 1980. Ecotope differentiation in a guild of sap-feeding insects on the salt-marsh grass, *Spartina patens*. – Ecology 61: 702–714.

Denno, R. F. and Kaplan, I. 2007. Plant-mediated interactions in herbivorous insects: mechanisms, symmetry, and challenging the paradigms of competition past. – In: Ohgushi, T. et al. (eds), Ecological communities: plant mediation in indirect interaction webs. Cambridge Univ. Press, pp. 19–50.

Denno, R. F. et al. 1995. Interspecific interactions in phytophagous insects: competition reexamined and resurrected. – Annu. Rev. Entomol. 40: 297–331.

Dixon, A. F. G. 1998. Aphid ecology: an optimization approach. – Chapman & Hall.

Futuyma, D. J. and Moreno, G. 1988. The evolution of ecological specialization. – Annu. Rev. Ecol. Syst. 19: 207–233.

Gause, G. F. 1934. The struggle for existence. – Williams and Wilkens.

Groeters, F. R. 1989. Geographic and clonal variation in the milkweed-oleander aphid, *Aphis nerii* (Homoptera, Aphididae), for winged morph production, life-history, and morphology in relation to host plant permanence. – Evol. Ecol. 3: 327–341.

Hajek, A. E. and Dahlsten, D. L. 1986. Coexistence of 3 species of leaf-feeding aphids (Homoptera) on *Betula pendula*. – Oecologia 68: 380–386.

Holmes, E. E. and Wilson, H. B. 1998. Running from trouble: long-distance dispersal and the competitive coexistence of inferior species. – Am. Nat. 151: 578–586.

Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. – Princeton Univ. Press.

Hutchinson, G. E. 1961. The paradox of the plankton. – Am. Nat. 95: 137–145.

Karban, R. 1989. Community organization of *Erigeron glaucus* folivores: effects of competition, predation, and host plant. – Ecology 70: 1028–1039.

Karban, R. and Baldwin, I. T. 1997. Induced responses to herbivory. – Univ. of Chicago Press.

Knight, J. D. et al. 1986. Insect cold hardiness: supercooling points and prefreeze mortality. – Cryo-Letters 7: 194–203.

Lawton, J. H. and Strong, D. R. 1981. Community patterns and competition in folivorous insects. – Am. Nat. 118: 317–338.

MacArthur, R. H. 1972. Geographical ecology; patterns in the distribution of species. – Harper & Row.

Malcolm, S. B. 1986. Aposematism in a soft-bodied insect: a case for kin selection. – Behav. Ecol. Sociobiol. 18: 387–393.

Malcolm, S. B. 1991. Cardenolide-mediated interactions between plants and herbivores. – In: Rosenthal, G. A. and Berenbaum, M. (eds), Herbivores, their interactions with secondary plant metabolites. Academic Press, pp. 251–296.

Malcolm, S. B. and Zalucki, M. P. 1996. Milkweed latex and cardenolide induction may resolve the lethal plant defense paradox. – Entomol. Exp. Appl. 80: 193–196.

Martel, J. W. and Malcolm, S. B. 2004. Density-dependent reduction and induction of milkweed cardenolides by a sucking insect herbivore. – J. Chem. Ecol. 30: 545–561.

McPeek, M. A. 1996. Tradeoffs, food web structure, and the coexistence of habitat specialists and generalists. – Am. Nat. 148: S124–S138.

Mondor, E. B. et al. 2002. Cornicle length in Macrosiphini aphids: a comparison of ecological traits. – Ecol. Entomol. 27: 758–762.

Mooney, K. A. 2006. The disruption of an ant-aphid mutualism increases the effects of birds on pine herbivores. – Ecology 87: 1805–1815.

Mooney, K. A. 2007. Tritrophic effects of birds and ants on a canopy food web, tree growth and phytochemistry. – Ecology 88: 2005–2014.

Mooney, K. A. and Agrawal, A. A. in press. Plant genotype shapes ant-aphid interactions: implications for community structure and indirect plant defense. – Am. Nat. 168.

Nishida, R. 2002. Sequestration of defensive substances from plants by Lepidoptera. – Annu. Rev. Entomol. 47: 57–92.

Omkar and James, B. E. 2004. Influence of prey species on immature survival, development, predation and reproduction of *Coccinella transversalis* Fabricius (Col., Coccinellidae). – J. Appl. Entomol. 128: 150–157.

Rathcke, B. J. 1976. Competition and coexistence within a guild of herbivorous insects. – Ecology 57: 76–87.

Reader, T. et al. 2006. Aggregation, intraguild interactions and the coexistence of competitors on small ephemeral patches. – Oikos 115: 321–333.

Rothschild, M. et al. 1970. Cardiac glycosides in oleander Aphid, *Aphis nerii*. – J. Insect Physiol. 16: 1141–1145.

Schluter, D. 2000. The ecology of adaptive radiation. – Oxford Univ. Press.

Shingleton, A. W. and Foster, W. A. 2001. Behaviour, morphology and the division of labour in two soldier-producing aphids. – Anim. Behav. 62: 671–679.

Sih, A. et al. 1998. Emergent impacts of multiple predators on prey. – Trends Ecol. Evol. 13: 350–355.

Smith, R.A. et al. in press. Coexistence of three specialist aphids on common milkweed *Asclepias syriaca*. – Ecology 89:.

Stadler, B. and Dixon, A. F. G. 2005. Ecology and evolution of aphid-ant interactions. – Annu. Rev. Ecol. Evol. Syst. 36: 345–372.

Strathdee, A. T. et al. 1995. Cold-hardiness of overwintering aphid eggs. – J. Insect Physiol. 41: 653–657.

Takada, H. and Miyazaki, M. 1992. Occurrence of sexuales of *Aphis nerii* B De F (Homoptera, Aphididae) in Japan. – Appl. Entomol. Zool. 27: 117–124.

Thaler, J. S. et al. 1996. Exogenous jasmonates simulate insect wounding in tomato plants (*Lycopersicon esculentum*) in the laboratory and field. – J. Chem. Ecol. 22: 1767–1781.

Tilman, D. 1982. Resource competition and community structure. – Princeton Univ. Press.

Van Zandt, P. A. and Agrawal, A. A. 2004a. Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). – Ecology 85: 2616–2629.

Van Zandt, P. A. and Agrawal, A. A. 2004b. Specificity of induced plant responses to specialist herbivores of the common milkweed, *Asclepias syriaca*. – Oikos 104: 401–409.

Zalucki, M. P. et al. 2001. Detrimental effects of latex and cardiac glycosides on survival and growth of first-instar monarch butterfly larvae *Danaus plexippus* feeding on the sandhill milkweed *Asclepias humistrata*. – Ecol. Entomol. 26: 212–224.

Zar, J. H. 1999. Biostatistical analysis. – Prentice Hall.

Zehnder, C. B. and Hunter, M. D. In press. Interspecific variation within the genus *Asclepias* in response to herbivory by a phloem-feeding insect herbivore. – J. Chem. Ecol. 33: 2044–2053.