

The susceptibility of *Echinacea angustifolia* to a specialist aphid: eco-evolutionary perspective on genotypic variation and demographic consequences

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Summary

1. Plants and their herbivores may influence each other's fitness and, hence, genetic dynamics, as well as their demography. Conversely, variation in fitness-related traits may influence the occurrence or intensity of the interaction. Disentangling the fitness consequences of an interaction in nature from the influence of fitness variation on it is challenging, but important to clarifying the ecological and evolutionary dynamics of plants with their herbivores.

2. As part of a larger effort to elucidate eco-evolutionary dynamics in a population of *Echinacea angustifolia* that is subject to severe fragmentation, we initiated an experiment in 2001 to evaluate differences in fitness among plants of three genotypic classes resulting from matings of plants from different remnant populations ('between', *B*), plants randomly chosen from the same remnant ('within', *W*) and maternal siblings ('inbreds', *I*). The experiment was planted into a field undergoing restoration to a prairie community. Fitness components of individual plants were recorded through 2012. During 2004–2010, each plant was also monitored for its load of *Aphis echinaceae*, a specialist insect herbivore.

3. Within a season, aphid-load depended consistently on a plant's location and on its load the previous season. Further, flowering individuals generally harboured more aphids than non-flowering plants. In analyses of overall plant fitness, within each genotypic class, fitness was greatest for plants with the greatest aphid-loads, consistent with the preference of aphids for flowering individuals. Inbreeding depression was severe, with *I* plants producing 60% fewer achenes than *W* or *B* plants, and varied with aphid-load. To distinguish the role of aphid choice from the effect of aphid herbivory in the relationship between plant fitness and aphid-load, we evaluated how components of fitness varied with prior aphid-load. Notably, genotype *I* plants with high aphid-loads the previous year produced far fewer achenes per flower head than those that carried fewer aphids.

4. Synthesis. Sibmating reduces individuals' demographic contribution by 60% over the first 12 years. Outbred individuals tolerate this aphid; each produces on average about 200 achenes per head in a year, despite a heavy aphid-load the previous year. However, inbreeding, which is greater in severely fragmented prairie habitat, results in poor tolerance. Aphid herbivory exacerbates inbreeding depression, further reducing the contribution of those individuals to population growth. This study illustrates an approach that helps to distinguish fitness-dependent attraction of herbivores from the effects of herbivory on plant fitness and demography, a goal that is critically important to eco-evolutionary understanding.

Key-words: aster modelling, eco-evolutionary dynamics, habitat fragmentation, herbivory, inbreeding depression, plant–herbivore interactions

Introduction

It is well-established that the demography of a population can both influence and be influenced by its genetic composition

(Antonovics 1976). Additionally, genetic differences among individuals can influence their interactions with associated species, potentially affecting dynamics and genetic composition of the population (Levin & Udovic 1977; Antonovics 1992). Despite recognition that such eco-evolutionary feedbacks are prevalent, it remains challenging to disentangle

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them to assess how consistent they are, to evaluate the magnitudes of their effects and to predict their consequences. This is especially true for long-lived perennial plants. In this case, genetic differences among individuals can result in differences in their components of fitness, that is their demographic contribution to population maintenance or growth. These fitness components also generally differ among years and seasons as individuals develop and respond to changing abiotic aspects of the environment. Further, associated organisms, including herbivores, pollinators and pathogens, may differentially attend genotypically differing plants and affect them differently. Moreover, the evolutionary dynamics and growth of those associated species may vary in relation to the genotypic composition of host plants. Accurate assessment of ecological and evolutionary consequences of interplay between species will depend on quantitative evaluation of feedbacks such as these and their effects in realistic conditions. Individual fitness, that is the number of offspring an individual contributes to the next generation, taking into account its survival through reproduction, mediates the interplay between demography and evolutionary change. Accordingly, it is the ideal metric of these effects.

Studies of plants and their herbivores have demonstrated key elements of this interplay, showing that herbivores may respond to genetic differences in the host, including variation in inbreeding. Herbivores may also affect host individuals differentially depending on their inbreeding, as would be expected if inbreeding reduces plant defence, tolerance of herbivory, plant size or fitness. Experimental addition or exclusion of a herbivore is an especially informative approach to conclusively distinguish its attraction to particular genotypes of the plant from its effects on the plant. Such experiments conducted in the controlled conditions of a glasshouse have indicated the potential for these effects in nature and have also shown that inbreeding of the plant host may strongly affect the outcome of the interaction. For example, feeding by spittlebugs on inbred *Mimulus guttatus* plants exacerbated inbreeding depression of both biomass and flower production (Carr & Eubanks 2002). Plant inbreeding also affected spittlebug development, but the effects differed between the two populations studied. In another case, moth larvae consumed more leaf tissue when placed on inbreds of *Vincetoxicum hirsutaria* than on outbred plants (Muola *et al.* 2011); even so, the mass of larvae reared on inbred vs. outcross plants did not differ significantly. While these findings suggest that herbivory may reduce inbreds' fitness more than that of outbreds, it remains unclear whether this is the case and, if so, the magnitude of this effect.

The causes and the demographic consequences of genotype-specific herbivory are likely to depend on environmental context. Accordingly, experiments carried out in realistic field conditions are likely to yield still greater insight into these effects in nature. In field experiments comparing progeny from selfed and outcrossed matings of *Mimulus guttatus* in their responses to experimentally imposed spittlebug herbivory, Ivey, Carr & Eubanks (2004) found that inbreeding depression with respect to biomass was significantly more

severe for plants subject to herbivory compared to protected control plants. Similarly, the disadvantage of inbreds with respect to probabilities of survival and bolting tended to be greater under herbivory, whereas the probability of flowering showed less consistent responses to these treatment combinations. Likewise with *Datura stramonium*, inbreeding increased herbivore damage in the field (Bello-Bedoy & Núñez-Farfán 2011). The effect was modest (4%), exacerbating inbreeding depression by 10% relative to plants partially protected from herbivory by insecticide treatment. More substantial and consistent effects have been found for the largely self-incompatible *Solanum carolinense*. Inbreds attracted generalist herbivores in greater abundance than plants that were not inbred (Kariyat *et al.* 2012). Further, when experimentally damaged by lepidopteran larvae, inbreds attracted fewer predators and parasitoids. Overall, inbreds suffered more severe herbivory and produced well under half as many seeds per ramet (Kariyat *et al.* 2011; see also Campbell, Thaler & Kessler 2013), implying ongoing selection favoring the maintenance of outcrossing. These findings under semi-natural conditions overturned the conclusion reached through a glasshouse experiment, that inbreeding depression was insufficient to maintain the outcrossing mating system (Mena-Alí, Keiser & Stephenson 2008), illustrating the crucial importance of ecological context to evolutionary change.

Notwithstanding the value of field manipulation of herbivory in assessing the evolutionary feedbacks between a plant and its herbivore and their demographic consequences, manipulative experiments are not always feasible. Such experiments are especially challenging for long-lived plants subject to herbivores that vary substantially in their abundance over years, though this has been accomplished for 1–2 year periods (Muller 2013; Mohl 2014). In the absence of experimental manipulation, causation and consequences cannot conclusively be inferred from associations between herbivory and plant attributes. However, assessment of herbivore loads in relation to genetic and phenotypic attributes of hosts, including their fitnesses, can be informative.

Here, we contribute insights from an experiment on a long-lived perennial, *Echinacea angustifolia*, whose once extensive populations in tallgrass prairie are now severely fragmented. Using data on each pedigreed plant in the study population, together with an 8-year record of infestation by a specialist aphid, *Aphis echinaceae*, we compare the aphid loads of three genotypic classes of progeny, across a range of inbreeding levels likely to occur in fragmented habitat. Moreover, we take advantage of detailed records of fitness components to assess the impact of the aphid on the fitness of plants and therefore on their demographic and genetic contributions to the next generation. We thus illustrate how an evolutionary change in populations of *E. angustifolia* resulting from habitat destruction feeds back to affect the populations' demography, with implications for their further evolutionary change. We also demonstrate a key empirical challenge for eco-evo research: disentangling effects of an herbivore on plant fitness from fitness-dependent attraction of herbivores – a particularly acute challenge in a long-lived plant host. We

illustrate how aster modelling can be used to distinguish these effects.

Materials and methods

STUDY SYSTEM

Echinacea angustifolia (narrow-leaved purple coneflower, hereafter *Echinacea*) is an herbaceous perennial native to the tallgrass prairie and plains of North America. Its characteristics typify much of the prairie flora. Plants are long-lived, rarely flowering before their fourth year and not necessarily every year thereafter (Wagenius *et al.* 2012). Spring burns promote summer flowering. Plants tend to produce a single inflorescence (head), though sometimes more, which are pollinated by native generalist bees (de Nettancourt 1977; Wagenius & Lyon 2010). *Echinacea*'s strict sporophytic self-incompatibility bars inbreeding via self-pollination. Pollen dispersal distances are generally short (Ison & Wagenius 2014; Ison *et al.* 2014). Thus, fertilization declines with isolation of individuals (Wagenius 2004, 2006). *Echinacea* does not reproduce or spread vegetatively; each new plant arises solely from seed. Consequently, not only its evolutionary dynamics but also its demography depend closely on seed production. The seeds are borne in uniovulate fruits (achenes), typically 100–200 per head. Achenes are dense and have no specialized dispersal mechanism and do not form a persistent seed bank.

Echinacea is common in undisturbed prairie west of the Mississippi River, ranging from Texas to Canada. Compared to its extent at the time of European settlement, tallgrass prairie is now severely reduced to small remnants that are sparsely scattered. In our 6400-ha study area located in rural western Minnesota (centered near 45° 49' N, 95° 42.5' W), the entire landscape, apart from lakes and wetlands, offered suitable habitat for a continuously distributed population of this plant prior to European settlement, which began about 1870. Now, 45 remnants of the population persist. The largest is on a 45-ha virgin prairie preserve owned and managed by The Nature Conservancy with 1000–6000 flowering plants per year. The smallest remnants have 0–4 flowering plants per year (Wagenius 2006). We have demonstrated genetic differences among these remnant populations with respect to intrapopulation mating compatibility (Wagenius, Lonsdorf & Neuhauser 2007) and fitness expressed in common conditions (Geyer, Wagenius & Shaw 2007).

In our study area, we have observed many herbivorous insects on *Echinacea*, but an aphid specializing on it is the most common. These aphids, *Aphis echinaceae* Lagos (Lagos & Voegtlin 2009), including winged migrants, appear on *Echinacea* leaves in the spring, as well as on stems and heads of flowering plants, reaching densities exceeding 100 individuals on many plants in mid- to late-summer. Aphids transferred to other species of Asteraceae native to Minnesota do not survive (L. Hobbs and H. Lyons, unpubl. data). Aphids did survive, though not to reproduction, when transferred to *Echinacea pallida*, a non-native species (C. Shorb, unpubl. data). Thus, this aphid appears to depend entirely on *E. angustifolia*. The aphids are tended by several species of ant, including *Formica obscuripes* and *Lasius alienus*. Ants frequently build structures of thatch and soil on the undersides of leaves that harbour aphids. We rarely observed predators of aphids.

EXPERIMENTAL DESIGN

The present study is based on an experiment initiated in 1999. We have previously reported details of its design and results (Wagenius

et al. 2010; Ridley *et al.* 2011). Briefly, this experiment was designed to compare fitness and other attributes of *Echinacea* plants differing in their degree of inbreeding, ranging from plants derived from mating between plants from different prairie remnants less than 8 km apart (between remnant, *B*), random mating between plants from the same prairie remnant (within remnant, *W*) and sib-mating (inbred, *I*). Whereas *W* matings seem most likely to represent matings before fragmentation became severe, the population genetic structure of small remnant populations now exhibits patterns of substantial relatedness between near neighbours (Wagenius 2000). Consequently, although self-incompatibility prevents seed production via selfing, local foraging of pollinators can often result in biparental inbreeding, such as mating between siblings or between parent and offspring. Between-remnant crossing may also have increased in frequency as pollinators travel farther in search of suitable plants. Using paternity assignment, Ison *et al.* (2014) documented pollen movement between two small *Echinacea* populations separated by 247 m. Thus, all three genotypic classes are now expected to be prevalent in our fragmented prairie landscape.

Parental plants originated as seed collected in 1995 from remnant prairies in our study area, all within 5 km of an experimental field and were planted into it in 1996. This field had previously been in agricultural production but has been undergoing restoration to a prairie community and has been burned in 2002, 2004, 2006, 2008, 2010 and 2013. It is now dominated by native, warm-season prairie grasses but also contains many plant species introduced from Europe.

Hand pollinations to produce seeds in the three genotypic classes were done in 1999 and 2000. We germinated progeny seed in 2001 obtaining 323, 95 and 139 germinants in the between, within and inbred genotypic classes, respectively ($n = 557$). In May 2001, we planted 508 seedlings into an experimental plot adjacent to the one in which parental plants were growing, each seedling at a randomly assigned location with 50 cm spacing between seedlings in four rows 1 m apart.

ANNUAL MEASUREMENTS

With the goal of evaluating the relationship between plant fitness and aphid-load, as well as temporal variation in the relationship and spatial influences on it, we made the following observations on individual plants in the experimental population. In July or August of each year starting in 2004, we assessed the primary components of fitness: survival, flowering status and the number of heads each plant produced. All measurements were taken blind in the sense that measurers did not know any plant attributes except location. We also assessed aphid abundance by inspecting foliage and recording the abundance of aphids in five categories: 0, 1, 2–10, 11–80, > 80 individuals. We used the same categories to record aphid-load separately for each flowering stem. For the analysis, we obtained a measure of aphid-load for each plant in a given year by aggregating the information from parts of plants. Thus, for the analysis, aphid-load had four levels: none – no aphids on any part of the plant, low – at least one but no more than ten aphids on any part of the plant, medium – 11–80 aphids on the basal leaves or on at least one of the flowering stems, or high – over 80 aphids on basal leaves or at least one flowering stem. Later each year, all seed heads were removed from plants as they matured but before dispersing achenes (mid-August through September). We removed all achenes from each receptacle and scanned them with a flatbed scanner to count achenes. Achene count per plant per year closely represents each plant's annual ovule production. Fertilization of achenes depends on receipt of compatible pollen. Seed

set, the proportion of achenes fertilized, varies dramatically in nature, in relation to the size of the population fragment (Wagenius 2004). In this experimental field, median seed set was 40%, 60% and 50% in 2005, 2006 and 2007, respectively (Ison & Wagenius 2014). Plants that produced no achenes in a year were scored as not flowering that year. Using plant fitness data recorded 2001–2008, Wagenius *et al.* (2010) reported differences in fitness dependent on genotypic class. Ridley *et al.* (2011) reported on differential infestation by aphids and ants, as well as relationships between elemental composition of plants and their infestation in 2005. Kittelson *et al.* (2015) reported differences among the genotypic classes in physiological attributes.

STATISTICAL ANALYSIS

Aphid-load

To investigate how aphid-load, considered as an ordered categorical response, varies spatially and in relation to attributes of individual plants, including their flowering status and prior aphid-load, we used proportional odds logistic regression (*polr*) in the MASS package of R (Venables & Ripley 2002; R Core Team 2014; Ripley *et al.* 2014). Models initially included five predictors: genotypic class, flowering status, aphid-load in the previous year, row (E-W coordinate treated as a factor) and position (N-S coordinate treated as continuous). All of their two-way interactions were also included. We included row and position of each individual in the planting design to account for spatial heterogeneity within the plot. Flowering status could influence aphid-loads either because individuals in flower are more apparent or because aphids prefer them. We used aphid-load in the previous year as a predictor to evaluate how consistently plants are infested over successive years, as expected if the dispersal of aphids is highly restricted. Aphids start infesting flowering stems in early summer after the development of the heads has established all florets (Wagenius 2004). Therefore, aphid infestation cannot influence achene count for that year; however, we hypothesize that herbivory by aphids affects subsequent components of fitness, for example overwinter survival and components of reproduction the following year. We did not analyse aphid-load data from 2004 to 2011, because we had no previous load information for 2004, and in 2011 loads were so low there was insufficient variation to conduct analyses. Aphid-loads during 2005–2010 were modelled separately for each year, because a single model

including year as a factor did not have comparable factor-level combinations among years; each year had distinct combinations of prior abundance and flowering rates, together with current aphid abundance, resulting in numerous instances of 0 or 1 fitted probabilities. We used likelihood ratio tests via backwards elimination to identify minimal adequate models for each year (Crawley 2005). In all years, aphid-loads showed no significant relationship ($p > 0.05$) with the following two-way interactions: genotypic class with each other factor, previous load with each other factor except position, and flowering status with each other factor except row. Accordingly, these interactions were eliminated from further consideration. For each year, we obtained maximum likelihood estimates of expected aphid-loads for plants with all combinations of attributes included in the model. These estimates accounted for covariates by reporting values for a typical hypothetical individual in a middle row and at a median position.

We conducted a generalized linear model analysis with incidence of at least one aphid as a binomial response and year (2005–2009) as a factor, along with the factors in the *polr* analysis above, to investigate relationships between aphid-load and plant attributes in a single model.

Plant fitness

To evaluate the relationship between fitness and the predictors, genotypic class and aphid-load, we analyzed the components of fitness using aster models (Geyer, Wagenius & Shaw 2007; Shaw *et al.* 2008; www.stat.umn.edu/geyer/aster/), which are implemented in the aster package of R (Geyer 2014; R Core Team 2014). Aster models employ a suitable probability distribution for each fitness component, as well as a graphical model that specifies the dependence of each component on an earlier component, to yield a comprehensive assessment of overall fitness in relation to predictors of interest. For this study, the graphical model includes, for each individual at the start of the period under consideration, the fitness components observed in each year: its survival, whether it flowered, the number of heads and the number of achenes (Fig. 1); the first two of these are modelled as Bernoulli variables, and the last two as zero-truncated Poisson conditional on the immediate predecessor in the graphical model.

Aster modelling can be used to make inferences about unconditional fitness from its underlying components, that is survival of an

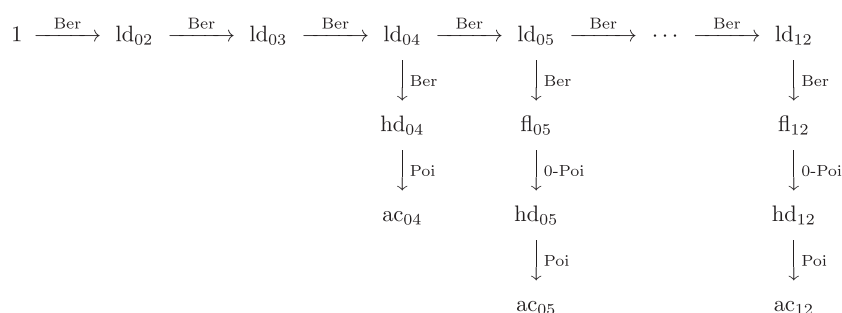


Fig. 1. Aster graph for the first analysis. Nodes of the graph correspond to response variables (components of fitness), arrows to conditional distributions. Subscripts indicate year, 02 for 2002, etc. Variables named *ld* are survival (zero for dead, one for alive); those named *fl* are flowering indicators (zero for no flower heads, one for one or more flower heads); those named *hd* are flower head counts; those named *ac* are achene counts. The node marked 1 indicates the constant 1 so the graph describes data for one individual (all individuals have the same graph). *Ber* indicates a conditional Bernoulli distribution; *Poi* indicates a conditional Poisson distribution, 0-*Poi* indicates a conditional zero-truncated Poisson distribution. The ellipsis indicates that every year between 2005 and 2012 has variables and arrows that look just like years 2005 and 2012. The combination of a Bernoulli arrow followed by a zero-truncated Poisson arrow is a zero-inflated Poisson distribution (the conditional distribution of *hd*₀₅ given *ld*₀₅ = 1 is zero-inflated Poisson). The *fl*₀₄ variable is omitted because in 2004 every individual had at most one flower head, so *hd*₀₄ is zero-or-one valued and hence must be conditionally Bernoulli given *ld*₀₄ = 1.

individual from juvenile stages throughout the life span, as well as all progeny produced from each reproductive bout. Unconditional models are generally recommended for aster analysis because they provide a multivariate monotonic relationship between unconditional mean value parameter estimates and predictors (see appendix of Shaw & Geyer 2010). That is, unconditional aster models estimate the direct relationship between the expected value of the chosen measure of overall fitness and the predictors in the model. One key aspect of unconditional aster models is that predictors are not modelled as affecting components of fitness singly. Rather, predictors are modelled as influencing the designated measure of overall fitness, including the effects on it of all the components that precede it in the graphical model.

Our operational measure of fitness through 2012 is the expected total number of achenes for a plant from its initial planting as a sprout; thus, this measure takes into account not only the number of achenes produced by plants that flowered, but also the probability of not producing achenes, whether because the plant did not flower or because it had died. Using this approach, we first present an aster analysis comparing the genotypic classes with respect to their mean fitness through the 12th year, including as a covariate the plant's position within the row. This analysis updates the fitness comparisons of Wagenius *et al.* (2010) with five additional years of data.

This unconditional aster analysis cannot validly be used to assess the effects of aphids on plant fitness because those effects are confounded with the effect of a plant's fitness on its aphid-load, whether via its attractiveness to aphids or its effect on proliferation of aphids once they arrive. The dependence of aphid-load on plant size and flowering status is clear in the results of the *polr* analysis below. Moreover, unconditional aster analysis is not appropriate when considering a predictor that varies over years, as aphid-load does, because of its aforementioned property of modelling the designated measure of fitness taking all preceding fitness components into account; conditional aster accommodates time-varying predictors, while also enabling inferences about components of fitness. Therefore, to disentangle the fitness consequences of aphid-load from the dependence of aphid-loads on plant fitness while accounting for differences in individuals' aphid-load over years, we carried out a second aster analysis.

When considering a time-dependent predictor such as aphid-load, it is appropriate to assess its influence on the individual components of fitness, that is, to obtain estimates of dependence on the predictor for each component of fitness, conditional on reaching the immediately previous stage of the life history (Fig. 1). For this analysis, we focus on the subset of plants surviving in 2004, when the annual record of aphid-load began, to evaluate the relationship between the fitness components of those plants and their aphid-loads. This second aster analysis also included position, row and genotypic class as predictors. Because aphid-loads on a given plant were similar from 1 year to the next, models including effects of both current and previous year aphid-loads on achene number would have suffered from collinearity. This conditional analysis enabled estimation of expected values of unconditional fitness for plants in each genotypic class and subject to each aphid-load across years (Fig. 3) in addition to expected values for each of the components of fitness for plants in every combination of genotypic class and aphid-load (Fig. 4).

To summarize, our first aster analysis of the full data set, yields estimates of unconditional mean value parameters for fitness (expected achene count) of plants through their 12th year, while our second aster analysis, of plants remaining alive in 2004, evaluates the relationship of fitness to aphid-load through conditional aster analysis.

This analysis yields estimates of both unconditional expected fitness and estimates of components of fitness, conditional on reaching the immediately previous stage in the graphical model (Fig. 1), in relation to aphid-load.

Results

APHID-LOAD

Overall levels of aphid-load varied substantially during 2004–2010, but a plant's flowering status, load the previous year, and location within the experimental plot consistently predicted aphid-load (Fig. 2 and see Table S1 and Figure S1 in Supporting Information).

Flowering plants had greater aphid-loads than non-flowering plants ($P < 0.05$) in every year except in 2007, when aphid-loads did not differ significantly ($P > 0.05$), and in 2005 when non-flowering plants were more likely than flowering plants to be infested at every level ($P < 0.05$). The probability of the highest load was usually 2–5 times greater in flowering compared to basal plants within a year. In 2005, a year with few aphids and few flowering plants, a similarly large difference existed, but in the opposite direction (2–4% in flowering plants, 6–12% in non-flowering).

In each year, a plant's load the previous year predicted load, though not significantly so in 2010. Generally higher load in 1 year followed high load in the previous year. The spatial distribution of aphids was also non-random. In every year, aphid-load varied within the experimental plot; either a plant's row or position was a significant predictor in the proportional odds logistic regression analysis, and in most years both were.

A plant's genotypic class was associated with aphid-load only in 2009 ($P = 0.015$), when *I* had the lowest proportions of plants carrying aphids for all aphid-load categories compared to *W* and *B* plants, which had similar loads. This same trend was observed in 2004 ($P = 0.06$).

The generalized linear model analysis with incidence of at least one aphid as a binomial response and year as a factor provided no evidence ($P = 0.16$) that genotypic classes differed in whether or not an individual plant had any aphids (data not shown). The results corroborated those of the *polr* analysis demonstrating associations of aphid occurrence with plant flowering status, location and previous load, as evidenced by a main effect of previous year's aphid-load ($P < 0.0001$) and significant interaction terms of year with row ($P = 0.002$), position ($P = 0.008$) and flowering status ($P < 0.0001$).

RELATING FITNESS TO GENOTYPIC CLASSES

After 12 years, fitness, quantified by expected achene count per seedling planted at the outset of the experiment, estimated with the unconditional aster model, approximately doubled compared to total fitness in these plants after 7 years (Wagenius *et al.* 2010). Although absolute fitness increased between years 7 and 12, the relative fitnesses among the three geno-

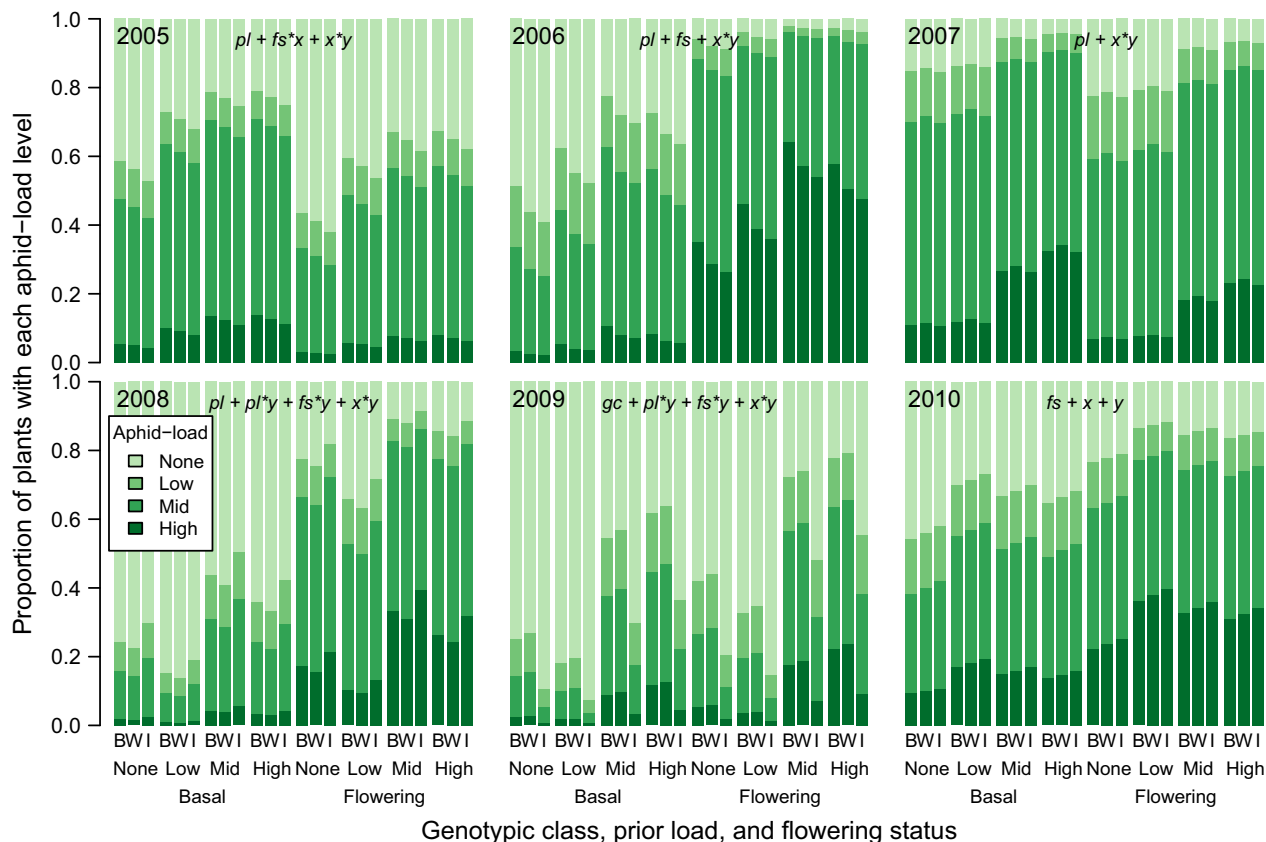


Fig. 2. Expected proportion of plants in each category of aphid-load during 2005–2010 taking into account all combinations of the attributes genotypic class (*gc* in three categories *B*, *W* and *I*), aphid-load the previous year (*pl*, in 4 categories, none, low, mid, high) and flowering status (*fl* yes or no). Aphid-load categories are based on largest count of aphids present on any above-ground plant part: foliage, a stem or a head. To facilitate comparison among years, all maximum likelihood estimates result from a *polr* model with the above main effects, the spatial coordinates of the plants within the plot, *x* and *y*, and the following two-way interaction terms: *pl***y*, *fs***x*, *fs***y* and *x***y*. Formulae of minimal adequate models, selected via backward elimination, for each year are shown in each panel (see Table S1 in Supporting Information). In 2005 living plants included progeny from crosses between remnants “*B*” (*N* = 249), within remnants “*W*” (*N* = 63), and between sibs “*I*” (*N* = 82). In 2010, sample sizes were 174, 42 and 51, respectively.

typic classes remained similar, with inbreds (*I*) about 60% lower than the progeny of within-remnant crosses and 55% lower than those of between-remnant crosses (mean achene counts (se): *B*, 178 (17); *W*, 197 (28); *I*, 80 (12)). As after 7 years, the mean fitness of *B* plants was about 10% lower than that of *W* plants.

RELATING PLANT FITNESS TO APHID-LOAD

Fitness, quantified as the expected number of total achenes produced during 2004–2012 per individual alive in 2004, ranged widely among the combinations of genotypic class and aphid-load in the previous year, from 44 to 560 (Fig. 3). Both factors and their interaction were highly significant ($P < 0.001$). In general, within a given genotypic class, greater aphid-loads had larger estimates of unconditional fitness. This is consistent with the above finding that aphids were more prevalent on plants that were flowering and also reflects that aphids cannot occur on plants that died previously. Inbreeding depression, the reduction in fitness of *I* plants relative to *W* plants, was apparent even for the lowest

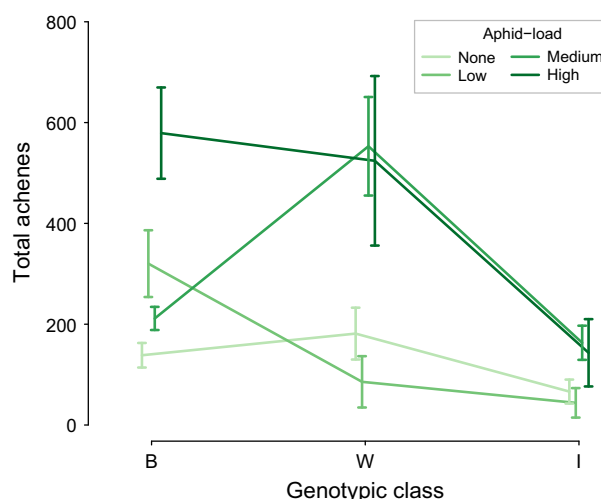


Fig. 3. Expected overall fitness (\pm SE), quantified as sum of achene counts over years as the measure of fitness, during 2004–2012 per individual alive in 2004 in relation to genotypic class and aphid-load in the previous year. Estimates were obtained from an aster analysis based on the graphical model in Fig. 1, thus accounting for mortality and failure to flower.

aphid-loads (no aphids: 0.63, low aphid-load: 0.49) but greater for the highest two aphid-loads, reaching 0.7 (medium: 0.7, high: 0.73). Whereas non-inbreds had fitnesses exceeding 500 achenes per plant, despite high aphid-loads, the inbreds produced fewer than 200 achenes, regardless of aphid-load. The expression of fitness of between-remnant crosses was complex. Heterosis, that is higher fitness in *B* relative to *W* plants, was apparent at the lowest aphid-load, though not for plants lacking aphids altogether. At higher aphid-loads, there appeared to be outbreeding depression, though not at the highest aphid-loads.

In these comparisons, the effects of aphids on plant fitness are fully confounded with the effects of plant fitness both on aphids' choice of host individual and on aphid reproduction, as noted above. In an effort to isolate the effects of aphids on plant fitness, we further considered the estimates of the components of fitness conditional on the plant reaching the state that immediately precedes that component in the graphical model for fitness (Fig. 1). This focused perspective reveals how relationships between fitness components and aphid-load in the previous year differed among genotypic classes (Fig. 4). The probability of survival was high in most years, and relatively insensitive to aphid-load and to genotypic class. Probabilities for both survival and flowering tended to be higher for plants infested in the previous year with greater numbers of aphids, again consistent with the tendency of aphids to choose flowering individuals, to proliferate more on them, or both. However, for the number of flower heads given that a plant flowered, this effect was less apparent, and for the number of achenes per head, it was absent. For achene number, conditional on flowering, inbreeding depression was most severe at the highest aphid-load (0.42), whereas inbreeding depression was weaker for plants lacking aphids in the previous year (0.04). Outbreeding depression, seen as striking reductions in achene counts for *B* plants relative to *W* plants, was also evident at the two highest aphid-loads.

Discussion

Herbivores in nature are notoriously variable in their abundance, and *Aphis echinaceae* is no exception, as our 9-year sequence of aphid observations in this experiment shows (Fig. 1). Despite this variability, we have detected clear differences among plants in their susceptibility to aphid feeding. The non-random distribution of aphids among the plants complicates evaluation of the effects of aphids on plant fitness. The expected unconditional fitness, here defined as the number of achenes a plant produced throughout the period 2004–2012, accounting for mortality, is greatest for plants with the highest aphid-loads (Fig. 3). In isolation, this relationship suggests the possibility that aphids enhance plant fitness, but an equally valid interpretation, supported by the proportional odds logistic regression (*polr*) analysis, is that aphids are attracted to flowering plants and proliferate more rapidly on them. Statistical control for aphid choice and growth is obtained through estimation of fitness components, conditional on the previous component, for plants with different

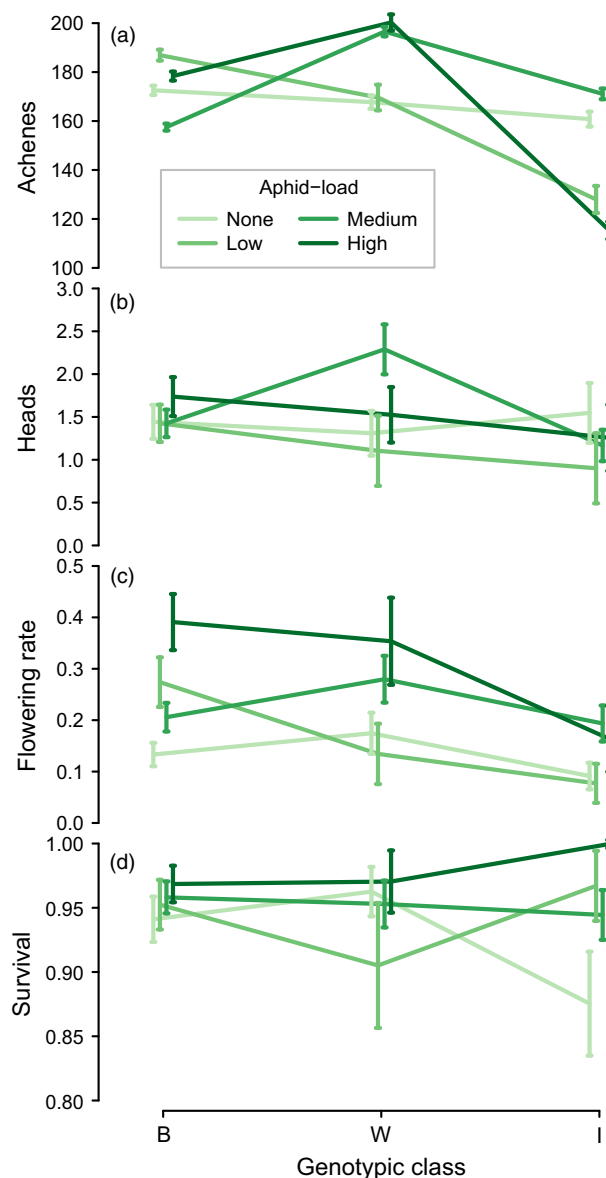


Fig. 4. Expected values (\pm SE) for four components of fitness of plants alive in 2004 in relation to genotypic class and aphid-load in the previous year: (a) number of achenes produced per flowering head, (b) number of heads per flowering plant, (c) probability of flowering per living plant and (d) probability of surviving from previous year. These estimates were obtained from the same aster analysis that yielded the results shown in Fig. 3, but these are estimates for each life-history state, conditional on reaching the previous state, as shown in the graphical model in Fig. 1. As a representative example of the years 2004–2012, the values displayed are for 2009.

aphid-loads in the previous year. Thus, conditional aster modelling is a valuable tool for disentangling eco–evo causes and consequences. The conditional estimates give a more nuanced view of the interdependence of aphid feeding and plant fitness, implying that the genotypic classes differ in their tolerance of high aphid-loads, such that high aphid-loads exacerbate inbreeding depression of *Echinacea*, impairing the contribution of these individuals to both growth and genetic composition of the population. Below, we consider each of

these findings more fully and underscore their import for understanding of eco-evolutionary dynamics.

The results of our analyses of aphid presence on plants imply that plants in flower are more attractive to aphids than non-reproductive plants. Beyond this, flowering may promote reproduction in aphids contributing to the observed variation in aphid-loads late in the season in each of 8 years. Aphid-load in 1 year also consistently predicts infestation by aphids in the following year (Fig. 2). This finding could indicate that aphids induce greater susceptibility to future aphid infestation. Alternatively, it may simply reflect that movement of aphids tends to be extremely limited, even between years.

Aphid-loads show no steady trend in relation to genotypic class, though in 2009, they were significantly lower on *I* genotypes and in 2004, nearly so. These cases are consistent with the general finding of lower loads on smaller, basal individuals, given that inbreds tend to be smaller and are less likely to flower (Shaw *et al.* 2008; Wagenius *et al.* 2010), two plant attributes that consistently predict lower aphid-loads (Ridley *et al.* 2011, this study). In 2005, however, when aphid-loads were assessed in June, aphids were twice as likely to be on *I* and *B* plants and in greater numbers, compared to *W* plants (Ridley *et al.* 2011). Moreover, aphid-loads early in the season were positively associated with foliar nitrogen and also with plant size, and they were negatively associated with foliar concentration of phosphorus, which tended to be greater in inbred plants (Ridley *et al.* 2011). These latter patterns remained apparent in August of 2005, whereas by then, when 72% of plants harboured aphids, aphid-loads did not differ significantly among the genotypic classes (Fig. 2, first panel). It may be that that year's early disparities in aphid-loads reflect preference of aphids for *I* and *B* plants as well as more rapid growth on them; whereas, by late that season, aphid numbers had grown sufficiently for spread to plants without regard to aphid preference among the plant genotypes and to reach similar loads across the genotypic classes. More recently, Kittelson *et al.* (2015) found numerous physiological differences among plants attributable to genotypic class, including lower photosynthetic rates, water use efficiencies, and specific leaf areas, as well as higher trichome numbers, % C and % N of inbreds compared to outbred individuals. The abundance of aphids in the year of that study was too low to detect associations with these plant attributes.

Through frequent monitoring in 2 years (biweekly in 2011, monthly in 2012), Muller (2013) found that aphid-loads peaked in each year over a narrow interval of time. We note that our longer term records of aphid-loads were taken over a period of just a few days late in the summer of each year and are therefore expected to reflect well the inter-individual differences in aphid-load at the time of assessment. However, these observations likely did not coincide with the peak each year; this could obscure relationships between plant attributes and aphid-loads. We also note that our categories of aphid-loads, chosen for feasibility of assessment, are coarse and may therefore limit statistical power. These issues likely undermine detection of a relationship between plant genotypic class and aphid-load.

Evaluating the consequences of herbivory for fitness of a perennial plant is challenging. Experimental manipulation of aphid-loads would most informatively disrupt the confounding between the effect of plant fitness on host choice and the response of plant fitness to herbivory, which may be expressed in subsequent years. In a nearby planting of *Echinacea*, Muller (2013) conducted aphid additions and removals on the same individuals in two successive years and assessed plant responses, finding that leaves of plants in the addition treatment senesced significantly more rapidly in the second year but also were subject to less foliar herbivory in the first year, possibly due to protection by ants, which are positively associated with aphids (Ridley *et al.* 2011). It remains to be determined how these effects of aphids on the plants would balance if the treatments were sustained over more years. The experimental population used in Muller's study did not include plants of differing inbreeding levels and so does not bear on the question of differences in tolerance among the genotypic classes considered here.

The aster analysis yielding conditional estimates of fitness components illustrates how it can help distinguish effects of aphids on plant fitness from differential attraction of aphids. Over the 8 years during which aphid-loads were observed aster detected a highly significant dependence of the components on the interaction between genotypic class and aphid-load in the previous year. This analysis cannot fully address the confounding of effects of plant fitness on aphid choice with response of plant fitness to aphid herbivory; for example, the generally higher probability of survival and flowering of plants with the greatest aphid-loads may simply reflect the preference of aphids for larger, more robust plants and their greater proliferation on them, together with the greater probability of survival of those plants. The drastically reduced number of achenes per head for *I* genotypes with the greatest aphid-loads, compared to inbreds bearing fewer aphids, is not consistent with this effect, however. It suggests that inbreds tolerate high aphid-loads poorly, relative to *W* genotypes that sustain high and moderate aphid-loads and tolerate them sufficiently well to produce nearly 200 achenes per head in the year following severe infestation. The tolerance of *B* genotypes of high aphid-loads appears to be intermediate. Thus, regardless of the extent to which the genotypic classes are differentially susceptible to herbivory, differences in their tolerance of high aphid-loads appear to result in differences in fitness among the plant genotypes.

Our finding that aphids most severely reduce achene output of inbreds represents an evolutionary demographic impact of the aphids on *Echinacea* populations, reducing inbreds' contributions of offspring to the next generation. In today's smallest prairie fragments, few *Echinacea* individuals remain and are likely to be closely related. In this context, pollen transfer often fails to produce seeds, due to the incompatibility system (Wagenius 2006; Wagenius & Lyon 2010). When seeds do result, the case we have focused on here, new recruits are highly likely to be inbred and to express inbreeding depression with respect to fitness, with reduction in survival and reproduction, further impairing the maintenance and

growth of the resident populations. Infestation of inbreds by aphids drives their achene production still lower and thereby further reduces population growth rate – or hastens population decline, increasing the chance of extirpation. This expectation contrasts sharply with that for the large populations prior to fragmentation, which primarily consisted of offspring of matings between less closely related plants. Even with typical germination proportions as low as 1–5% (Wagenius *et al.* 2012), our finding that flowering *W* individuals produced almost 200 achenes per head in a year following severe aphid infestation the previous year implies that these historic populations maintained themselves or grew, despite feeding by this aphid. To the extent that inbreds did infrequently arise in these historically predominantly outcrossing populations, the primary effect of aphid infestation to reduce inbreds' achene output would have enriched those populations with progeny of outbred individuals. However, for remnant populations increasing in the frequency and degree of inbreeding, our finding that achene production of inbreds following severe aphid infestation was reduced to about 120 achenes per head (Fig. 4) suggests that aphids, in conjunction with inbreeding, may compromise the growth or maintenance of these remnant populations.

More generally considering the bearing of this and related studies of feedbacks between evolution and ecology of populations of long-lived *Echinacea angustifolia*, we recall that fragmentation of the prairie and conversion of land to agriculture has altered mating patterns, increasing biparental inbreeding and also, perhaps to a lesser extent, crossing at large distances. The fitness decrement of inbreds, in terms of achene production through 12 years, of approximately 60% is extreme; if extrapolated to the case of selfing, as inbreeding depression is usually evaluated, this corresponds to inbreeding depression of at least 90%, exceeding levels found in most studies (reviewed in Wagenius *et al.* 2010). Our estimate of overall fitness of inbreds represents severely limited capacity of these individuals to contribute to population persistence and growth. Inbreeding depression, even for plants lacking aphids altogether, is extremely severe (i.e. 0.63), but it is even more so for plants with high aphid-loads (0.73). A tendency towards greater susceptibility of inbreds to aphid infestation early in the season, as found in Ridley *et al.* (2011), would likely compound their detrimental effect on these plants.

The dependence of plant fitness on mating patterns, the role of aphids in mediating it, and the consequences for individuals' demographic and genetic contributions to remnant populations of *Echinacea* can be expected to vary substantially among prairie fragments in the study area, not only because they range widely in the number, sparseness, and relatedness of *Echinacea* plants flowering in them, but also because the abundance of *Aphis echinaceae* has been found to vary dramatically among them (Muller 2013). Even so, the general implication of the eco-evolutionary feedbacks documented here is that small populations are at risk of extirpation through the combined effects of inbreeding and herbivory.

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Data accessibility

Shaw, R., Wagenius, S. & Geyer, C. Data from: The susceptibility of *Echinacea angustifolia* to a specialist aphid: eco-evolutionary perspective on genotypic variation and demographic consequences. *Journal of Ecology*, <http://dx.doi.org/10.5061/dryad.3s85r>

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. ANOVA tables comparing models of aphid-load 2005 – 2010 using stepwise model simplification via backward elimination.

Figure S1. Expected aphid-loads based on minimal adequate models 2005 – 2010.