

**INBREEDING DEPRESSION AND PARTITIONING OF GENETIC LOAD
IN THE INVASIVE BIENNIAL *ALLIARIA PETIOLATA* (BRASSICACEAE)¹**ALICIA A. MULLARKEY², DIANE L. BYERS, AND ROGER C. ANDERSON³

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- *Premise of the study:* Invasive species are nonnative species that enter novel environments, establish sustained populations, and can negatively impact native species. Here we assess a potential weakness of invasive species (genetic load) and show how species might overcome genetic barriers. Colonization of novel habitats by invasive species typically involves few individuals, exposing populations to founder effects. We empirically tested a central Illinois population of an invasive biennial plant, *Alliaria petiolata*, for evidence of a founder effect by assessing the pattern of genetic load.
- *Methods:* To estimate genetic load, we assayed offspring from three cross types (self-pollinated, outcrossed within- and between-populations) in a greenhouse. Vegetative and reproductive traits were measured on first-year plants grown with or without intraspecific competition.
- *Key results:* We found substantial genetic load in this population of *A. petiolata*, which can mostly be attributed to genetic drift (founder effect) and not inbreeding depression. Between-population heterosis was expressed more than inbreeding depression under intraspecific competition.
- *Conclusions:* Inbreeding may be adaptive for *A. petiolata* in its introduced range by providing reproductive assurance, with limited inbreeding load. Nevertheless, most of the genetic load in this population of *A. petiolata* is due to fixation of deleterious alleles. Drift load is expected, given that this population is near the edge of its continuous geographic range in highly fragmented habitats, and gene flow between isolated populations is likely highly limited. Preventing additional introduction and movement of propagules between isolated local populations should reduce heterosis and *A. petiolata* competitiveness.

Key words: *Alliaria petiolata*; Brassicaceae; founder effect; genetic load; drift load; inbreeding load; invasive species.

Invasive plant species can threaten the integrity of ecosystems they invade by altering ecosystem processes, causing changes in native plant community composition and structure, and by reducing overall diversity of native species (Mack et al., 2000). However, a genetic paradox exists with respect to invasive species because of their potential to experience genetic limitations (Kolbe et al., 2004; Frankham, 2005; Novak and Mack, 2005; Perez et al., 2006; Facon et al., 2011). Colonization of novel habitats by invasive species typically involves a small number of individuals. These small establishing populations are exposed to founder effects, which include genetic bottlenecks, genetic drift, and inbreeding (Sakai et al., 2001; Novak and Mack, 2005; Dlugosch and Parker, 2008) and subsequent reductions in genetic variation and evolutionary potential (Nei et al., 1975;

Genton et al., 2005). Assessing potential genetic weaknesses of invasive species could have implications for the management of these species.

The mating system of an invasive plant species can play an important role in determining genetic effects of colonization and directly influences the capacity for founding individuals to successfully reproduce (Baker, 1955; Bazzaz, 1986; Daehler, 1999; Novak and Mack, 2005). A preadaptation for reproductive assurance through self-fertilization can increase the likelihood of success for invasive plant species entering novel habitats where there may be few conspecifics and pollinators are low in abundance or unreliable (Baker, 1955; Daehler, 1999; Cano et al., 2008). Nevertheless, inbreeding can lead to fitness reductions due to inbreeding depression (Darwin, 1889; Baker, 1955; Charlesworth and Charlesworth, 1987; Waller, 1993), which has been attributed to increased frequency of homozygous individuals, causing increased expression of hidden deleterious recessive alleles (genetic load) in inbred populations (Charlesworth and Charlesworth, 1987; Waller, 1993). Inbreeding depression may initially limit spread of invasive plant populations and lower the probability that populations becoming established will persist (Daehler, 1999).

However, theoretical models predict that inbreeding depression decreases with increased selfing, because selection will cause highly recessive alleles of large effect to be purged from the population (Lande and Schemske, 1985; Charlesworth et al., 1990). Thus, inbreeding depression may be reduced when invasive plant populations undergo genetic bottlenecks, by increasing the rate of inbreeding and exposing genetic load to selection (Kirkpatrick and Jarne, 2000). However, the extent of purging may depend on numerous population and genetic factors, making

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it difficult to predict whether inbreeding depression will occur in natural populations (Charlesworth et al., 1990; Waller, 1993; Byers and Waller, 1999; Prentis et al., 2008).

In invasive plant populations with few initial founders, genetic drift can overwhelm natural selection causing stochastic fluctuations in mildly deleterious recessive alleles, resulting in fixation of certain deleterious alleles of small effect and loss of others (Keller and Waller, 2002; Paland and Schmid, 2003). Fixation of deleterious recessive mutations due to drift can increase the magnitude of genetic load in founder populations (Cano et al., 2008). Isolated or structured populations will tend to fix different deleterious alleles that mask each other when brought together by among population crossing (Ingvarsson and Whitlock, 2000; Keller and Waller, 2002). Thus, the amount and fitness-reducing effects of deleterious mutations or genetic load in invasive plant populations will depend on the strength of selection to purge deleterious recessive mutations brought to a homozygous state following inbreeding (inbreeding load) and the fixation of deleterious recessive alleles through genetic drift (fixed drift load) (Keller and Waller, 2002; Paland and Schmid, 2003).

Examining inbreeding depression and between-population heterosis, defined here as the increased mean fitness of individuals from among population crosses as compared to individuals from within-population crosses (Keller and Waller, 2002; Busch, 2006), can be useful in assessing the relative importance of selection and genetic drift operating in invasive plant populations. This information can lead to important insights into the impacts that founder effects and inbreeding have on these introduced populations.

To determine the extent and source of genetic load, we examined the offspring resulting from selfing and interpopulation pollination in the highly invasive plant, *Alliaria petiolata* (M. Bieb.) Cavara and Grande (Gleason and Cronquist, 1993) under different experimental conditions. This Eurasian biennial herb (Chapman et al., 1952) is among the most widespread herbaceous invaders in the ground layer of eastern deciduous forests of North America (McCarthy, 1997; Carlson and Gorchov, 2004; Slaughter et al., 2007; Rodgers et al., 2008). *Alliaria petiolata* exhibits a mixed mating system, specifically delayed autogamy (Cruden et al., 1996), which provides reproductive assurance when pollinators are inefficient and allows a single individual to colonize a new area.

Populations of *A. petiolata* are genetically highly structured in its native and introduced range, and they exhibit high genetic variation among populations and relatively low genetic variation within-populations (Meekins et al., 2001; Durka et al., 2005). A genetic marker study showed that *A. petiolata* has high inbreeding coefficients and likely undergoes high rates of inbreeding, although the degree of inbreeding varies among populations (Durka et al., 2005). The species has high reproductive output and most flowers produce viable seeds (Anderson et al., 1996; Cruden et al., 1996). Introduced populations of *A. petiolata* show reductions in genetic diversity as compared to native populations, indicating that *A. petiolata* may have undergone a genetic bottleneck in its introduced range (Durka et al., 2005). Nevertheless, comparisons of the genetic diversity of introduced and likely source region populations of *A. petiolata* indicate that the introduced populations likely have originated from multiple locations in Europe resulting in substantial genetic variability in the introduced range (Meekins et al., 2001; Durka et al., 2005).

Dense stands of *A. petiolata* can build up over a relatively short period of time (Baskin and Baskin, 1992; Anderson et al.,

1996; Cruden et al., 1996; Rodgers et al., 2008). Rapid establishment of the species results from (1) high seed production, varying from 19 800 to 9522 seeds·m⁻² depending on location (Cavers et al., 1979; Nuzzo, 1993; Anderson et al., 1996), (2) limited dispersal with *A. petiolata* seeds typically falling within a few meters of the mother plant (Drayton and Primack, 1999; but see Nuzzo, 1999), and (3) approximately 70% of seeds germinating within the first year (Baskin and Baskin, 1992). Thus, intraspecific competition can place selective pressure on progeny, increasing the likelihood that deleterious recessive mutations (genetic load) could be expressed and potentially purged.

The objectives of this study were to examine the fitness effects of selfing and interpopulation pollination on *A. petiolata* plants and to examine how genetic load is partitioned in a central Illinois *A. petiolata* population. It has been assumed that *A. petiolata* likely escapes negative effects of inbreeding depression due to purging of deleterious recessive mutations from populations via selection (Durka et al., 2005; Rodgers et al., 2008). However, genetic load may remain in populations due to fixation of deleterious alleles following founder events. No studies have experimentally examined the fitness effects of selfing or interpopulation pollination in this species. This study aims to answer the following questions in a representative *A. petiolata* population: (1) Is there evidence for inbreeding depression from comparisons between selfed progeny and within-population outcrossed progeny? (2) Is there evidence for heterosis indicated by comparisons of results for within-population outcrossed progeny and among-population outcrossed progeny? (3) Does intraspecific competition affect the expression and magnitude of inbreeding depression and between-population heterosis? (4) What are the relative contributions of inbreeding load and fixed drift load to overall genetic load in the population tested?

MATERIALS AND METHODS

Study species distribution and life history—In North America, *A. petiolata* is most abundant in northeastern and midwestern United States and in southeastern Canada (Blossey et al., 2001). Although *A. petiolata* was first introduced to North America in 1868, its wide distribution across the introduced range is relatively recent. *Alliaria petiolata* seeds germinate in early spring and persist through the following winter as a basal rosette. Regardless of size, all plants surviving winter bolt, produce inflorescences during the spring of the second year of growth, set fruit and subsequently die (Cavers et al., 1979; Anderson et al., 1996). *Alliaria petiolata* is capable of both self-fertilization and cross-fertilization and seed production is similar for self-pollinated and cross-pollinated flowers (Anderson et al., 1996; Cruden et al., 1996).

Parental plant sampling and site descriptions—The focal population we used to test for the expression of genetic load was the ParkLands Foundation Merwin Nature Preserve (Merwin or MNP). Two additional populations of *A. petiolata* were used as pollen donors to provide pollen for between population crosses. We chose to focus this study on the Merwin population as it is typical of the populations in our region, occurring in a forested area along a waterway. Similar to other populations in the region, this site has had a population of *A. petiolata* since the 1980s, and at the time of this study, the population had about 300 to 400 individuals. The history and ecology of the population in Merwin is known as reflected by nine published studies (Anderson et al., 1996; Dhillon and Anderson, 1999; Roberts and Anderson, 2001; Myers and Anderson, 2003; Myers et al., 2005; Anderson et al., 2010; Bauer et al., 2010; Engelhardt and Anderson, 2011; Herold et al., 2011). Two other sites were used as pollen donors, which were located beyond the typical distance of the pollinator movement from Merwin. While assessment of the genetic load in all three populations from the three sites would be of interest to assess a broader pattern of genetic load, this is beyond the scope of the current study and was not feasible given the availability of greenhouse space. We chose to have a well-designed study that used a sufficiently large number of maternal lines and two environmental

conditions as both maternal and environmental effects can impact expression of genetic load (Dudash, 1990; Paland and Schmid, 2003). Adding assessment of genetic load in multiple populations would have required us to weaken aspects of our experimental design. We recognize that these conditions limit our ability to make broad generalization that will have applicability across *A. petiolata*'s geographic range in North America.

Second-year rosettes of *A. petiolata* were randomly selected along transects between 20 March and 7 April 2006 to serve as either target plants (pollen recipients) or pollen donors from ParkLands Foundation Merwin Nature Preserve (284 ha). During the same period, randomly selected plants along transects at Ewing Park (6 ha) and Funks Grove Nature Preserve (300 ha) were chosen to function as pollen donors. Ewing Park, in Bloomington, Illinois (40.46°N 88.93°W), and Funks Grove Nature Preserve (40.36°N 89.12°W) are located 25 km and 45 km southwest of the Merwin Nature Preserve (40.56°N 89.30°W), respectively.

Crossing design—The selected plants from the described sites were excavated and transported to a Illinois State University greenhouse in Normal, Illinois where they were planted in pots (12.7 cm diameter; 11.1 cm deep) filled with 91 cm³ of Metro-mix 510 potting soil (Sun-Grow, Vancouver, Canada). Stratified random sampling was used to select 30 plants from Merwin (MNP), which were designated as target plants (received pollination treatments and offspring were used for assessment of genetic load). Ten plants randomly selected from each population served as pollen donors. Pollen donors from MNP at least 5 m from the target plant were used for the within-population outcross. The between-population crosses were made using pollen from Ewing Park and Funk's Grove plants.

We examined expression and magnitude of inbreeding depression and between-population heterosis in *Alliaria petiolata*, by performing three types of crosses on 30 maternal plants from MNP in May 2006. Crosses were applied acropetally on each raceme after plants bolted. The order of the cross treatments was randomized and held constant for each individual target plant and all maternal plants were hand pollinated. Three types of crosses were applied to each target plant with a bee stick (honey bee thorax glued to a toothpick): (1) Self (S)—pollen donor flower on the target plant from MNP, (2) Within-population outcross (W)—pollen donors from MNP, and (3) Between-population outcross (B)—pollen donor from Ewing Park or Funk's Grove, where one flower on each maternal plant received pollen from Ewing Park and another flower received pollen from Funk's Grove. We used two populations for the between-population cross-type to assure greater genetic diversity among the donors. Offspring resulting from Ewing Park vs. Funks Grove did not differ for most traits (e.g., seed mass 2.3 ± 0.02 vs. 2.4 ± 0.02 mg). Thus we pooled the data from these crosses for all analyses.

We emasculated flowers receiving outcross pollen prior to anther dehiscence in the flower bud to prevent self-pollination, which can occur before the flower bud opens (Anderson et al., 1996; Cruden et al., 1996). We applied pollen to the stigma of the target flower directly after emasculation. Mixed pollen from at least four pollen donors from the appropriate population was applied to ensure that pollen was compatible. Self-pollinated flowers were not emasculated. Pollen from the target flower was applied to the stigma during anthesis while anthers were undergoing dehiscence. Mature fruits were collected from each maternal plant in June 2006 and air-dried.

Inbreeding depression and between-population heterosis—Seed number and seed mass—We randomly selected 18 of the 30 pollinated maternal plants to be used in the selection of progeny; from these target plants, 2–3 fruits per cross-type were randomly selected from all fruits on each plant. We counted the number of seeds per fruit and weighed each seed to the nearest 0.001 mg using a Cahn microbalance (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Stratification and germination—From each maternal plant, 24 seeds per cross type were randomly chosen to produce offspring for the next generation. As a precautionary step, four times as many seeds as were necessary for the experiment were stratified for each cross type. Seeds were then randomized and placed in 400-well plug flats filled with Fertilome seedling and cutting starter mix (Voluntary Purchasing Groups, Bonham, Texas, USA). Competitor seeds were randomly selected from remaining fruits from all inflorescences on all maternal plants. Eight hundred competitor seeds per cross type were planted in eight 400-well plug flats filled with the above germination mix.

We covered all flats with black shade cloth to prevent seeds from escaping their wells and placed them outside in Normal, Illinois on 11 November 2006

in a sheltered backyard with a partial tree canopy for 119 d of cold/moist stratification. Seeds were brought into an Illinois State University (ISU) greenhouse on 9 March 2007 to germinate. For each seed, days to germination (emergence), days to first true leaf emergence, and cotyledon length at first true leaf emergence were recorded. Percentage germination was determined by cross type for each maternal plant.

Effect of intraspecific competition on inbreeding depression and between-population heterosis—Expression of inbreeding depression can be dependent on the environmental condition of the assay (Dudash, 1990; Uyenoyama et al., 1993; Wolfe, 1993; Cheptou et al., 2001; Fox and Reed, 2010) and in the field *A. petiolata* experiences significant intraspecific competition (Baskin and Baskin, 1992; Anderson et al., 1996; Cruden et al., 1996; Rodgers et al., 2008; Bauer et al., 2010; Herold et al., 2011). Consequentially, we assessed the effect of cross-type using *A. petiolata* seedlings planted with and without intraspecific competition (high and low-density, respectively).

In April 2007, three replicates per four cross types from each of the 18 maternal plants were randomly selected and assigned to each competition treatment (3 replicates \times 2 density treatments \times 4 cross types \times 18 maternal plants = 432 target plants). We started the competition treatment with young seedlings to ensure all of the pots would have the correct number of plants at the beginning of this part of our study. We transplanted seedlings into round, plastic azalea pots (16.5 cm in diameter and 12.7 cm deep) filled with 102 cm³ of Metro-mix 510 potting soil (Sun-Grow). Target plants in each high-density treatment pot were surrounded by four garlic mustard plants that were comprised of progeny from each of the three cross types, including one seedling from each of the two between-population crosses. A single target plant was planted in a pot in the low-density treatment.

Plants were grown in the greenhouse from 9 March to 5 October 2007. Each pot was initially assigned a random position on a greenhouse bench. Benches were rotated every 7 d and pots within a bench were randomized every 14 d to minimize potential microclimate effects on plant growth. Plants were fertilized as necessary.

Rosette size—To assess the effects of cross type, competition, and their interactions, we measured rosette size (sum of the width of all leaves) in the spring (mid-April), midsummer (mid-July) and in the fall prior to overwintering (late September). Measuring the sum of the width of all leaves allowed for nondestructive sampling and has been shown to be strongly correlated with aboveground biomass in *A. petiolata* rosettes (Byers and Quinn, 1998).

Cold stratification treatment—Plants were moved outside of the greenhouse into a concrete enclosure from 5 October 2007 to March 2008 to receive cold treatment over the winter (required for flowering). We located each pot in a random position in the enclosure and placed it into wood mulch that was approximately 20 cm deep. Plants were watered as needed until the soil was frozen. Plants were brought back into the ISU greenhouse in mid-March before plants bolted and flowered. While we lost a few plants to overwintering mortality, many plants died due to fungal infection once in the greenhouse. Due to this high mortality with limited remaining plants, we were unable to use data collected on second-year growth and reproduction traits.

Data analysis—Cross-type effect—The percentage germination (arcsine square-root transformed) of the different maternal lines within each cross-type, was assessed for cross-type effect using an ANOVA. These and all following data met or were transformed to meet the assumptions implicit to MANOVA/ANOVA, normality, and homogenous distribution of residuals, unless explicitly stated otherwise.

Mean individual seed mass per fruit was determined by summing the mass of all seeds within a fruit and dividing the total mass by the number of seeds for that fruit. Mean individual seed mass/fruit and seed number/fruit are not likely to be independent, so we used MANOVA and multivariate orthogonal contrasts to analyze these data. Similarly, the seedling traits (days to emergence, first leaf emergence, cotyledon length) were also analyzed together using MANOVA and multivariate orthogonal contrasts as these traits are likely not independent. Data transformations did not improve normality or homogeneity of variance for seedling traits of cotyledon length, emergence day or leaf emergence day, thus nontransformed data were used for these variables.

We used ANOVA and univariate Tukey multiple comparisons of these traits as needed to determine the expression of inbreeding depression by comparing the offspring from self crosses vs. the offspring from within-population outcrosses. Similarly, we assessed heterosis using the same analysis but by comparing

within-population outcrosses vs. between-population outcrosses. For determining the expression of inbreeding depression and heterosis in rosette size, MANOVA was used by analysis of the differences in rosette size between the initial and ending times for the two growth period (April to July and July to September). Cross type and density were fixed effects and maternal plant was a random effect. Multivariate orthogonal contrasts with standardized canonical coefficients were used to assess the interaction of cross type and density. We log-transformed individual measurements of rosette size to meet the assumptions of MANOVA before the differences were calculated.

Competition effects—In the above MANOVA of rosette size, the interaction of the fixed effects, density treatment and cross type enabled us to address whether competition affects the expression of inbreeding depression and between-population heterosis (our second question). We used multivariate contrasts to compare the means for growth, where this interaction was significant.

Relative performance and partitioning of genetic load—To examine the relative contributions of inbreeding load and fixed drift load to overall genetic load (our third question) in *A. petiolata* populations, we obtained population-level estimates of inbreeding load (IL), fixed drift load (FL) and genetic load (GL) (Paland and Schmid, 2003) by calculating the relative performance of the cross types (RP) according to Agren and Schemske (1993).

Inbreeding load (IL)—Estimates of IL, the amount of genetic load due to recessive deleterious alleles segregating within the population, were calculated by comparing selfed progeny to the within-population outcross progeny (relative performance, RP_{S/W}). Estimates of total genetic load (GL) due to deleterious recessive alleles both segregating and fixed within the population, were calculated by comparing selfed progeny to the between-population outcross progeny (RP_{S/B}). According to Agren and Schemske (1993):

$$RP = 1 - (W_{\text{selfed}} / W_{\text{outcross}}), \text{ where } W_{\text{outcross}} > W_{\text{selfed}}, \text{ and}$$

$$RP = (W_{\text{outcross}} / W_{\text{selfed}}) - 1, \text{ where } W_{\text{selfed}} > W_{\text{outcross}},$$

where W_{selfed} is the mean value of a character for selfed progeny and W_{outcross} is the mean value of a character for outcrossed progeny (within-population or between-populations). In this measure, relative performance ranges from -1 to 1 for all traits and can therefore be used to make comparisons among traits and treatments. Positive values indicate that outcross progeny outperformed inbred progeny for a particular trait, while negative values indicate that inbred progeny outperformed outbred progeny. Note this interpretation assume that a greater value in the trait measured indicates a more robust plant. While true for most traits in the study, it is not the case for the number of days for the first leaf to emerge. Thus, this aspect needs to be considered when interpreting the results. A value of zero for RP_{S/W} or RP_{S/B} indicates that the performance of inbred progeny equaled that of outbred progeny.

Fixed drift load (FL)—Fixed drift load (FL) measures the amount of genetic load due to fixed deleterious recessive alleles (Paland and Schmid, 2003). Population-level estimates of fixed drift load (FL) were obtained by calculating the relative performance of within-population outcross progeny and between-population outcross progeny (Paland and Schmid, 2003):

$$RP_{W/B} = 1 - (W_{\text{within}} / W_{\text{between}}), \text{ where } W_{\text{between}} > W_{\text{within}}, \text{ and}$$

$$RP_{W/B} = (W_{\text{between}} / W_{\text{within}}) - 1, \text{ where } W_{\text{within}} > W_{\text{between}},$$

where W_{within} is the mean value of a character for within-population outcross progeny and W_{between} is the mean value of a character for between-population outcross progeny. The values of RP_{S/W}, RP_{W/B}, and RP_{S/B} were determined for each trait measured. If RP_{S/W} is of similar magnitude to RP_{S/B} (IL = GL), then genetic load (GL) is entirely due to segregating alleles (Paland and Schmid, 2003). Alternatively, if RP_{W/B} is of similar magnitude to RP_{S/B}, (FL = GL), then genetic load is entirely due to fixed deleterious alleles (Paland and Schmid, 2003).

Formal assessment of relative performance—To determine whether there were significant differences in the relative performance values (RP_{S/W}, RP_{W/B}, and RP_{S/B}) and assess the relative contribution of inbreeding load (IL) and fixed drift load (FL) in overall genetic load (our third question), ANOVAs were used. To allow for formal statistical analyses of the different RP's, our mean fitness values (W values) were means by cross type within maternal plant. Traits that

were significantly affected by cross type or an interaction with cross type were included in these analyses. Traits included were individual seed mass, cotyledon length, days to leaf emergence, and rosette growth. Density and the interaction with RP-type were also included in the model for rosette growth. Maternal lineage was used as the unit of replication to compare relative performance among treatments and thus not included as a response variable. Tukey multiple comparison follow up tests were used tests when appropriate. Relative performance coefficients for seed mass were ln-transformed to meet the assumptions for ANOVA.

For all of the statistical analyses, the Proc GLM and Proc Mixed procedures in SAS version 9.1 (SAS Institute, 2004) were used. In all analyses, cross type, density, and cross \times density were analyzed as fixed effects. Maternal plant and all interactions with maternal plant were analyzed as random effects. When the response variable was combined across maternal lines such as seeds per fruit, maternal plant and any interactions with maternal plant were not included.

RESULTS

Inbreeding depression and between-population heterosis—Percentage germination was highest for seeds produced by between-population outcross ($76.2 \pm 1.4\%$), followed by within-population outcross ($74.7 \pm 2.9\%$), and self-fertilized seeds ($73.6 \pm 2.7\%$), but these differences were small and not statistically significant ($F_{2,69} = 0.33, P = 0.719$); thus, inbreeding depression or between-population heterosis does not appear to be expressed for this trait.

There were significant effects of cross type and maternal plant but not for the interaction between cross type and maternal plant in the multivariate analysis of mean seed mass and seed number per fruit. Seed mass contributed more to the significant differences than seed number according to the canonical coefficients (Table 1A). Estimate of the variance for maternal plant is zero for seed number per fruit from the univariate analysis. Thus, the significant effect in the MANOVA is due to seed mass, where maternal plant explains 15.7% of the variation (absolute variance = 0.0169 ± 0.005447). Multivariate contrasts indicated significant differences among cross types for combinations of these seed traits (Table 1A). Univariate tests ANOVA (Table 1B) and post hoc Tukey multiple comparison tests (Table 1C) indicated self-fertilized fruits produced significantly more seeds per fruit than within- and between-population crossed fruits, but seeds for self and within-population crossed had significantly less mass than progeny from the between-population outcross. The greater mass for outcrossed seeds could be due to heterosis.

Seedling traits were significantly affected only by cross-type (Table 2A). There were no significant differences in seedling traits between progeny produced by self-fertilization and within-population cross-fertilization, suggesting that inbreeding depression is not expressed for seedling traits. Standardized canonical coefficients for the contrasts indicate that cotyledon length contributes the most to the differences between cross types. Between-population cross type had significantly longer cotyledon length and earlier first true leaf emergence than selfed and within-cross types (Table 2B, 2C), further supporting between-population heterosis.

Effect of intraspecific competition on inbreeding depression and between-population heterosis—Rosette size in *A. petiolata* plants grown without intraspecific competition increased in size at a significantly faster rate than plants growing with competitors, but the extent of increased growth depends on the cross type (Table 3A). Thus, expression of genetic load depends on intraspecific competition, which provided increased environmental stress. In the presence of intraspecific competition (high density), between-population outcross progeny had a significantly

TABLE 1. Results of analysis of cross type (self, within, or between) and maternal plant on seed traits.

Source	df	Pillai's trace	F ratio	P	Standardized canonical coefficients	
					Mean seed mass	Seed number
Cross type	4, 68	0.9703	16.02	<0.0001	1.3866	-0.2095
Maternal plant	34, 68	0.9328	1.75	0.0255	1.3637	-0.3171
Cross × maternal plant	68, 282	0.3923	1.01	0.4561	-0.1262	1.0967
Contrasts between cross types						
Self vs. within	2, 33	0.2994	7.05	0.0028	-0.1110	0.9554
Self vs. between	2, 33	0.6079	25.59	<0.0001	1.2919	-0.4741
Within vs. between	2, 33	0.5992	24.67	<0.0001	1.3526	0.0943
B. Univariate (ANOVA) tests for seed traits.						
Trait and source	df	F ratio	P			
Mean seed mass						
Cross type	2, 34	29.07	0.0001			
Seed number						
Cross type	2, 34	7.37	0.0022			
C. Means and their standard errors for seed traits with Tukey multiple comparisons. Different letters indicate a statically significant difference between pairs of means.						
Trait	Cross-type					
	Self		Within		Between	
	Mean	SE	Mean	SE	Mean	SE
Mean seed mass (mg)	2.12 ^B	0.033	2.11 ^B	0.04	2.38 ^A	0.034
Seed number	12.3 ^A	0.7	8.5 ^B	0.66	10.0 ^B	0.5

more rapid rate of rosette growth than self-fertilized progeny or within-population outcross progeny. By comparison without intraspecific competition (low density), the within-population outcrossed and selfed progeny had faster rosette growth than the between-population outcrossed progeny (Table 3B), although this relationship was significant in the multivariate contrasts, only for within and between-population outcrosses. For the significant main effect (density), standard canonical coefficients for early and late grow, respectively, were 3.089 and 0.888 and for the significant interaction (density × cross-type) the same values were 2.726 and 0.421, respectively. Standard canonical coefficients for the significant contrast indicated that differences among cross types were also more strongly influenced by early rosette growth than the late growth (Table 3C).

Relative performance and partitioning of genetic load—For all traits included in this analysis, cross type was significant indicating the different sources of genetic load, and namely that drift and inbreeding load did not equally contribute to genetic load (Table 4).

Relative performance coefficients for selfed and within-population outcross progeny ($RP_{S/W}$) for most of the assessed traits was low, indicating limited inbreeding depression in this population (Fig. 1, Table 4). However, if an inbred population shows little or no differences between selfed and within-population cross progeny, these results could reflect fixation within the population for several deleterious alleles. An estimate of inbreeding load (IL) is provided by ($RP_{S/W}$), and it was significantly less than $RP_{S/B}$, the overall estimated fixed genetic load due to deleterious recessive alleles (GL) for most of these traits.

Analyses on the effect of relative performance type on relative performance coefficients for seed mass, cotyledon length, and leaf emergence indicate that the relative performance of

$RP_{S/B}$, the measure of genetic load due to deleterious recessive alleles (GL), is not statistically different from $RP_{W/B}$, the measure of fixed drift load (FL) (Fig. 1). Thus $FL = GL$ and most of the genetic load for these traits can be attributed to drift load given the larger relative performance coefficients for the within-population and between-population outcross progeny ($RP_{W/B}$). This demonstrates that most of the genetic load in this population of *A. petiolata* is due to the fixation of deleterious alleles within the population.

Analysis of rosette growth also included density, enabling us to determine whether the source of genetic load is dependent on the environmental context. There was a significant interaction between relative performance type and density for relative performance coefficients of rosette growth indicating environmental dependence (Table 4). $RP_{W/B}$ and $RP_{S/B}$ were significantly greater than $RP_{S/W}$ when plants were grown with intraspecific competition than when they were grown alone (Fig. 1D). There were no significant differences among RP-types in the low-density treatment. These results indicate that the expression of genetic load for rosette growth is greater in more stressful environments and can be attributed to drift load.

DISCUSSION

Our study population of *A. petiolata* has substantial genetic load, but it can mostly be attributed to genetic drift (founder effect) and not inbreeding depression, as indicated by comparisons of relative performance values. Our greenhouse density treatment significantly impacted rosette growth and expression of genetic load, as heterosis was more evident with competition, which created more stressful conditions.

TABLE 2. Results of effect of cross type and maternal plant on seedling traits.

A. MANOVA results for effect of cross type and maternal plant on seedling traits. Planned contrasts are presented.							
Source	df	Pillai's trace	F ratio	P	Standardized canonical coefficients		
					Emergence day	Cotyledon length	Leaf emergence day
Cross type	6, 66	0.7641	6.80	<0.0001	0.5547	1.1215	-0.3299
Maternal plant	51, 102	1.1573	1.26	0.1647	0.3951	-0.5454	0.4015
Cross × maternal	102, 2982	0.0973	0.98	0.5380	-1.0141	0.5232	1.6030
Contrasts							
Self vs. within	3, 32	0.0273	0.30	0.8259	1.1718	0.8628	-0.2313
Self vs. between	3, 32	0.6909	23.84	<0.0001	0.5989	1.1252	-0.3298
Within vs. between	3, 32	0.6326	18.36	<0.0001	0.4881	1.1126	-0.3292
B. Univariate tests (ANOVA) of cross type for seedling traits.							
Trait and source	df	F ratio	P				
Emergence day							
Cross type	2, 37	0.66	0.5232				
Cotyledon length							
Cross type	2, 37	40.83	0.0001				
Leaf emergence day							
Cross type	2, 37	9.23	0.0006				
C. Mean and their standard errors for seedling traits with Tukey multiple comparisons. Different letters indicate a statistically significant difference between pairs of means.							
Trait	Cross type						
	Self		Within		Between		
	Mean	SE	Mean	SE	Mean	SE	
Emergence day	4.91	0.088	4.99	0.093	4.86	0.062	
Cotyledon length (mm)	17.69 ^A	0.170	17.78 ^A	0.179	19.20 ^B	0.120	
Leaf emergence day	13.18 ^A	0.096	13.19 ^A	0.101	12.77 ^B	0.068	

Hybridization between small, isolated populations can allow them to escape a fixed mildly deleterious mutational load (Ellstrand and Schierenbeck, 2000; Keller and Waller, 2002; Coutellec and Caquet, 2011), and is thought to be an important factor in allowing invasive species to overcome founder effects and genetic bottlenecks (Ellstrand and Schierenbeck, 2000; Lavergne and Molofsky, 2007; Geiger et al., 2011). The increases in *A. petiolata* progeny success that results from interpopulation crossing, as found in our study, is consistent with different alleles becoming fixed in distinctly separated populations (Paland and Schmid, 2003; Busch, 2006). Among-population gene flow may effectively bring these alleles together, masking the effects of one another, increasing progeny fitness, and resulting in between-population heterosis (Richards, 2000; Keller and Waller, 2002; Paland and Schmid, 2003; Zhou et al., 2010). In our study, interpopulation crossing resulted in positive between-population heterosis for many size traits in *A. petiolata*, with increased fitness of progeny produced by between-population outcrosses as compared to progeny from within-population outcrosses.

Mean seed number was the only trait for which self-fertilized fruits differed significantly from within-population and between-population outcrossed fruits, with self-fertilization producing more seeds per fruit than cross-fertilization, which could be interpreted as outbreeding depression. However, significantly reduced seed number per fruit may be due to experimental manipulation of flowers resulting in stigmatic or ovule damage or reduced pollen loads compared to nonemasculated, self-pollinated flowers. Experimental conditions may have also contributed to lower seed mass for the selfed treatment compared to the between-population cross, with allocation of resources to individual seeds increasing when there are fewer

seeds per fruit. Several studies have reported that emasculation prior to hand pollination did not negatively affect seed set or mass compared to nonemasculated flowers (Culley et al., 1999 [viable seeds per capsule]; Fischer and Matthies, 1997 [seed number and mass]; Eckert and Schaeffer, 1998 [seed set]; Fan and Li, 2012 [number of seeds and fruits]). However, other studies have reported opposite results and found that emasculation reduced seed set due to mechanical damage to floral parts (Young, 1982; Vaz et al., 1998). In our study, observations during hand pollination of the stigmas showed that pollen loads were sufficient to fertilize all of the available ovules.

Seed mass in *A. petiolata* is highly variable (Susko and Lovett-Doust, 2000), and standard canonical coefficients indicated seed mass differences were more important than seed number in causing differences among cross types and there is a potential trade-off. However, for the within- and between-population crosses, coefficients for seed number and mass were positively correlated across treatments and the number of seeds per fruit was not significantly different, indicating no apparent tradeoff between seed number and seed mass. Differences in seed mass between the within- and between-population crosses are likely due to heterosis, because flowers of both groups were emasculated and hand-pollinated.

In this study, we found no evidence for inbreeding depression in seedling traits. Similarly, relative performance coefficients were low for selfed and within-population crossed progeny. However, heterosis was expressed for cotyledon length and leaf emergence day, with between-population crosses having longer cotyledon length and earlier leaf emergence than the self- and within-population crosses.

TABLE 3. Results from effect of cross type, density, and maternal plant on rosette growth (sum of width of all leaves (cm) as amount of increase in size between measurements (July–April and September–July).

A. MANOVA of effect of cross type, density, and maternal plant on rosette growth and multivariate contrasts for cross type \times density interaction.

Source	df	Pillai's Trace	F ratio	P
Cross (C)	4, 68	0.0284	0.24	0.9114
Density (D)	2, 16	0.9949	1589	<0.0001
Maternal (M)	34, 68	0.6334	0.93	0.5869
C \times D	4, 68	0.5409	6.30	0.002
C \times M	68, 68	0.9879	0.98	0.5393
D \times M	34, 68	0.5935	0.84	0.7017
C \times D \times M	68, 640	0.2003	1.05	0.3731
Contrasts				
Low density				
Self vs. within	2, 33	0.0904	1.64	0.2091
Self vs. between	2, 33	0.1507	2.93	0.0674
Within vs. between	2, 33	0.3744	9.88	0.0004
High density				
Self vs. within	2, 33	0.0078	0.13	0.8788
Self vs. between	2, 33	0.2412	5.25	0.0105
Within vs. between	2, 33	0.3023	7.15	0.0026

B. Means and standard errors for rosette growth (cm^2) for cross type \times density interaction term.

Cross type	Low density		High density	
	Mean	SE	Mean	SE
Early rosette growth (April–July)				
Self	1101.9	41.69	54.9	10.48
Within	1174.2	34.53	47.5	9.78
Between	1081.0	28.96	98.5	9.02
Later rosette growth (July–September)				
Self	273.7	50.35	58.7	15.91
Within	257.6	35.24	49.0	13.44
Between	238.6	32.74	59.7	10.50

C. Standard canonical coefficients for significant multivariate contrasts.

Source	Early growth	Late growth
Low density		
Within vs. between	2.72	0.42
High density		
Self vs. between	2.72	0.40
Within vs. between	3.08	0.88

Intraspecific competition and genetic load—Intraspecific competition significantly increased the severity of genetic load for rosette growth, suggesting that genetic load, particularly drift load, may be density dependent in *A. petiolata*. These results are consistent with the maintenance of a small amount of genetic load in the population due to fixation of deleterious recessive mutations of mild effect (Coutellec and Caquet, 2011).

First-year *A. petiolata* occurred in densities of $8.3/\text{dm}^2$ during the spring in central Illinois, but plant survivorship declined rapidly during the first year, and only 7.5% of plants survived to maturity (Anderson et al., 1996). In addition, *A. petiolata* grew more actively from April to July (early growth) than from July to September (late growth) (Anderson et al., 1996). Thus, there would be greater intraspecific competition for resources and higher stress in the early period than the later period of growth. More active early growth may explain why standard canonical coefficients indicated that early growth was more important in influencing differences among cross types than later growth. The density of plants used in our experiment is probably lower

TABLE 4. Individual ANOVAs for effect of relative performance-type ($\text{RP}_{\text{W/S}}$, $\text{RP}_{\text{W/B}}$, or $\text{RP}_{\text{S/B}}$) and density on relative performance values for the following traits: seed mass; cotyledon length; leaf emergence day; and rosette growth. See Fig. 1 for means and results of the Tukey multiple comparisons tests.

Trait and source	df	F ratio	P
Seed mass			
RP type	2, 51	13.93	<0.0001
Cotyledon length			
RP type	2, 51	13.65	<0.0001
Days to first leaf emergence			
RP-type	2, 51	3.95	0.0255
Rosette growth			
RP type	2, 102	3.74	0.0271
Density	1, 102	8.81	0.0037
RP type \times Density	2, 102	7.71	0.0008

than *A. petiolata* densities found in the biennial's first spring and early summer in the natural environment, but was more reflective of intraspecific competition in late summer and in spring of the second year, following high mortality of plants.

Inbreeding load did not contribute significantly to overall genetic load in this *A. petiolata* population, even when plants were grown at higher density. Interspecific and intraspecific competition can influence the degree to which inbreeding depression is expressed in various species (e.g., Schmitt and Ehrhardt, 1990; Uyenoyama et al., 1993; Wolfe, 1993; Garcia-Serrano et al., 2008). However, many studies have involved self-incompatible species with high outcrossing rates, which increases the likelihood of inbreeding depression being expressed at greater magnitudes than in species with high selfing rates, such as *A. petiolata* (Wolfe, 1993; Daehler, 1999; Garcia-Serrano et al., 2008). Other studies found that the magnitude of inbreeding depression did not increase with increased stress (Mayer et al., 1996; Cheptou et al., 2001; Heschel et al., 2005; Cano et al., 2008; Waller et al., 2008). Nevertheless, studies comparing selfed and outcrossed progeny in more stressful conditions, such as those found in the natural environment, have shown increased expression of inbreeding (Dudash, 1990; Armbruster and Reed, 2005).

Genetic load dynamics of an invasive species—Inbreeding depression and between-population heterosis need to be measured together to determine the architecture of genetic load in subdivided or isolated populations (Keller and Waller, 2002; Paland and Schmid, 2003). Our results suggest that inbreeding depression is expressed at low levels in *A. petiolata*, as evidenced by limited superior performance of within-population outcross progeny compared to self-fertilized progeny for most traits measured. However, examination of inbreeding depression and between-population heterosis in *A. petiolata* suggested a much greater level of fixed genetic load than inbreeding load within the population studied, presumably resulting from drift and/or founder effects fixing deleterious alleles within the population, decreasing both the expression of inbreeding and resulting in an overall decrease in population fitness. These findings are consistent with the prediction that inbreeding depression decreases following genetic bottlenecks in invasive species (Kirkpatrick and Jarne, 2000; Facon et al., 2011), which is expected because increased inbreeding during a bottleneck should expose inbreeding load to selection and subsequently be purged from the introduced population. During bottlenecks, genetic drift can overwhelm selection,

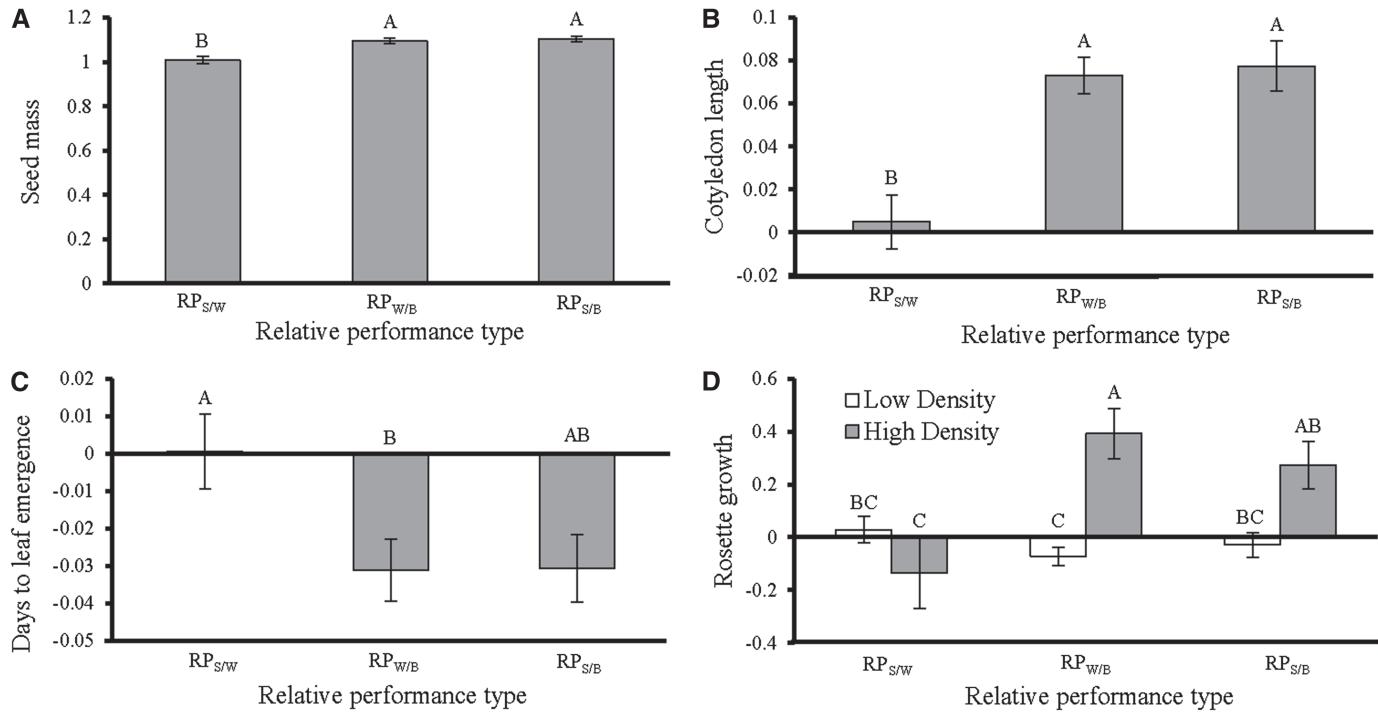


Fig. 1. Mean (standard error) relative performance coefficients comparing progeny from self to within-population cross (RP_{S/W}), within-population cross to between-population cross (RP_{S/B}), and within-population cross to between-population cross (RP_{W/B}) showing the relative magnitude of inbreeding load (IL), genetic load (GL), and fixed drift load (FL) for (A) seed mass, (B) cotyledon length, (C) leaf emergence*, and (D) rosette growth from April to September. Positive values for RP_{S/W} and RP_{S/B} indicate that outcross progeny outperformed inbred progeny for a particular trait (W or B > S), while negative values indicate that inbred progeny outperformed outbred progeny (S > W or B). Similarly, positive values for RP_{W/B} indicate that B > W for a particular trait, and negative values indicate instances in which W > B. Mean values for seed mass were back-transformed. Means with the same letter are not significantly different as determined by Tukey multiple comparison tests. For rosette growth, the ANOVA showed a significant interaction between RP type and density, thus the Tukey multiple comparison tests results indicate which of the means for this interaction differ significantly. *For the trait, days to first true leaf emergence, a lower value would be considered a more robust plant; thus, the more negative values for RP_{S/B} and RP_{W/B} indicates a greater success of the offspring from between-population crosses and a significant genetic load.

resulting in fixation of mildly deleterious mutations, thus increasing genetic load (Kirkpatrick and Jarne, 2000). Our results suggest purging of genetic load of large effect due to segregating alleles (inbreeding load) in early life cycle traits of *A. petiolata* and support the prediction of Durka et al. (2005) that there would be little expression of inbreeding depression in *A. petiolata*, owing to the high rates of selfing in this species. However, our results are also consistent with a founder effect. Keller and Waller (2002) indicated that fixation can occur for several deleterious alleles in inbreeding populations, so few or no differences would be displayed by progeny resulting from inbred or outbred matings.

It is not possible to generalize about the pattern of genetic load based on the assessment of one population, particularly because of the stochastic nature of genetic drift, likely differences in the extent of population isolation (Busch, 2006), differences in the intensity of selection in various habitats and populations, and variations in population size (Paland and Schmid, 2003). Further examination of inbreeding depression and between-population heterosis across a greater range of populations of different sizes and/or comparing more recently colonized populations to populations that have existed for a long period of time will be necessary to provide more insight into the relative contributions of genetic drift and selection in the maintenance of genetic load in this species.

Conclusions—Inbreeding appears to be adaptive for *Alliaria petiolata* in its introduced range. Self-compatibility provides the benefits of reproductive assurance, while expression of inbreeding load appears to be negligible indicating no large fitness cost. Populations are experiencing appreciable fitness reductions due to fixation of mildly deleterious recessive alleles indicating a potential weakness in this *A. petiolata* population. This genetic weakness is likely due to a combination of founder effects, genetic bottlenecks, autogamy, and reduced gene flow among isolated populations owing to habitat fragmentation, limited seed dispersal, and movement of pollinators (Baker, 1955; Richards, 2000; Meekins et al., 2001; Coates et al., 2007).

Reduced allelic diversity resulting from founder effects and genetic bottlenecks may reduce the adaptive potential of *A. petiolata* to some extent, but multiple introductions and dispersal among locally isolated populations may restore genetic variation resulting in increased population fitness. The extent of genetic exchange among isolated local populations is unknown, but our results suggest that such an exchange may increase genetic diversity and offspring vigor through heterosis especially under stressful conditions. Preventing additional introduction and movement of propagules between isolated local populations should reduce heterosis and *A. petiolata* competitiveness.

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