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How functional traits, herbivory, and genetic diversity interact in *Echinacea*: implications for fragmented populations

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Abstract. Habitat fragmentation produces small, spatially isolated populations that promote inbreeding. Remnant populations often contain inbred and outbred individuals, but it is unclear how inbreeding relative to outbreeding affects the expression of functional traits and biotic interactions such as herbivory. We measured a suite of 12 functional traits and herbivore damage on three genotypic cross types in the prairie forb, *Echinacea angustifolia*: inbred, and outbred crosses resulting from matings within and between remnant populations. Inbreeding significantly affected the expression of all 12 functional traits that influence resource capture. Inbred individuals had consistently lower photosynthetic rates, water use efficiencies, specific leaf areas, and had higher trichome numbers, percent C, and percent N than outbred individuals. However, herbivore damage did not differ significantly among the cross types and was not correlated with other leaf functional traits. Leaf architecture and low physiological rates of the inbred compared to outbred individuals imply poorer capture or use of resources. Inbred plants also had lower survival and fitness relative to outbred plants. Our results show that inbreeding, a phenomenon predicted and observed to occur in fragmented populations, influences key functional traits such as plant structure, physiology and elemental composition. Because of their likely role in fitness of individuals and ecological dynamics plant functional traits can serve as a bridge between evolution and community or ecosystem ecology.

Key words: Echinacea; *fitness; functional traits; habitat fragmentation; herbivory; inbred; outbred; photosynthetic rate (*A_{max}*); remnant populations; specific leaf area (SLA); water use efficiency (WUE).*

Introduction

The formation of small habitat islands during conversion of contiguous habitat tends to reduce the size of populations that remain (Saunders et al. 1991, Lienert and Fischer 2003). Isolation may continue to erode population size by decreasing gene flow and the probability of favorable demographic transitions (Benitez-Malvido 1998, Aguilar et al. 2006). These numerical effects can be compounded by genetic alterations. Small, isolated populations generally contain less genetic variation than larger interconnected populations (Honnay and Jacquemyn 2007) because random genetic drift and inbreeding are more likely (Aguilar et al. 2008); consequently increased homozygosity can result in inbreeding depression (Ellstrand and Elam 1993, Young et al. 1996). Depending on the strength of selection, population size, and distance, isolated populations also can be locally adapted (Nagy and Rice 1997, Etterson 2004, Leimu and Fischer 2008) and in these cases, reproduction between plants from distant remnants may result in outbreeding depression (Barrett and Kohn 1991, Waser and Price 1994).

Plant populations post-fragmentation likely comprise a mix of inbred and outbred plants. Despite the importance of understanding how populations respond to globally pervasive habitat fragmentation, few studies have measured how these genetically variable populations may respond demographically (Harrison and Bruna 1999, Lienert 2004, but see Wagenius et al. 2010). Even fewer have investigated variation in functional traits and interspecific interactions in relation to genetic differences due to inbreeding vs. outcrossing, and how these responses may be related to fitness (Kingsolver et al. 2001, Geber and Griffen 2003). Because habitat fragmentation can change patterns of reproduction, the prevalence of both inbred and outbred individuals in remnant populations can increase. Thus it becomes increasingly important to understand how genotypic classes express functional traits differently, and how these differences might influence the ecology and evolution of remaining populations. While many ecologists may take for granted that inbreeding depression results from compromised resource acquisition, little evidence exists for this.

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Functional traits directly affect resource uptake and use, and can influence growth, survival, and reproduction (Geber 1990, Donovan and Ehleringer 1994). Functional traits can be physiological or morphological in nature and include photosynthetic rates, water use efficiency, photosynthetic nitrogen use efficiency, leaf area, and percent nitrogen. The expression of these traits can vary among genotypes by as much as 25–50% (Sultan and Bazzaz 1993, Sandquist and Ehleringer 2003). While physiologists have examined genotypic variability in functional traits, many studies are on agronomic or weedy species (Condon et al. 1993, Geber and Dawson 1997, Alonso-Blanco et al. 2009), annuals (Farris and Lechowicz 1990, Dudley 1996), or among plants growing in greenhouses; less is known about how functional traits vary within populations of perennial species and particularly how inbreds differ from outbreds in native, fragmented populations of perennial species (McGraw and Wulff 1983, Geber and Griffen 2003).

How inbreeding or outbreeding may differentially affect damage by insects also merits additional research. Inbreeding can increase susceptibility (Campbell et al. 2013) or resistance (Strauss and Karban 1994), or it can have no apparent effect (Nuñez-Farfan et al. 1996). Differential expression of functional traits also can mediate herbivory (Bello-Bedoy and Nuñez-Farfan 2011, Campbell et al. 2013). For example, foliar nitrogen or carbon may be strongly associated with herbivore susceptibility (Mattson 1980, Carmona et al. 2011). The thickness and hairiness of leaves also are strong adaptive defenses against herbivory (Agrawal and Fishbein 2006). Examining the dependence of herbivory on leaf functional traits and inbreeding can shed light on how plant populations respond to habitat fragmentation.

Isolated prairie remnants are a good system to evaluate how ecological and evolutionary processes influence native population persistence because they once covered vast regions of North America, but only 1–4% now remain (Samson et al. 2004) and the biome is endangered (Hoekstra et al. 2005). Our research focuses on the perennial forb, *Echinacea angustifolia* (hereafter *Echinacea*). Inbreeding is likely to occur in isolated remnants (Wagenius 2000), and inbred *Echinacea* have 20% lower survival rate and ~66% lower fitness than non-inbred plants (Wagenius et al. 2010). However, it remains unknown whether functional traits or foliar herbivory differ among inbred and outbred *Echinacea*, and what relationship these traits might have to fitness.

Our research explores how leaf herbivory and functional traits vary among three genotypic classes of *Echinacea* plants differing in their degree of inbreeding. The three classes include plants derived from mating between plants from different prairie remnants (B), random mating between plants from the same prairie remnant (W), and sib-mating (I). Genotypic class W is likely typical of populations prior to fragmentation, while inbred plants (class I) may currently predominate in small, highly isolated remnants, and class B may become increasingly common because pollinators must travel farther to forage and because humans move seeds for restoration and other purposes. All three genotypic classes are now expected to be prevalent in our fragmented study area. The plants all grow in two experimental plots within a field undergoing restoration to a prairie community. Specifically, we address (1) How does a suite of functional traits differ in inbred and outbred plants, and how do these morpho-physiological traits relate to one another? (2) To what extent do plants from different cross types undergo different levels of foliar herbivory, and what interactions may exist between functional traits and leaf herbivory? We also discuss the relationship of these traits to existing measures of *Echinacea* fitness, which can yield insight into mechanistic processes that may underlie survival, reproduction, and adaptation (Ackerly et al. 2000, Caruso et al. 2005, Agrawal et al. 2008).

METHODS

Study organism, cross types, and experimental plots

Narrow-leaved purple coneflower, *Echinacea angustifolia* (DC.), is an herbaceous perennial forb native to the Great Plains of North America. Conversion of prairie to agriculture has resulted in significant habitat loss for *Echinacea*. Small remnant populations that persist primarily in roadside ditches or areas inaccessible to farming can contain as few as three and up to hundreds of individuals (Wagenius 2000). *Echinacea* is self-incompatible and pollinated primarily by native solitary bees (Wagenius 2000, Wagenius and Lyon 2010). Populations may be isolated $\geq$1000 m from other remnant populations and successful pollination is significantly lower for spatially isolated *Echinacea* (Ison 2010, Wagenius and Lyon 2010). Fecundity is low in small populations often because compatible pollen donors are few (Wagenius 2004, 2006, Wagenius and Lyon 2010) and because flowering time is asynchronized (Ison 2010). Genetic differentiation exists within and among many *Echinacea* populations (Baskauf et al. 1994, Wagenius 2000, Kapteyn et al. 2002, Still et al. 2005, Ison 2010). Genetic diversity can be high in some western Minnesota prairie remnants suggesting little inbreeding in these sites (Ison 2010). Recruitment is limited only to seeds that gravity disperse. In early spring, plants sprout from a single taproot to form one to many rosettes. First flowering occurs at least three years after germination and then intermittently throughout an individual's lifetime. Plants generally make one inflorescence during the growing season. Seasonal senescence occurs in fall.

To assess how variation in mating affects plant traits and fitness, seedlings were generated from three types of crosses: (1) between maternal half sibs (inbred), (2) within a remnant, but not sharing a maternal or paternal parent (outbred), and (3) between individuals from different remnants (also outbred). Individuals from six remnant populations served as maternal parents, and were mated to multiple pollen donors from the same six remnants. We refer to individuals from these cross types as inbred (I), within remnant (W), and between remnants (B), respectively. Two experimental plots were established in prairie restoration on a former agricultural field in Douglas County, Minnesota (near 45°49′ N, 95°42.5′ W) from greenhouse grown seedlings in 2001 (INB1; $N = 518$) and 2006 (INB2; $N = 1136$). Following germination, seedlings of each cross type were randomly planted to positions 0.5 m apart and in rows 1 m apart. Management includes biyearly spring burns, mowing, periodic weeding of exotic invasive species and hand broadcasting of two native grasses. See Wagenius (2000), Wagenius et al. (2010), and Ridley et al. (2011) for more information on the species, crosses and study site.

Functional traits

We measured a suite of functional traits on *Echinacea* leaves growing in the two plots to address how inbred and outbred plants differed in functional trait expression. In late June/early July, and again in August 2013, we measured photosynthetic rates on an area basis (expressed as $A_{\max}$; μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) on the third-most basal leaf on the largest rosette using a Li-Cor 6400XT (Li-Cor, Lincoln, Nebraska, USA; $n = 110$ 11-year-old plants in INB1; $n = 448$ 7-year-old plants in INB2). Chamber conditions were set to ambient CO_2 (400 μL/L) and a saturating photon flux density (1600 μmol quanta$\cdot m^{-2} \cdot s^{-1}$). Air temperature tracked ambient. We took measurements from 10:30–15:00, when $A_{\max}$ peaked and plateaued in our diurnal response curves (data not shown). Leaves were allowed to equilibrate in the chamber and readings were recorded only after stability criteria were met for $A_{\max}$, conductance, and transpiration (~30–100 s). Instantaneous water use efficiency (WUE) was calculated by dividing $A_{\max}$ by transpiration/10 000 (mol H_20). The leaf was marked with a twist tie so that other functional trait measurements of the same leaf could be obtained.

Trichome density of marked leaves was determined in late July based on the better of two digital photographs taken on black cardstock. Each photograph was analyzed using ImageJ 64 (*available online*).[6] Edge trichomes along a 2-cm transect were counted starting 1 cm from the tip. We averaged two separate counts of trichomes. If the difference between the two counts was greater than 15, both counts were made again. Edge trichomes positively correlated with trichomes on the leaf blade (Pearson's correlation, $r = 0.80$, df $= 17$, $P < 0.001$), so we used edge trichome data only.

After recording photosynthetic rates during peak flowering (12–15 Aug), we removed the marked leaf to calculate leaf dry matter content (LDMC; mg/g), specific leaf area (SLA; m^2/g), and photosynthetic rate on a mass basis (A_{mass}; μmol $CO_2 \cdot g^{-1} \cdot s^{-1}$). After removal, leaves were saturated and weighed within 15–60 min (following Wilson et al. [1999] and Garnier et al. [2001]). Each sample was immediately pressed. Leaves were dried to a constant dry mass (g) at 45°C. Average leaf area to the nearest 0.001 cm^2 was found by averaging two measurements from a calibrated Li-Cor 3100. Then, we dried leaves again and ground each sample for analysis of percent N, percent C, and C:N at the University of Nebraska Ecosystems Analysis Lab. Photosynthetic nitrogen use efficiency (PNUE) was calculated as μmol CO_2/mol N. The length of longest leaf (basal or cauline if no basal leaves present) was used as a relative estimate of overall plant size.

Interactions between herbivory and genetic diversity

Herbivore damage was measured on the same plants once in late July 2013 by rating leaf damage as none, 0% missing; minor, 1–25% of leaf removed or chewed; half, 25–75% of leaf missing or chewed; major, 75–95% of leaf missing; and >95%. Overall damage (%) was calculated by summing the midpoint for each leaf (0%, 12.5%, 50%, 85%, and 97.5%) and then finding the average for all the leaves on the plant. Aphid load also was recorded using the following categories for each leaf: 1 aphid, 2–10 aphids, 11–80 aphids, and >80 aphids.

Statistical analyses

Factor analysis was implemented in JMP (SAS Institute, Cary, North Carolina, USA) to group potentially correlated leaf traits as response variables so we could perform subsequent analyses on uncorrelated factors (Browne 2001). In factor analysis a principal component analysis is first done to reveal a correlation structure that explains the most variance in our 13 response variables (June $A_{\max}$, August $A_{\max}$, August A_{mass}, June WUE, August WUE, percent N, percent C, C:N, LDMC, SLA, PNUE, average trichome count, and herbivore damage) by each successive principal component. Next, a scree plot was used to determine the minimum number of components required to account for the majority of the variation in the response variables. We then used an orthogonal Varimax method to rotate the principal components axes to better identify underlying factors that contribute to response variables. The rotation revealed clustering of correlated variables (factors) and the correlation of each response variable to its factor (factor loading). Traits were considered members of a factor when the factor loading was >0.7 and if that trait was associated with only that factor. Each factor was completely uncorrelated with other factors.

To evaluate the extent to which cross type (I, W, B) affected expression of the functional traits and herbivory, we performed ANCOVAs with the factors identified in the analysis previously described as response variables and the following predictors: experimental plot (INB1

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[6] http://rsbweb.nih.gov/ij/

TABLE 1. ANCOVA data from the three factors identified by factor analysis, which tested the effects of location (plot, row, position), maternal family, cross type (inbred, between-, and within-remnant crosses), longest leaf length, and their interactions.

Trait	Plot	Row (plot)	Position (plot)	Maternal population	Cross	Longest leaf	Plot × cross	Cross × longest leaf
Factor 1	**7.629**	**2.568**	**3.733**	1.509	**43.88**	**5027**	**4.720**	0.341
Factor 2	0.021	**3.767**	**3.981**	1.670	**8.005**	**152.0**	**3.469**	0.201
Factor 3	0.546	**6.172**	1.216	1.819	2.735†	**24.18**	1.287	1.492

Notes: F values are shown (df = 24, 520); boldface type denotes values that are significant at $P < 0.05$. Factor 1 includes June average photosynthetic rate on an area basis ($A_{\max}$), August $A_{\max}$, June instantaneous water use (WUE), August WUE; Factor 2 includes average photosynthetic rate on a mass basis (A_{mass}), percent C, leaf dry matter content (LDMC), specific leaf area (SLA), and photosynthetic nitrogen use efficiency (PNUE); Factor 3 includes percent N and C:N ratio.

† $P = 0.06$.

vs. INB2), row (plot), position (plot), maternal population, cross type, longest leaf length, and two-way interactions of cross × leaf length and cross × plot (using JMP). Interaction terms test whether effects of cross type on functional traits depended on longest leaf length and whether the effects of cross type on leaf traits depended on the experimental plot (environment and age of plants). We specified leaf length and position as continuous variables while the remainder were categorical variables. If a response variable was not identified as part of a factor (see *Results*), then we did a separate ANCOVA with that variable as the response using the same model described above. We also did a separate ANCOVA to investigate the effect of cross type on the length of the longest leaf by using length as a response variable.

Relationships between the leaf functional traits and herbivory were compared using partial correlation coefficients from the factor analysis. Herbivory also was analyzed using a logistic regression of herbivore damage (0 or 1) as a function of continuous leaf traits (using JMP).

RESULTS

Three factors, linear combinations of the 13 response variables, accounted for 64% of the variation. Factor 1, which accounted for 26% of the total variation, showed high loading for the physiological traits June $A_{\max}$, August $A_{\max}$, June WUE, August WUE (factor loadings = 0.87, 0.88, 0.79, and 0.72, respectively). Factor 2, which accounted for 23% of the total variation, showed high loading for A_{mass}, percent C, LDMC, SLA, and PNUE (factor loadings = 0.87, 0.68, –0.63, 0.87, and 0.82, respectively). Factor 3 comprised percent N and C:N (factor loading 0.98 and –0.93, respectively) and accounted for 16% of the variation. Herbivore damage and average trichome number did not group with any other variable to form a factor.

Cross type significantly affected factor 1 functional traits, as did longest leaf length, location (row, position, and plot) and the cross by plot interaction (Table 1). Inbred (I) *Echinacea* had the lowest photosynthetic rate (Fig. 1a) and WUE (Fig. 1b) followed by within remnant crosses (W). Between remnant crosses (B) were highest, but in some cases W were not different statistically from either I or B cross types (differences among cross types $P < 0.001$; Fig. 1).

The factor 2 grouping, comprising leaf morphological and metabolic traits, also were affected by cross type, longest leaf length, location, and a cross by plot interaction (Table 1). Inbred plants expressed the lowest composite values for factor 2 relative to B or W cross types, regardless of plot (Table 2). PNUE, SLA, and A_{mass} were each ~10% lower in inbred crosses than B or W, while percent C, LDMC, were 1–4% higher in inbred plants.

The main effects on factor 3, percent N and C:N, were longest leaf and location (row; Table 1). Cross type affected factor 3 marginally (Table 1); inbred cross types grouped on their own with a mean (±SE) of 0.118 ± 0.095 while composite values of both the B and W cross types overlapped strongly with one another (–0.149 ± 0.012 and –0.131 ± 0.078, respectively), but not with I. On average percent N was at about 1.5% higher for inbred plants compared to B and W.

Three distinguishable groups of *Echinacea* cross types appeared after plotting ellipsoids that captured 70% of the variation in the three factors, and after accounting for plant size and location (Fig. 2). The between remnant cross types were relatively distinct from the inbred while the within remnant cross types were intermediate in the bivariate and three-dimensional plots (Fig. 2).

As noted, an interaction between cross type and experimental plot (INB1 or 2) contributed to differences in the composite functional traits represented by factors 1 and 2. Inbred plants expressed much lower composite values of factor 1 and factor 2 traits in the INB2 experimental plot with 7-year-old plants, than in INB1 plot, which comprised older 12-year-old plants (Table 2). However, the relative ranking of cross types remained the same in each experiment; inbred crosses always expressed the lowest composite scores followed by within and between remnant crosses. The experimental plot did not explain differences among plants with regard to factor 3 ($P > 0.05$) or interact significantly with cross type (Table 1).

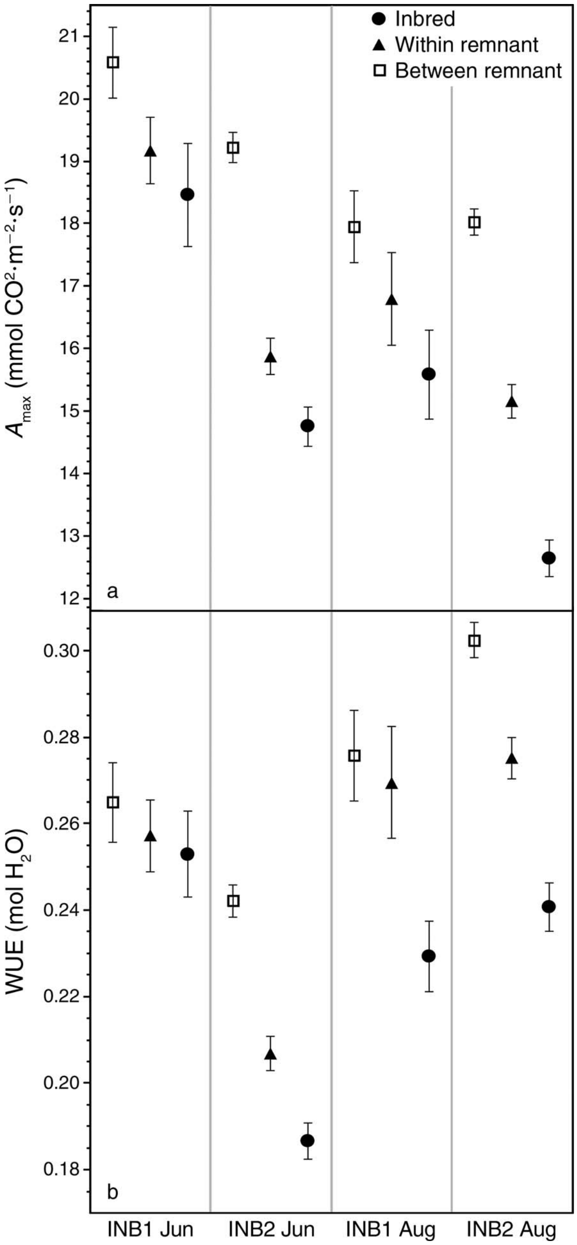

FIG. 1. (a) Average photosynthetic rates on an area basis (A_{max}; mean ± SE) and (b) instantaneous water use efficiency (WUE; mean ± SE) for three cross types of *Echinacea angustifolia* (inbred, between and within remnant cross types) in both experimental plots (INB1 and 2) early summer (27 Jun–12 Jul) and during peak flowering (12–15 Aug). Regardless of month or plot, between-remnant cross types always had the highest A_{max} and WUE, while inbred crosses had the lowest, and within-remnant cross types were intermediate.

Average trichome density did not correlate with other factors (Table 1) or the other functional traits (factor analysis partial correlation coefficients, $P > 0.05$). However, average trichome density did vary significantly as a function of cross; inbred plants had, on average, 1 more trichome per 2 cm than W, and 3 more than B. Inbred and within remnant plants had more trichomes (4–7 more trichomes per 2 cm on average) in the older INB1 garden than INB2, and had more trichomes than between remnant crosses.

Overall foliar herbivore damage (percentage of the total) was low in 2013; over 36% of the plants had zero damage. The proportion of plants with damage to any leaf was 64%, 66%, and 62% for between, within, and inbred crosses, respectively. Aphid occurrence was exceptionally low compared to other years (Ridley et al. 2011, Shaw et al., *in press*); aphids were found on only five of 590 plants, and on plants derived from all three cross types. Leaf damage did not load onto any factor, and neither cross type nor length of longest leaf explained patterns in *Echinacea* mean herbivore damage (ANCOVA, $F_{24,520} = 1.12$, $P = 0.318$). The proportion of leaves without damage vs. those with damage also did not differ as a function of cross or any functional trait (logistic regression, $P > 0.05$ for cross type and each trait). Herbivore damage was not correlated with any functional trait, such as nitrogen, A_{max}, or trichomes (partial correlation coefficients, $P > 0.05$). Longest leaf length also was not significantly different among the three cross types (ANCOVA, $F_{21,547} = 3.47$, $P = 0.33$).

DISCUSSION

We found that the three *Echinacea* cross types differed significantly in the expression of functional traits that influence resource capture and plant performance. Inbred plants had the lowest composite means for the three factors, and for specific traits such as A_{max}, WUE, and PNUE, while between remnant cross types had the highest composite and individual means (Figs. 1 and 2; Table 2). Thus, our measurements of functional traits showed no negative effect of crossing between remnants; instead, the effect on metabolic rates and functional traits was positive (Figs. 1 and 2). Within-remnant cross types tended to express intermediate traits (Fig. 2).

High A_{max}, WUE, and PNUE like those for between-remnant crosses suggest better capture or use of resources, which has translated into greater growth or reproductive success of other species (Geber and Dawson 1990, Dawson and Ehleringer 1993). For example, lower photosynthetic rates in *Amaranthus*

TABLE 2. Composite least-square means (±SE) for factor 1 and 2 for each cross type (B, between-remnant crosses; W, within-remnant crosses; and I, inbred) and experimental plot (INB1, INB2).

Cross type, plot	Factor 1	Factor 2
B, INB1	0.549 ± 0.185	0.118 ± 0.196
B, INB2	0.686 ± 0.079	0.269 ± 0.080
W, INB1	0.056 ± 0.207	−0.063 ± 0.219
W, INB2	−0.042 ± 0.079	0.093 ± 0.081
I, INB1	−0.170 ± 0.164	−0.074 ± 0.174
I, INB2	−0.625 ± 0.082	−0.451 ± 0.174

Note: Factor 1 is primarily the physiological traits (June and August A_{max} and A_{mass} and WUE), while factor 2 is a composite of A_{mass}, percent C, LDMC, SLA, and PNUE.

mutants were directly related to lower survivorship and fertility than those with higher photosynthetic rates (Arntz et al. 2000). Greater reproductive success also was observed in high-WUE *Xanthium strumarium* than for plants with low WUE (Farris and Lechowicz 1990). Greater WUE was correlated with higher survivorship of *Encelia farinosa* during drought (Ehleringer 1993), and high WUE was selected for in dry treatments of *Cakile edentula* (Dudley 1996). *Echinacea* cross types B and W also appear to conserve water better while photosynthesizing at a relatively high rate (Fig. 1), which could lead to greater survivorship and reproduction, especially in the dry periods that often challenge tallgrass prairie perennials (Weaver and Albertson 1936, Clark et al. 2002). It is important to note that our study occurred in one growing season, which represents a fraction of the lifetime of our plants. The consistency of functional traits within individuals across years merits attention (Arntz and Delph 2001, Geber and Griffen 2003), particularly in plants such as *Echinacea*, where aboveground structures resprout each year from a taproot.

Echinacea leaf architecture was significantly related to physiological rates, which is a pattern found in other studies (Reich et al. 1997, Poorter and Garnier 1999). Inbred plants had smaller SLA, which on average also had lower mass- and area-based rates of photosynthesis, and PNUE. Inbred *Echinacea* also had high percent C and LDMC, which was negatively related to photosynthetic rates (A_{mass}), possibly because the additional molecules that build a heavier leaf were non-photosynthetic (Wilson et al. 1999). The fact that the length of the longest leaves were not different among the cross types indicates that inbred and outbred plants may possess externally similar leaves but that the leaves differ in terms of quality (percent C, LDMC and SLA). Higher LDMC, like in the inbred *Echinacea*, can indicate resource limitation induced by abiotic factors or competition (Kittelson et al. 2008). Gonzalo-Turpin and Hazard (2009) found that patterns in SLA explained survivorship and reproduction of *Festuca eskia* along an elevation gradient, specifically higher SLA was correlated with higher values of fitness. Higher SLA and lower LDMC, like those measured in plants from between remnant crosses rather than inbred *Echinacea*, are also associated with higher growth rates (Navas and Moreau-Richard 2005, Thébault et al. 2011). While there were slight differences among cross types for some of the specific factor 2 traits, even small differences in leaf traits may affect subsequent development and fitness (Dawson and Ehleringer 1993, Dudley 1996). Thus, we might expect that lower expression of morphological and physiological traits associated with factor 2 contributes to the reduced fitness of our same inbred *Echinacea* individuals found by Wagenius et al. (2010).

Inbred *Echinacea* leaves had slightly more N and lower PNUE than leaves from non-inbred plants. Among plants of the same size, inbred *Echinacea* may require slightly more N to maintain a similar photosynthetic rate and foliar biomass, and/or allocate foliar N into more non-photosynthetic constituents such as cell walls relative to non-inbred plants (Lambers and Poorter 1992, Hikosaka 2004). While efficient use of nitrogen can increase plant fitness (Hikosaka 2004), the small differences in foliar nitrogen among *Echinacea* cross types may be less biologically meaningful. However, our study found patterns in foliar N and C similar to those documented for the same INB1 plants by Ridley et al. (2011), which suggests that patterns in *Echinacea* leaf N and C are robust across years. Long-term differences in leaf N among different genotypes may influence processes such as litter decomposition, e.g., more inbred leaves with higher N could increase leaf decomposition rates (Cornwell et al. 2008, Kaproth et al. 2013) or higher C:N leaves of B and W remnant plants may result in slower rates, such that the nutrients remain available to promote plant growth the following spring (Hobbie 1992, Kaproth et al. 2013).

Cross type altered functional traits, which had the potential to influence species interactions, especially herbivory. However, in 2013, there was relatively little insect damage on *Echinacea*, and cross type did not explain the slight differences in foliar damage. We also did not find any relationship between leaf damage and functional traits, even traits often associated with plant susceptibility such as foliar percent N, trichomes, or LDMC (Pontes et al. 2007, Dalin et al. 2008). It is unlikely that 2013 herbivore damage strongly influenced measurements of *Echinacea* functional traits. Interestingly, variation in aphid infestation among *Echinacea* cross types has been found. In June of 2005, Ridley et al. (2011) found significantly more aphids on inbred individuals and between remnant individuals in INB1 *Echinacea* vs. within-remnant crosses, and aphid loads positively correlated to leaf N. While 2013 leaf damage did not depend on cross type or other foliar traits, and did not influence plant functional traits, there can be temporal variation in aphid loads both within a year (Ridley et al. 2011) and among years (Shaw et al., *in press*), and an accumulation of herbivory could affect *Echinacea* survival and reproduction over time. The interaction of functional traits and herbivores remains an open question that will need to be answered in a year when herbivores are more prevalent.

Functional traits in factors 1 and 2 and trichome number differed between the plots (cross by plot interactions), and along dimensions of the plots, as expected if functional traits are sensitive to environmental variation. Instantaneous gas exchange and leaf size can be influenced strongly by environmental variation (Geber and Griffen 2003, McKown et al. 2014). Regardless, inbred plants consistently had the lowest values even if the relative magnitude of the differences among the crosses differed between experimental plots (Table 2). Despite the influence of environment, cross

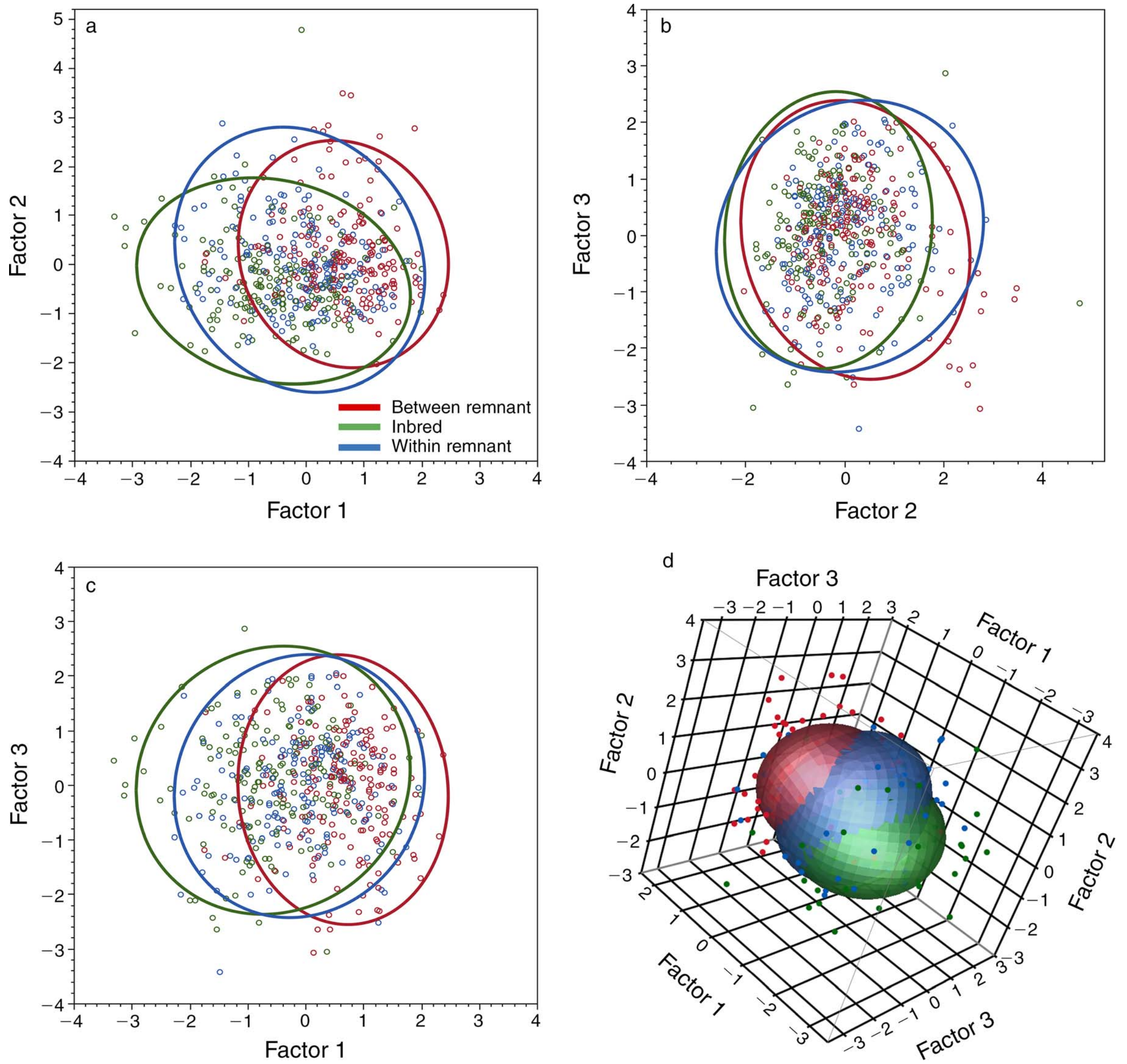

FIG. 2. Factor representation of the three *Echinacea* cross types comparing each factor in pairwise plots (a–c) and using a scatterplot depicting all three factors (d). Each cross type is depicted by a colored ellipsoid that captures 70% of the data. Factor 1 includes June $A_{\max}$, August $A_{\max}$, June WUE, August WUE; Factor 2 includes A_{mass} (average photosynthetic rate on a mass basis), percent C, leaf dry matter content (LDMC), specific leaf area (SLA), and photosynthetic nitrogen use efficiency (PNUE); Factor 3 includes percent N and C:N ratio.

type has a strong effect on plant functional traits, suggesting that selection on genetic variability in functional traits could occur (see Geber and Griffen 2003). The higher mean values for functional traits of inbred plants in the older INB1 compared to younger INB2 (Table 2) is consistent with selection during the course of the experiment having eliminated more of the plants with low values from the older cohort. Differences in ontogeny may also contribute to the trait differences. While it is rare that a single genotype will do well in all environments and variation in traits may change with environmental contexts (Sultan and Bazzaz 1993), our results show that, regardless of location, inbreeding impairs physiological traits, reducing the capture and assimilation of resources and resulting in different ways leaves are constructed, and these alterations in functional traits may translate into plants with lower fitness (Wagenius et al. 2010).

Taken together our results suggest that inbreeding strongly influences functional traits, and these measured differences have the potential to mediate fitness. For example, after seven years of growth, inbred plants in the INB1 plot had the lowest rate of survival, 20% and 35% lower than between and within cross types, respectively (Wagenius et al. 2010). We measured the survivors of these three cross types and fitness of our same group of inbred plants from INB1 was ~66% less than B and W cross types up to 2010 (Wagenius et al.

2010), and ~60% less at the end of 2012 (Shaw et al., *in press*). Other studies have found that functional traits were related to fitness correlates in annuals, e.g., populations of *Polygonatum arenastrum* flowered earlier when $A_{\max}$ was high (Geber 1990, Geber and Dawson 1997) and higher physiological rates in cocklebur was positively related to reproduction (Farris and Lechowicz 1990). Variation in functional traits also may indirectly influence fitness correlates (Dudley 1996, Arntz and Delph 2001). It is surprising that our measure of plant size, length of the longest leaf, did not differ among cross types in 2013, given that plant fitness has varied so strongly. On one hand, length of longest leaf is a coarse measure of size that could miss other key aspects, such as rosette or leaf number. On the other hand, perhaps *Echinacea* leaf length is not as clearly related to past and present fitness as the other functional traits we measured, such as leaf quality. For perennial species, it can take years to estimate fitness and *Echinacea* is no exception because there is little variation in survival and reproduction in a single year; the vast majority of plants survive and only a small proportion of plants reproduce. Our experiment is unique, however, because for INB1, we can relate functional traits to direct measures of fitness that were calculated from a nine year dataset, allowing us to reveal potential mechanisms that underlie the inbreeding depression observed in *Echinacea*. The positive relationship between functional traits and past fitness suggests that functional traits may be a good single-year predictor of survival and reproduction in subsequent years.

Little is known about how functional traits vary within populations, let alone whether patterns in reproduction and functional trait expression influence fitness in fragmented populations. Our combined results indicate that to the extent that habitat fragmentation increases the prevalence of inbred plants in populations, the traits of these individuals could reduce resource capture and assimilation, and have potential to mediate impaired survival and reproduction. In our study, physiological performance and persistence of inbred plants is lower than outbred progeny derived from between and within remnant crosses. Inbred individuals simply operate more poorly than outbred plants.

Severe habitat reduction can result in small plant populations, which can lead to inbreeding within remnants as well as outbreeding. Outbreeding occurs when pollinators move long distances among fragments and when people move plants and seed, as is increasingly common in our study area. It remains unclear what patterns might exist for other species common to remnant populations since few studies have examined the effect of in- and outbreeding on underlying functional traits. However, assuming our results are transferable to other long lived perennials, then inbred plants in fragmented populations might express a group of functional traits that can reduce persistence and potentially fitness, thereby exacerbating the numerical and genetic effects of habitat fragmentation on populations. Some restorations collect and use only locally sourced seed from few remnants to retain locally adapted genotypes (Broadhurst et al. 2008, Leimu and Fischer 2008) while bolstering population size, but this practice could result in inbred populations with compromised functional traits, especially if restored populations remain small. Other restorations use seeds from distant sources, much greater than the 6 km distance between our farthest sites of origin. While we found no adverse effect of outcrossing among remnants on functional traits, we cannot generalize that between remnant crosses at substantially greater distances would perform similarly. Regardless, our results suggest that restoration or active management needs to consider balancing local adaptation with avoidance of inbreeding.

Plant functional traits bridge evolution and community dynamics or ecosystem processes. This study shows that inbreeding, a phenomenon predicted and observed to occur in fragmented populations, influences key organismal traits, such as plant structure, physiology, and elemental composition. These traits are known to influence nutritional quality for herbivores, competitive interactions, and soil–microbe dynamics providing evidence that widespread changes in the genetic composition of remnant plant populations have potential to influence community and ecosystem dynamics.

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