SCALE DEPENDENCE OF REPRODUCTIVE FAILURE
IN FRAGMENTED *ECHINACEA* POPULATIONS

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Abstract. I investigated reproduction in a three-year study of *Echinacea angustifolia*, purple coneflower, growing in a fragmented prairie landscape. I quantified the local abundance of flowering conspecifics at individual-based spatial scales and at a population-based spatial scale. Regression analyses revealed that pollen limitation increased while seed set and fecundity decreased with isolation of individual plants. Isolation, defined as the distance to the kth nearest flowering conspecific, was a good predictor of pollen limitation, for all nearest neighbors considered ($k = 1$–33), but the strength of the relationship, as quantified by R^2, peaked at intermediate scales ($k = 2$–18). The relationship of isolation to seed set and fecundity was similarly strongest at intermediate scales ($k = 3$–4). The scale dependence of individual density effects on reproduction (density of flowering plants within x meters) resembled that of isolation. Analyses at a population-based scale showed that pollen limitation declined significantly with population size. Seed set and fecundity also declined with population size, but significantly so only in 1998. Whether quantifying local abundance with population- or individual-based measures, reproductive failure due to pollen limitation is a consistent consequence of *Echinacea* scarcity. However, individual-based measures of local abundance predicted pollen limitation from a wider sample of plants with a simpler model than did population size. Specifically, the largest site, a nature preserve, is composed of plants with intermediate individual isolation and, as predicted, intermediate pollen limitation, but its large population size poorly predicted population mean pollen limitation.

Key words: Allee effect; bee; density dependence; Echinacea angustifolia; *habitat fragmentation; isolation; pollen limitation; resource limitation; self-incompatibility; spatial scale; style persistence.*

INTRODUCTION

Habitat fragmentation increases the risk of extinction for many remnant populations via diverse mechanisms: reduction of genetic diversity (Newman and Pilson 1997, Saccheri et al. 1998), extirpation of interactants (Rathcke and Jules 1993), alteration of ecosystem functioning (Leach and Givnish 1996), and stochasticity (Menges 2000). Reproduction can be particularly sensitive to fragmentation due to alteration of spatial distributions of potential mates and, in plants, changes in pollinator abundance and behavior. Empirical investigations have supported predictions that pollination and reproduction decrease with fragmentation yet synthesizing the literature has been difficult because studies use disparate methodologies at different spatial scales (reviewed in Aizen et al. 2002). For example, fragmentation effects on local plant abundance can be estimated for populations and individuals. Investigating relationships among local abundance, pollination, and fecundity for individual plants at multiple spatial scales would shed light on the mechanisms and consequences

of widely observed reproductive failures (Burd 1994, Roll et al. 1997, Ashman et al. 2004). Comparing the same relationships at both population- and individual-based levels in a single study would provide evidence about the quality of inferences from previously observed population patterns (Aizen et al. 2002) to hypothesized individual processes (Haig and Westoby 1988, Ashman et al. 2004).

Causes of low seed set are broadly classified as pollen and resource limitation (Bierzychudek 1981). Pollen limitation occurs when seed set in plants is limited by receipt of compatible pollen. Pollen limitation is a major factor in reducing seed set of flowering plants in diverse contexts including habitat fragmentation (Burd 1994, Larson and Barrett 2000, Aizen et al. 2002). Characterizing the spatial pattern of pollen limitation is necessary to understand its causes. Indicators of unfertilized and unvisited flowers (e.g., style persistence and untripped flowers) can quantify pollen limitation in individuals with little disruption to natural spatial patterns, but only if variation in pollen limitation is present and only in some species (Aizen and Feinsinger 1994, Parker 1997, Wagenius 2004). Pollen supplementation, another method for distinguishing and quantifying causes of low seed set, requires moving pollen which might obliterate natural patterns of reproduction in small populations (Young and Young 1992, Wagenius 2004).

When characterizing spatial patterns to assess the consequences of fragmentation, researchers often focus either on populations or on individuals. The extent of habitat fragmentation can be assessed by quantifying the local abundance of flowering plants on either an individual or population basis (Kunin 1997a). Population-based measures of local abundance include population size (number of individuals), mean population density, or distance to nearest population (Aizen et al. 2002). Individual-based measures of local abundance include: isolation, defined as the distance to the nearest neighbor (Allison 1990) or, more generally, to the kth nearest neighbor, and local density, defined within x meters of each focal plant (Roll et al. 1997). All of these individual-based measures are scale dependent, i.e., they can be defined at different spatial scales of resolution. Furthermore, individual- and population-based research perspectives address different hypothetical mechanisms of pollen limitation. For example, pollinator scarcity is a possible cause of pollen limitation by either an individual- or population-based mechanism. Plants with many nearby neighbors may get more pollinator visits compared to isolated plants because they attract more pollinators (Sih and Baltus 1987, Steven et al. 2003). Alternatively, plants in large populations may get more pollinator visits than plants in small populations because large populations support larger pollinator populations (Jennersten 1988, Rathcke and Jules 1993). Another example is scarcity of compatible mates, which also could cause pollen limitation by distinct population- or individual-based mechanisms (Byers and Meagher 1992, Byers 1995). Plants in large populations may receive more compatible pollen than plants in small populations because large populations have greater mating-type diversity (DeMauro 1993). Alternatively, plants with many nearby neighbors may receive a greater proportion of compatible pollen compared to plants with few nearby neighbors because nearest neighbors tend to be mating-incompatible (Levin 1989). Habitat fragmentation is expected to reduce the local abundance both of individuals and of populations, resulting in a correlation of fragmentation measures across scales (Kunin 1997b). Consequently, it is not possible to distinguish population- and individual-based hypothetical causes of pollen limitation with an experiment conducted at a single spatial scale (Kunin 1997b, Steffan-Dewenter et al. 2002).

In this paper, I report a study of patterns of reproductive failure at multiple spatial scales in *Echinacea angustifolia*, in a landscape fragmented by agricultural activity. To quantify distinct aspects of *Echinacea*'s reproductive biology, I quantify four measures of individual annual reproductive fitness: style persistence (an index of pollen limitation), seed set, floret production, and fecundity (Wagenius 2004). I present a three-year study with large spatial scope (23 populations in a 6400-ha study site) and with fine-scale resolution (quantifying pollen limitation in and mapping of >2000 individual plants). First, I investigate the scale dependence of all four reproductive fitness measures on two individual-based measures of local abundance (isolation and local density). Then, I test the hypothesis that population size influences reproduction. Finally, I compare the population- and individual-based methods of characterizing spatial patterns of pollen limitation.

METHODS

Study species and components of fitness

The narrow-leaved purple coneflower, *Echinacea angustifolia* (Asteraceae), hereafter *Echinacea*, is a common plant native to the tallgrass prairie and plains of North America. *Echinacea* is a long-lived perennial with a single taproot. Plants reproduce only by seed and, in the field, rarely flower before their third year. In my study area, a flowering plant usually produces one inflorescence or head per season (mean = 1.2), but may have >10 heads per season. The large, distinctly colored heads stand above surrounding vegetation and are conspicuous during flowering (July and early August). Plants produce basal leaves in years when they do not flower. *Echinacea* seeds have no specialized means of dispersal. *Echinacea* shares two reproductive features with many plants from large, continuous populations in unbroken prairie: self-incompatibility and pollination by generalist insects. The self-incompatibility system, attributable to a single genetic locus with many alleles, prevents fertilization by self pollen and by pollen from closely related plants (De Nettancourt 1977). *Echinacea* inflorescences are visited by many generalist insects including bees, butterflies, flies, and beetles (*personal observation*). In observations of >1000 bee visitors to *Echinacea,* I have observed native bees from four families, but never a bumble bee (*Bombus*), and only three times a honey bee (*Apis mellifera*).

Style persistence, SP, is a measure of pollen limitation that is determined independently of the other fitness components. In *Echinacea,* receptive styles persist until they receive compatible pollen, at which point they shrivel within 24 h. I calculated SP, the average duration in days of all rows of styles on all heads of a plant by observing or inferring the first and last days of receptivity for each style row during the flowering season, i.e., before seed set. For example, an SP value of one means that no row was ever observed to persist longer than one day, characteristic of well-pollinated plants. A value of six means that rows of styles on the plant remained receptive for an average of six days (methods detailed in Wagenius 2004).

Floret production and the proportion of seeds set are two distinct aspects of floral biology that can be attributed to resource availability and pollination, respectively; they are the two main components of annual reproductive fitness in *Echinacea* (Wagenius 2004). Floret production is the count of all florets on all heads of a plant and it increases with resource availability (Franke et al. 1997; *personal observation*).

Echinacea produces a determinate number of uniovulate florets before flowering begins and, regardless of pollination, the seedcoats expand and harden into "achenes." Floret production is estimated at the end of the flowering season by removal and direct count of achenes. If successfully pollinated, each disk floret produces one uniovulate fruit. The proportion of seeds set for a given plant is the fraction of achenes with an embryo, hereafter "seed set." I estimated seed set by germinating a sample of 45 achenes per head from each head on a plant using a standard protocol and then by dissecting all non-germinants to detect the presence of an embryo (Feghahati and Reese 1994, Wagenius 2004). Fecundity, or annual reproductive fitness, is the actual number of seeds produced per plant per year. This fitness measure is estimated as the product of floret production and seed set. I previously found that population mean fecundity is independent of floret production, but population mean seed set strongly predicts population mean fecundity, demonstrating that strong pollen limitation overrides the fundamental relationship between floret production and fecundity. I also found that SP is independent of floret production and that SP predicts seed set, and thus fecundity (Wagenius 2004).

Study site and sampling

The study area comprises 6400 ha of rural western Minnesota, USA (centered near 45°49' N, 95°43' W). Before European settlement in the 1870s, the entire area, except for lakes and wetlands, was potential *Echinacea* habitat. *Echinacea* and other prairie plants now persist in remnant populations on hillsides too steep for agricultural production, in fence corners inaccessible to farm machinery, along road and railroad rights-of-way, and on abandoned pastureland. I located and mapped all remnant prairie with *Echinacea* in the study area. Potential habitat for *Echinacea* was identified in aerial photos and by field observation. The largest population occupies a 45-ha virgin prairie preserve owned and managed by The Nature Conservancy. I used 22 remnant populations for detailed observations ranging in size from 1 to 5044 flowering plants per year (Appendix A). In each population, all flowering *Echinacea* plants were counted in exhaustive searches, except at the preserve. I estimated population size of the preserve by counting all flowering plants within randomly placed, 10 m wide, belt transects that spanned the preserve.

Sampling individuals for maps and quantifying SP.—I mapped all flowering plants from all remnant populations using standard, high-precision (±3 cm) surveying procedures. Each plant was uniquely identified with a numbered aluminum tag at its base and each head had an inconspicuous tag at the base of the peduncle. In the preserve, I mapped only plants flowering on a 600 m long, 5 m wide transect. In 1998, I also mapped all plants flowering within 30 m of the transect, but did not tag

them. To determine SP in 1996, an observer visited each plant twice each week during the flowering season and characterized the receptivity of all style rows on each head on every plant using previously described methods (Wagenius 2004). During the summers of 1997 and 1998, an observer visited every plant every third day. Observations totaled 630 plants in 1996, 1020 plants in 1997, and 536 plants in 1998.

Sampling individuals for fitness measurements.—In 1998, I selected six plants from each of 27 different remnant populations, and six plants from each of two management units on the preserve. Plants were chosen at random from a list of all flowering plants in each population. On the preserve, plants were chosen at random from all flowering plants on the transect. In remnants with six or fewer plants, I selected all flowering plants (total 142 plants). Each head was collected in late August or September after seeds had matured. Heads were stored individually in paper bags at 4°C until achenes were removed. From each head, I randomly selected 45 achenes for germination trials the following January. In 1997 the harvest and storage schemes were identical, but I sampled 167 plants from 19 remnant populations (Wagenius 2004).

Quantifying spatial pattern.—Using the database generated by detailed mapping of plants (see *Methods: Sampling individuals for maps*), I calculated three indices of the local abundance of flowering conspecifics. The first index, population size, was defined each year as the number of flowering plants in each population. Populations were delimited as groups of plants all visible from one location and separated from other populations by trees or >50 m of *Echinacea*-free habitat. Individual-based indices, isolation and density, were defined for each plant at multiple scales. Isolation was defined as the distance to the kth-closest flowering conspecific. Density was the density of flowering conspecifics within x m. Hereafter, all references to isolation and density refer to these individual-based, scale-dependent definitions. For all non-preserve flowering plants in 1996, 1997, and 1998, I determined isolation for $k = 1$–33 and density for $x = 1, 5, 10, 30, 50,$ and 100 m. Plants in small populations had some kth nearest neighbors in a different population. Additionally, for preserve plants in 1998 I determined density for $x = 1, 5, 10,$ and 30 m and isolation for $k = 1$–9 (fewer than 95% of the plants had their 10th nearest neighbor within 30 m).

Comparison of population- and individual-based methods

I compared the population- and individual-based methods of characterizing spatial patterns of reproduction in four ways. First, I examined the dependency of all four measures of reproduction on both individual-based measures of local abundance, isolation and density, at a range of scales over two years using the subset of plants for which I had determined seed set and floret production. To compare the strength of the relationship between the measure of local abundance
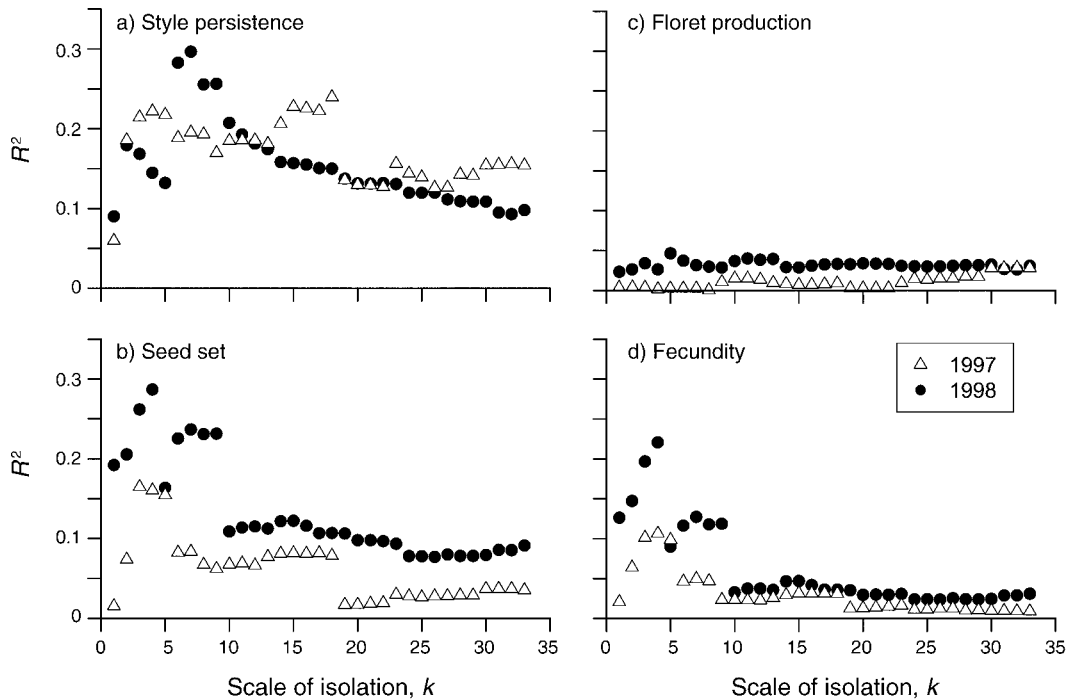
FIG. 1. Scale-dependent effects of isolation on four individual annual reproductive fitness measures over two years, showing R^2 values for simple linear regressions between the distance to the kth nearest flowering *Echinacea angustifolia* plant and four fitness measures. (a) SP is style persistence (pollen limitation) in days. (b) Seed set is the proportion of achenes with an embryo and is closely related to pollination. (c) Floret production is the count of uniovulate achenes produced per plant and is dependent, in part, on resource availability before the flowering season. (d) Fecundity, or annual reproductive fitness, is estimated as the product of floret production and seed set, two independent factors (1997, $n = 126$ plants; 1998, $n = 129$ plants).

and the measure of reproduction across scales, I use the coefficient of determination, R^2, which jointly reflects the strength of the local abundance effect, the variation in the measure of abundance, and, the goodness of fit of the simple linear regression model. Second, I conducted a parallel investigation of the dependency of reproduction on population size. Third, I used linear regression to assess the relationship between SP and each of the three measures of local abundance, over all plants over three years. Finally, to gauge how habitat fragmentation affected each measure of local abundance, I compared the distribution of values of each measure. In particular, I focused on the differences between the preserve site and the surrounding remnants. I also investigated how the distribution of values for each individual-based measure varied with the spatial scale. All statistical analyses were performed using R version 2.0.1 (R Development Core Team 2004).

RESULTS

Individual-based analyses

Individual isolation influenced three of the four reproductive fitness measures in this study in a scale-dependent fashion (Fig. 1). Isolation was a good predictor of pollen limitation, as quantified by SP, for all kth nearest neighbors considered ($k = 1–33$); when neighbors were closer, pollen limitation was lower. The strength of the isolation–SP relationship, as quantified by R^2, peaked with isolation to neighbors of intermediate proximity ($k = 2–18$) and was weakest when considering the closest neighbor ($k = 1$) and least-proximate neighbors ($k > 18$) (Fig. 1a). The relationship of isolation to seed set was also strongest at intermediate scales ($k = 3–5$ in 1997 and $k = 1–9$ in 1998), reflecting the isolation–SP relationship. In contrast to the other fitness measures, floret production showed little relation with isolation (Fig. 1c). The strength of the isolation–fecundity relationship was greatest for close proximity neighbors, but not the closest, in both years ($k = 2–8$ in 1997 and $k = 1–9$ in 1998), thus showing a pollen limitation effect, but over a narrower range of spatial scales than was observed in style persistence or seed set (Fig. 1d).

Viewing the data set at a single scale reveals the extent of variation among individuals, the size of the isolation effect, and, perhaps, the potential for biological relevance more completely than does a summary of R^2 values over multiple scales. The fourth nearest neighbor represents the intermediate scales where R^2 values peaked. In each year, at the fourth nearest neighbor scale, SP decreased with isolation (Fig. 2a, b). Predicted mean seed set varied from 40% to 50% for the least
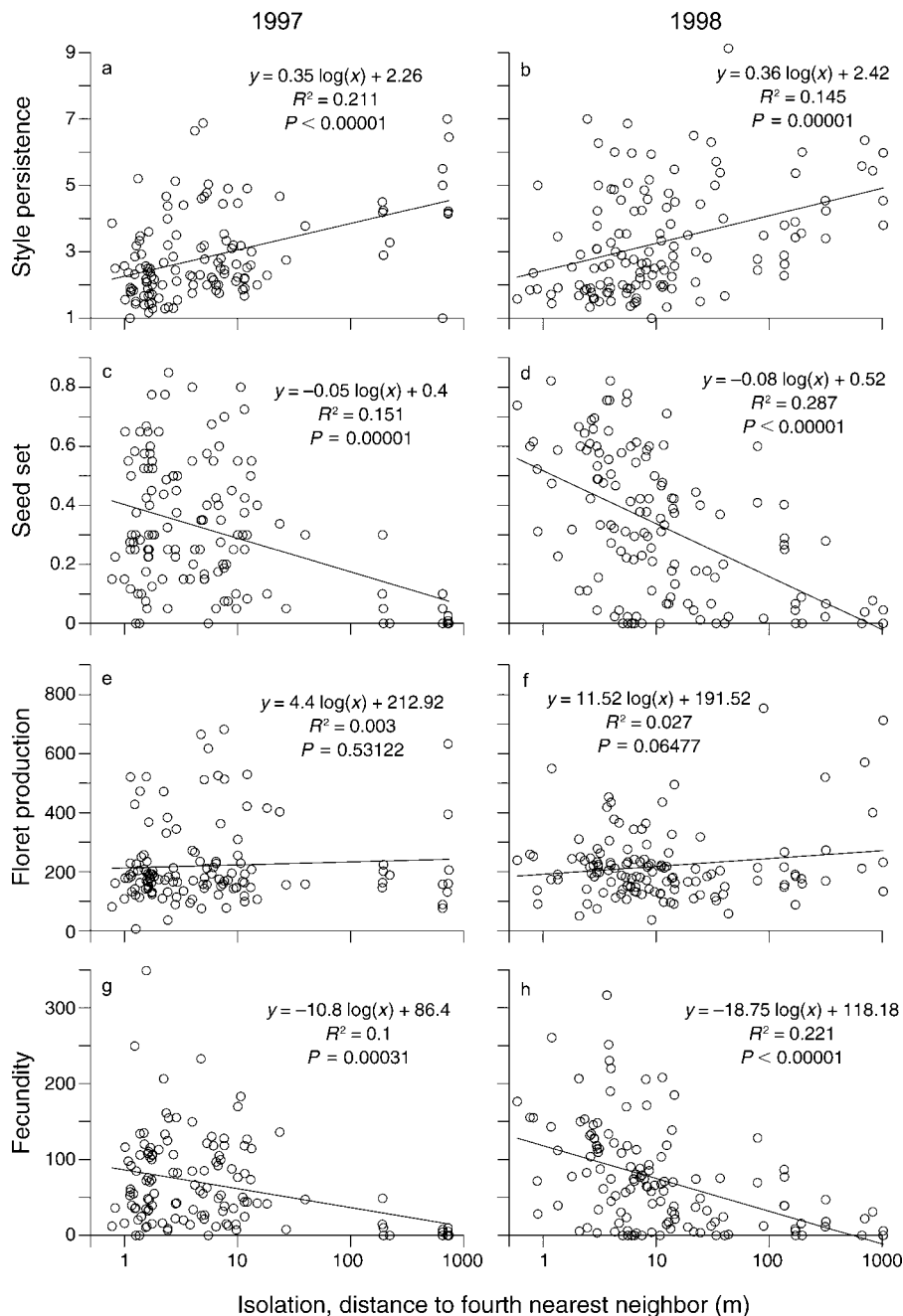
1997 1998

FIG. 2. The relationship between four annual reproductive fitness measures and individual plant isolation, quantified as the distance to the fourth nearest flowering neighbor, shown on a log scale (1997, $n = 126$ plants; 1998, $n = 129$ plants). Similar relationships hold for the first through the 33rd nearest neighbors; see Fig. 1. Preserve plants in 1997 are not included in this graph because flowering *Echinacea* plants off of the transect were not mapped that year.

isolated plants to seed set <10% for the most isolated plants (Fig. 2c, d). In contrast, individual floret production, a fundamental component of fecundity, did not vary with isolation or trended in the opposite direction (Fig. 2e, f). However, floret production did vary considerably, from 6 to 754 (three outliers had >1000). Predictions of fecundity for the least and most isolated plants differed by an order of magnitude (Fig.

2g, h). Note that the P values reported (Fig. 2) are valid for a regression at a single scale and are not corrected for multiple tests.

The scale dependence of the relationships between individual density and the reproductive fitness measures, for the most part, paralleled that of the isolation (Appendix B). SP, seed set, and fecundity were significantly predicted by density at scale x in both
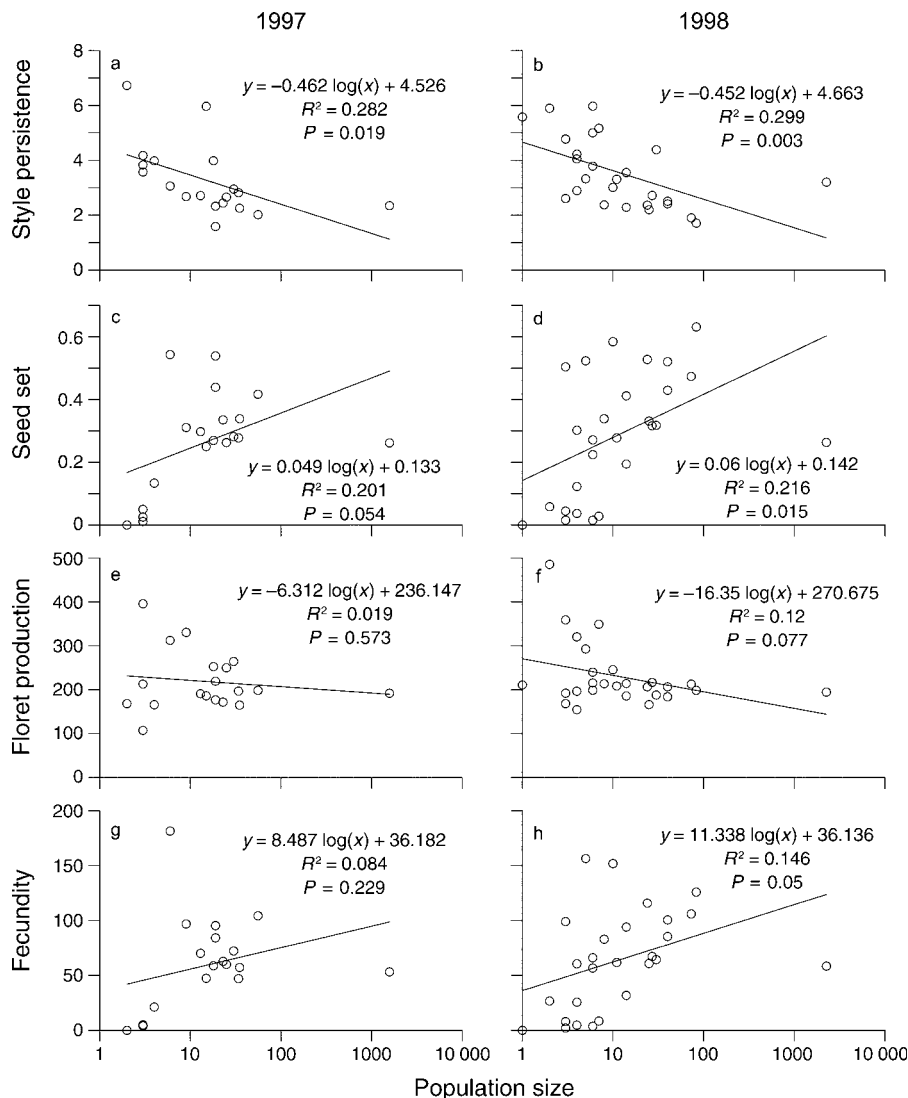
FIG. 3. The relationship between four mean annual reproductive fitness measures and population size (log scale) in *E. angustifolia* over two years, 19 populations in 1997 and 27 in 1998. The preserve, the rightmost point in each graph, is an influential observation in the seed set and fecundity graphs. When the preserve site is excluded, then all four relationships are significantly positive (Appendix C).

years for all *x* except *x* = 1 m in 1997. As with isolation, the strengths of the relationships with density peaked at intermediate radii, *x*, in 1998 with maximum R^2 for SP and seed set at *x* = 30 m, and with maximal R^2 for fecundity at *x* = 5 m. In 1997 the peaks were less distinct. Floret production in 1997 was not strongly related to density at any scale but, in 1998 for *x* = 30, 50, and 100 m, density was negatively related to floret production (Appendix B, Fig. B1). Note that estimates of density and isolation at a given scale are not independent of the measures on the same individual at a finer scale.

Population-based analysis

In both years there was a significant negative relationship between population size and pollen limitation, as measured by style persistence (Fig. 3a, b). Seed set and fecundity reflected the observed relationship between SP and population size, but significantly so only in 1998 (Fig. 3). When the preserve site was excluded, then all three relationships were significant (Appendix C). Population size was thus a good predictor of the annual reproductive fitness components influenced by pollen limitation for all populations except the preserve. The preserve site appeared to be an outlier in the distribution of population sizes in each year with >1000 flowering plants each year, but consisted of plants with intermediate means of SP, seed set, and fecundity. Unlike the other measures, floret production was related to population size only weakly in either year with or without the preserve included, but there was a consistent
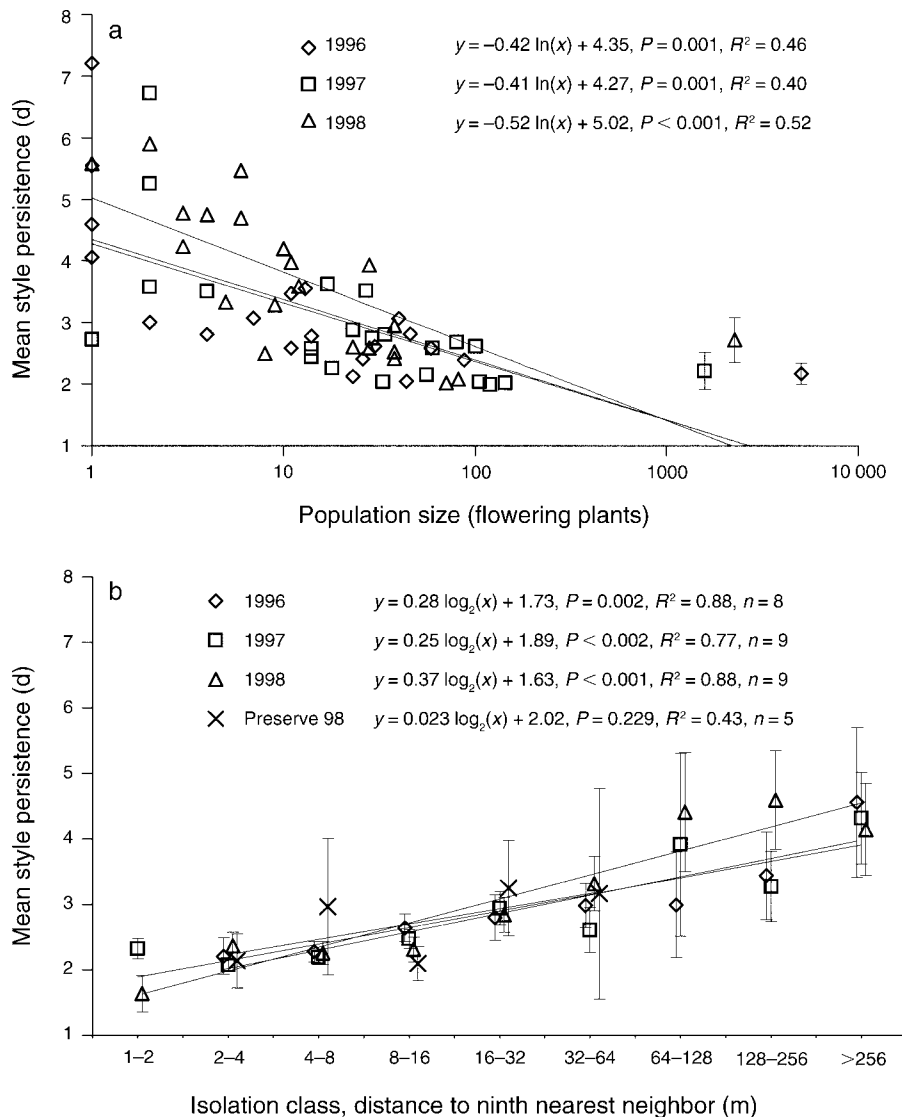
FIG. 4. The relationship between pollen limitation, quantified as mean style persistence (SP), and (a) population- and (b) individual-based measures of local abundance of flowering *E. angustifolia* plants over three years in 22 remnant populations in the study area. In (a), local abundance is quantified as the annual count of flowering plants in the remnant. The three rightmost points represent the preserve. In (b), local abundance is quantified as individual isolation, the distance to each plant's ninth nearest neighbor flowering *Echinacea* plant, grouped into isolation classes. SP is significantly positively related to isolation in each year where isolation is quantified as the maximum distance in each isolation class. Isolation for plants on the preserve was measured only in 1998, and this regression slope does not differ significantly from zero; however, within each isolation class, the mean pollen limitation of preserve plants is not significantly different from plants in surrounding remnants. All points in both panels show 95% confidence intervals, except in remnants other than the preserve, where mean SP was determined with a census of all plants.

trend that larger populations had lower floret production (Fig. 3e, f, Appendix C).

Comparison of population- and individual-based analyses

The role of the preserve site differs in the population- and individual-based analyses, even though both analyses reveal that local abundance of flowering plants strongly influences SP and that pollination is a major determinant of annual reproductive fitness. Over three years for all mapped plants, SP was negatively related to population size (Fig. 4a) and positively related to isolation of individual plants (Fig. 4b). The isolation–SP relationship for $k = 9$ is the greatest k encompassing 95% of the sampled preserve plants. In the population-based analysis, the preserve site appears to be an outlier in each year (Fig. 4a). In each year, the linear regression without the preserve accounts for more of the observed variation than when the preserve is included (compare

Fig. 4a with least square linear regressions excluding the preserve: 1996, $P < 0.001$, $R^2 = 0.57$; 1997, $P < 0.001$, $R^2 = 0.44$; 1998, $P < 0.001$, $R^2 = 0.72$). In the individual-based analysis, I separately determined the relationship between SP and plant isolation for plants on the preserve in 1998. Within each distance class, the mean SP of preserve plants did not differ significantly from plants in surrounding remnants, but the regression slope was not significant for plants on the preserve. The relationship between preserve and non-preserve plants for $k = 9$ (as shown in Fig. 4b) was qualitatively the same for $k = 1–8$. Thus, in the population-based analysis the preserve site appears as an outlier and its SP is not well predicted by population size, but the individual-based analysis reveals that plants from the preserve are not outliers and their isolation–SP relationship does not differ from non-preserve plants.

The univariate distributions of individual isolation and population size reveal that plants experience a wide variation in local abundances of flowering plants. For example, isolation of individual plants ranged from 10 cm to 765 m to the nearest neighbor (Appendix D, Fig. D1). Some plants in fragmented populations experienced extreme isolation but others were less isolated than the least isolated plants on the preserve transect (Appendix D, Fig. D1). The range of isolation ($k = 9$) for preserve plants was 2.95–28.35 m, while for non-preserve plants the range was 1.40–1037 m. The distribution of isolation in the preserve plants fits within the range of all others for all $k < 10$ but distributions varied with scale (Appendix D, Fig. D1) and for $k > 9$ this relationship is unlikely to hold. Population sizes also ranged widely (Appendix A), and to the extent that a comparison of the preserve to other remnants reflects the effect of habitat fragmentation on local abundances, habitat fragmentation has affected population size drastically compared to individual isolation.

A comparison between population size and individual-based density is similar to the above comparison with isolation (Appendix B). Likewise, the univariate distributions of density, both on and off preserve, parallel the distributions of isolation (Appendix D, Fig. D2).

DISCUSSION

For *Echinacea* plants within the study area, pollen limitation, as quantified by SP, consistently and strongly varies with the local abundance of flowering conspecifics. Regardless of how local abundance is characterized (population size, individual isolation, or local density), scarcity of neighbors is associated with greater pollen limitation in all three years of this study, resulting in reduced seed set and fecundity in isolated plants. Many of the effects of local abundance on annual reproduction are scale dependent. These results imply that reduced reproductive success is a consistent feature of isolated *Echinacea* plants in a prairie landscape fragmented by agriculture, and perhaps in many other

outcrossing prairie species (Molano-Flores and Hendrix 1999, Hendrix and Kyhl 2000).

Both individual- and population-based perspectives reveal that greater fragmentation reduces annual fecundity because of increased pollen limitation. However, individual-based models seem more informative in two critical ways. First, the simple linear regression on the population scale poorly predicts pollen limitation observed on the preserve (Fig. 4a). Modifications to the model, such as adopting a nonlinear saturating function or limiting its domain to exclude the preserve, would account for more of the observed variation, but at the expense of simplicity in the statistical model and in mechanistic explanations. Second, simple individual-based linear regression models readily account for the variation in pollen limitation observed within the preserve and among all plants in the study area (Fig. 4b). On the basis of interpreting pollen limitation from the widest sample of plants and of fitting the simplest statistical model, methods with individual-based measures are superior to a population-based method. In particular, the individual-based approach suggests that the SP–isolation relationship among preserve plants does not differ from the relationship among all other plants—a pattern undetectable with a population-based predictor such as population size or mean population density. Apart from the additional information that individual-based methods provide about this study system, the individual-based empirical perspective relates more closely to pollen limitation theory, which typically predicts evolutionary and ecological causes and consequences of pollen limitation for individual plants not populations (Haig and Westoby 1988, Kunin 1993, 1997b, Ashman et al. 2004). Because the individual-based empirical results show that the relationship between pollen limitation and isolation is scale dependent, it is helpful to consider the observed pattern of scale dependence when relating these findings to theory.

The scale dependence of the relationship between pollen limitation and individual isolation (or density) provides an explanation for the discrepancy between population- and individual-based perspectives on this relationship and also offers insight into the mechanistic basis of the relationship. The effects of individual-based measures of local abundance on SP peaked at close to intermediate spatial scales (Fig. 1), suggesting that relevant spatial scales are much finer than the dimension of the preserve, such that the abundance of nearby plants within the preserve is more important for pollination than plants farther away. This accounts for the discrepancy in individual and population perspectives. Specifically, pollination services, which depend on the spatial distribution of compatible mates and insect abundance and movement, must vary considerably within the preserve and also within smaller remnant populations. An additional aspect of scale dependence is that the most local abundance (within 1 m and the first closest neighbor) is a poor predictor of SP, suggesting

that the nearest flowering plants may be frequently incompatible (Byers and Meagher 1992). In spite of the many other factors that influence them, both seed set and fecundity have a strong pollen limitation signal in the scale dependence of their relationship with individual isolation and density.

Two broad mechanisms are often invoked as possible causes of pollen limitation among plants with reduced local abundance of flowering conspecifics: pollinator scarcity and mate scarcity. Each of these causes has distinct population- and individual-based mechanisms and each may contribute to pollen limitation in *Echinacea*. Although neither pollinator visitation or mate compatibility was measured in this study, theoretical and empirical work by others informs the interpretation of spatial patterns of pollen limitation in *Echinacea*.

Pollinator scarcity or pollinator limitation is frequently cited as a cause of pollen limitation (Rathcke and Jules 1993, Aizen et al. 2002). In both natural and experimental populations, investigators have studied mean density and population size effects on pollination (reviewed in Kunin 1997a), but few have quantified individual-based measures of abundance (but see Roll et al. 1997) or explicitly investigated multiple scales (Thomson 1981). In a study of pollinating insect guilds within a fragmented landscape, Steffan-Dewenter et al. (2002) found that the abundance of solitary bees attracted to arrays of potted plants was affected by the proportion of seminatural habitat within 250 m of the array, the finest scale measured in their study. Solitary bees responded to landscape structure at a markedly finer spatial scale than bumble bees and honey bees. They attribute these observations to the pattern of bee foraging, which depends on flying ability, the scales at which bees perceive variation in flower abundance, and the distribution of nest sites. *Echinacea* is pollinated by a similar guild of solitary bees, probably with similar foraging ranges. Reproduction in *Echinacea* is consistent with pollinator limitation and, in particular, the fine-scale variation in pollen limitation observed within the preserve corroborates the behavior of solitary bees in fragmented habitat observed by Steffan-Dewenter et al. (2002).

Mate scarcity is another possible cause of pollen limitation. Fragmentation increases distances between potential mates, reduces diversity of mating types in self-incompatible species, and increases selfing rates. In self-incompatible species, a low proportion of compatible mates has been implicated in pollen limitation of several species, especially in the Asteraceae (e.g., Byers and Meagher 1992, DeMauro 1993, Reinartz and Les 1994, Young et al. 2000). Based on a computer simulation study, Byers and Meagher (1992) predicted that small populations of self-incompatible plants should have lower seed set and that there should be greater variance of seed set rates in small compared to large populations. This is the case in *Echinacea* (Fig. 4a). Furthermore, this

proposed mechanism provides an explanation for the finding that local abundance at the finest spatial scales fails to predict pollen limitation: a close but incompatible first nearest neighbor will not contribute to fertilization. Reduced seed set could be due to either reduced fertilization rate or very early-acting inbreeding depression, each of which can be viewed as limitation on receipt of compatible pollen. Thus, pollen quality (apart from compatibility) may also reduce seed set via inbreeding, if fragmentation increases genetic similarity between pollen donors and recipients (Waser and Price 1991, Ramsey and Vaughton 2000). Reproduction of *Echinacea* in fragmented landscapes is likely limited by availability of compatible mates and perhaps also by pollen quality (Wagenius 2000).

The results of this study support hypotheses of pollen limitation in *Echinacea* by pollinator scarcity and mate scarcity. These results also highlight factors not investigated in this study that likely influence pollen limitation in *Echinacea*, namely: pollinator visitation rates and mate compatibility. Apart from these unaccounted factors that influence pollen limitation, other factors that affect seed set and fecundity are expected to lessen the strength of the relationship between pollen limitation and fecundity. In particular, where the effect of local abundance on floret production was significant, its effect was opposite that of pollen limitation. This is consistent with an interpretation that intraspecific competition for limited resources affects floret production. Given the great potential for variation in pollen limitation due to factors unrelated to the local distribution of flowering conspecifics and the countervailing spatial effect of resource limitation, the strength of the pollen limitation signal in fecundity is remarkable.

Studies of pollen limitation as a function of plant density should consider multiple spatial scales for three reasons. First, inferences about spatial patterns are suspect if the study is conducted at only one scale (Kunin 1997b), especially because it is difficult to know relevant spatial scales a priori (Fig. 1). Second, scale dependence in pollen limitation is likely to be common, given that pollinator movement varies among guilds and depends on landscape in a scale-dependent fashion (Steffan-Dewenter et al. 2002). Third, multiple-scale investigations facilitate comparisons among studies of spatial effects of pollination (Kunin 1997b, Aizen et al. 2002). The benefits of conducting a multiple-scale investigation must be weighed against the effort involved. Style persistence in this study was assessed on individual *Echinacea* heads ~30 000 times over three flowering seasons. Focusing on sampling individuals at a fine scale allows for later consideration of population means and coarser scales. Measuring the distance to the nearest neighbors ($k = 1$–3) is easier than estimating population sizes, at least in *Echinacea*. Also, knowing the spatial scales of pollinator movement or pollen flow and the scales with variation in density or isolation of flowering enables one to focus on the scales most likely

to be relevant. Additionally, sampling randomly from individual plants along a transect, instead of sampling from random locations will insure capturing variation in isolation or density.

This study quantifies a strong positive density-dependent spatial pattern in *Echinacea* reproduction, an Allee effect. Some models of Allee effects predict nonlinear or "threshold" effects of local abundance or connectivity on reproduction or population viability (With and Christ 1995, Fahrig 2002). Two empirical studies corroborate this. In a study of a patchily distributed roadside annual, Groom (1998) found that small patches suffered pollination failure when they were isolated past a certain threshold. In an experimental reintroduction of an endangered herb to fragmented grassland sites, Lennartsson (2002) found that, below certain connectivity thresholds, pollinator deficits reduced population viability via reduced seed production and inbreeding depression. In contrast, *Echinacea* appears to have no clear threshold in reproduction with regard to local abundance. Even so, reduced fecundity may have demographic consequences, but the effects of reduced seed production on seedling recruitment and population growth rate in *Echinacea* remain unknown.

Reproduction in this fragmented landscape depends on local abundance. Human activities, intentional and unintentional, have configured and will continue to alter the potential habitat for *Echinacea* and other native plants. The results of this work suggest that changing the distribution and abundance of potential *Echinacea* habitat can strongly influence *Echinacea* seed production. Small changes in population size for populations up to ~150 flowering individuals per year can strongly affect population mean fecundity. Within a population of any size, increasing the fine-scale local abundance of flowering plants could significantly increase fecundity. Management practices, such as prescribed burning, which increases the local abundance of flowering plants (Kuchenreuther 1996; *unpublished data*), should increase fecundity substantially.

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APPENDIX A

A table presenting counts of flowering plants in focal remnant *Echinacea* populations over three years (*Ecological Archives* E087-054-A1).

APPENDIX B

Figures showing the scale dependence of the relationship between individual-based *Echinacea* density and four individual annual reproductive fitness measures during two years (*Ecological Archives* E087-054-A2).

APPENDIX C

A table presenting regression coefficients of population size on four annual reproductive fitness measures when the preserve is not included (*Ecological Archives* E087-054-A3).

APPENDIX D

Figures showing the distribution of isolation and density of individual *Echinacea* plants in the preserve compared to those in surrounding remnants (*Ecological Archives* E087-054-A4).