Both flowering time and distance to conspecific plants affect reproduction in *Echinacea angustifolia*, a common prairie perennial

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Summary

1. In small fragmented plant populations, reproductive failure due to pollen limitation is often attributed to spatial isolation of individuals. While flowering time has been shown to affect seed set, its role in pollen limited fragmented populations is less understood.

2. In this study, we quantified near-neighbour distances, flowering phenology, and how they interact to affect seed set in individual plants. We followed the daily flowering phenology of over 2400 heads on over 500 *Echinacea angustifolia* individuals and quantified the resulting seed set during three consecutive flowering seasons. The study was conducted in an experimental plot where we randomized planting locations to eliminate spatial patterns of mate availability which are common in fragmented populations of *Echinacea*, a self-incompatible plant.

3. We found that individual flowering time had a larger and more consistent effect on seed set than did spatial location. Seed set in the earliest flowering plants exceeded seed set in the latest by 46–70% in all three years. The role of spatial isolation, characterized both by individual distance to conspecific plants and by location in the plot, was less consistent and showed a weaker relationship with seed set than did flowering phenology. The most isolated plants set 20–27% less seed than the least isolated plants in 2005–2006 with no difference in 2007.

4. In one year, we quantified seed set by floret position within a flowering head. We found significant positional effects; however, effects due to flowering time were much greater. These results were more consistent with the pollen limitation hypothesis than the resources limitation hypothesis.

5. *Synthesis.* Our results illustrate that flowering time and distance to neighbouring conspecifics can cause reproductive failure in fragmented populations, even in the absence of mate limitation caused by mating incompatibility. These findings suggest that flowering time may be an underappreciated contributor to reproductive failure in small fragmented populations.

Key-words: Allee effect, edge effect, flowering phenology, habitat fragmentation, pollen limitation, reproductive ecology, seed set, spatial isolation, tallgrass prairie

Introduction

The proportion of ovules that develop into seeds (i.e. seed set) is a measure of an individual plant's annual reproductive fitness. Many of the factors that affect reproduction, such as resource limitation, pollen limitation and seed predation, often vary with the density of flowering conspecific plants (e.g. Campbell & Halama 1993; Krupnick & Weis 1999; Knight *et al.* 2005). In addition, these factors often vary with the

flowering time of the individual (e.g. English-Loeb & Karban 1992; Gross & Werner 1983). Quantifying the relative and absolute contributions of these effects on reproduction is necessary to understand the demography and ongoing evolution in plant populations, especially in small and fragmented populations where reproduction is often limited by receipt of pollen (reviewed in Knight *et al.* 2005).

Small populations can have increased pollinator limitation compared with larger populations (e.g. Ågren 1996; Brys *et al.* 2004). Within a population, pollinator limitation often increases with isolation such that a low density of nearby flowering conspecifics reduces an individual's reproduction (e.g. Kunin 1997; Field, Ayre & Whelan 2005; Metcalfe & Kunin 2006; Wagenius 2006). This Allee effect is prevalent in insect and bird pollinated species because plants in low density areas

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often attract fewer or less effective pollinators than plants in higher density areas (Schmitt 1983; Grindeland, Sletvold & Ims 2005; Courchamp, Berec & Gascoigne 2008; Duffy, Patrick & Johnson 2013). However, higher plant densities may reduce visitation rates by increasing competition for pollinators (Steven *et al.* 2003), suggesting an intermediate density may be ideal for balancing pollinator attraction and saturation. Measuring distances to nearby neighbouring conspecifics quantifies the isolation of an individual from potential mates, capturing the local patch density that may result in an Allee effect (Wagenius 2006). An edge effect, distinct from isolation, may also affect seed set. Even if environmental conditions do not differ between the core and edge of a patch of flowering plants, the landscape of floral resources differs (Samson & Knopf 1994; Ricketts 2001). Pollinators likely perceive core and edge plants differently. For example, bees arriving from nests in or near the agricultural fields that surround a remnant prairie habitat encounter edge plants first but observe that they are not near the centre of a patch. Pollinators likely behave differently on plants in the core versus the edge with potential consequences for seed set (Fagan, Cantrell & Cosner 1999).

In self-incompatible plants, pollen limitation can be the result of either reduced pollinator visitations or reduced compatible pollen transfer (Byers 1995; Wagenius, Lonsdorf & Neuhauser 2007; Wagenius & Lyon 2010). This is because some pairs of individual plants are not compatible as mates owing to shared self-recognition alleles (de Nettancourt 1977). If the plant population has an underlying spatial genetic structure, neighbouring plants are more likely to share self-recognition alleles than plants that are farther apart (Wagenius, Lonsdorf & Neuhauser 2007). Mating incompatibility thus increases the likelihood of pollen limitation, especially in small fragmented populations with low genetic diversity and fine-scale spatial genetic structure. Reproduction is more often pollen limited in self-incompatible plants than in self-compatible plants (reviewed in Knight *et al.* 2005; Aguilar *et al.* 2006). When mating incompatibility increases with proximity, the effects of near-neighbour distances and mating compatibility on reproduction are confounded (Byers 1995; Aizen & Harder 2007). For instance, in remnant prairie populations of *Echinacea angustifolia*, both mate availability and compatibility increase with population size (Wagenius 2006; Wagenius, Lonsdorf & Neuhauser 2007). Therefore, to separate the effects of distances to neighbouring conspecifics on reproduction from the effects of mating incompatibility, the genetic structure of the population must be controlled.

Flowering time also plays an important role in plant reproduction. Early flowering plants often flower longer (Ollerton & Lack 1998) and produce more seed (Forrest & Thomson 2010; but see Thomson 2010). A recent meta-analysis found that selection typically favours early flowering plants, with stronger selection in temperate species (Munguía-Rosas *et al.* 2011). The extent to which variation in seed set among flowers within an individual contributes to this overall pattern is unknown, but resource limitation and positional effects frequently induce later flowers within a plant to produce fewer seeds than early flowers (Diggle 1997).

Similar to effects due to distances between neighbouring conspecific plants, the effect of flowering phenology on seed set can also depend on pollinator visitation. Individuals that flower during peak flowering may set more seed than those that flower off-peak by attracting more pollinators (reviewed in Elzinga *et al.* 2007). Alternatively, if at peak flowering the pollinator assemblage is saturated, then early or late flowering plants may set more seed because of reduced competition for pollinator services (Sabat & Ackerman 1996). If pollinator services change during the season, then we expect effects on seed set to be evident both among and within individuals.

Ison *et al.* (2014) investigated pollen movement within an experimental plot of the common self-incompatible perennial prairie plant, *Echinacea angustifolia* (hereafter *Echinacea*), by determining paternity of seeds from mapped plants using genetic markers. They found that pollen movement was not random; the likelihood of mating between a pair increased with their spatial proximity and with their flowering synchrony. Pollen movement distances also increased over the course of the flowering season. Their experimental plot eliminated the spatial genetic structure typical of nearby remnant populations (Wagenius, Lonsdorf & Neuhauser 2007). In the nearby remnants, from which the experimental plants originate, mating compatibility in a single population ranged from 26% to nearly 100% (Wagenius, Lonsdorf & Neuhauser 2007). In the experimental plot, a typical plant was compatible with over 85% of other individuals, and planting was randomized. In this plot, patterns of pollination resulting from pollen movement were not obscured by patterns of pollen rejection between incompatible pairs. Based on these findings, we expect that seed set will be higher in plants that flower synchronously with the rest of the population and in plants with many nearby neighbours.

In this study, we investigate the potential effects of non-random mating on seed set in *Echinacea* in the plot described above. Our three-year study contributes to the understanding of reproductive biology and within-population dynamics in this and other species that experience reproductive failure in small and fragmented populations (Wagenius 2006). Specifically, our study quantifies relationships between seed set and three hypothesized predictors of individual seed set: flowering phenology, location relative to edge and distances to conspecific flowering neighbours. Also, we quantify seed set within individual plants based on a floret's flowering time and position within the flowering head.

Materials and methods

STUDY SPECIES AND SITE

Echinacea angustifolia DC. (Asteraceae), the narrow-leaved purple coneflower, is widely distributed across the North American grasslands, ranging from Texas to Canada, and is long-lived, with an estimated generation time of 17–44 years (Hurlburt 1999). In Minnesota, an *Echinacea* plant typically flowers for the first time between three and eight years of age and may not flower each subsequent year; in non-flowering years, it persists as a basal rosette. Plants usually have

one flowering head in years when they flower, although they can have more than 10. Each head consists of 10–30 sterile ray florets, each of which produces a prominent ligule (petal) and 100–300 uniovulate disc florets (hereafter florets) that are arranged in regular circular rows. The florets develop regularly up the flowering head; the bottom row of florets produces anthers first; the top rows of florets produce anthers last. A style emerges through each anther the day after pollen is shed. A style remains receptive for up to 10 days unless it receives compatible pollen (Wagenius 2004). In *Echinacea*, annual seed set (total proportion of seeds produced by a plant) is independent from annual floret production, which is likely resource limited (Wagenius 2004). *Echinacea* has a sporophytic self-incompatibility system which prohibits self-fertilization (de Nettancourt 1977; Wagenius, Lonsdorf & Neuhauser 2007) and is pollinated primarily by a high diversity of native solitary generalist bees foraging for pollen (Wagenius & Lyon 2010).

This study was conducted in western Minnesota (near 45°49′ N, 95°43′ W) in an experimental plot (40 × 123 m) previously established as part of a fragmentation study (Wagenius *et al.* 2010). The plot is a former agricultural field containing *Solidago rigida, Bromus inermis, Medicago sativa* and native prairie grasses. The plot is surrounded by agricultural fields and a few trees. The nearest flowering *Echinacea* is in a smaller experimental plot 247 m away (20-100 flowering plants during this study). The nearest remnant populations of *Echinacea* were 420 m away (approximately 100 flowering plants) and 520 m away (<5 flowering plants).

Most of the *Echinacea* of reproductive age during the study were collected from 1995 to 1998 as seeds from remnant prairies within 5 km of the plot. Some individuals that started flowering in 2006 are progeny of the first generation plants described in Wagenius *et al.* (2010). *Echinacea* individuals were planted as seedlings in the plot; rows were separated by 1 m, and plants were 0.5 or 1 m apart, except for an 8 × 25 m section with 0.67 m between rows and 0.33 m between plants (Fig. 1). Within each planting year, genotypes were randomized, thereby removing the spatial genetic structure present in remnants (Wagenius, Lonsdorf & Neuhauser 2007). The plot is burned biennially during May of even-numbered years including once during this study (2006).

MONITORING FLOWERING PHENOLOGY
IN THE EXPERIMENTAL PLOT (2005–2007)

We visited each flowering plant daily in 2005 ($n = 204$) to count anthers on every head. In 2006 and 2007, we visited plants every second day ($n = 572, 529$). For each plant that flowered in each year, we determined start date, end date, flowering duration, peak flowering date and synchrony (Fig. 2). The cone-shaped head of *Echinacea* causes more florets to shed pollen early rather than at the end of the flowering period. Thus, we calculated peak flowering date for each plant as the mean date of flowering weighted by the number of florets shedding pollen on each flowering day. We calculated the flowering synchrony of each plant compared with all flowering plants in the plot (for equation see Table S1 in Supporting Information). In each year, we inadvertently did not have complete records for several plants (6, 4 and 3 in 2005, 2006 and 2007, respectively), and these were excluded from our analyses (see Table S2).

QUANTIFYING SEED SET

Achenes in *Echinacea* expand, even if they do not contain an embryo. We quantified the relationship between seed set and fruit mass by individually weighing, then X-raying (Faxitron LX-60, Tucson, Arizona, USA), 1541 achenes from the 2005 seed batch. In this trial, 98.4% of achenes 2.00 mg or heavier had an embryo, while 92.2% of achenes <2.00 mg were unfertilized. Based on this trial, we classified all achenes in this study ≥2.00 mg as fertilized.

For all flowering heads in 2005, we removed achenes based on their position in the seed head, thus sampling the entire range of flowering time in each plant. We removed achenes from the bottom, earliest flowering, 30 disc florets and then the top 30. The remaining achenes in each head were then removed, and we chose a random sample of 30. All sampled achenes were individually weighed.

In 2006 and 2007, we randomly sampled 489 flowering plants (with 557 heads) and 225 flowering plants (with 271 heads), respectively, from all plants that flowered each year (R Development Core Team 2011). In 2006, we counted the total number of achenes per head and then weighed a random sample of 30 disc achenes from the sampled seed head. Each sample was used to estimate seed set for the whole head. In 2007, we first counted all of the achenes and then used an aspirator to remove the lightest, clearly empty achenes. We then recounted the remaining achenes and weighed a random sample of 30 achenes. This sample was used to estimate seed set for the whole head, including achenes removed by the aspirator. For this study, a total of 71781 achenes were individually weighed.

DATA ANALYSIS

Individual seed set, distance to conspecifics, location and flowering time (2005–2007)

We used linear models and generalized linear models (GLMs) to test for potential effects of near-neighbour distance, edge effects, flowering time and their interactions on seed set. Seed set is best modelled as a binomial response (full or empty achenes) in a GLM. We used such models for 2006 and 2007. However, a GLM was not appropriate for 2005 because we calculated the proportion of seed set per head as the mean of each head position sample weighted by the number of florets in the sample. Thus, we used linear models with seed set logit-transformed (Warton & Hui 2011). We analysed each year separately because seed set was estimated differently in each year.

We focused our analysis on one phenological and two spatial predictors. Potential phenological predictors included start date, end date, peak flowering date, duration of flowering and synchrony. Start, peak and end flowering date were all significantly correlated (Spearman's rank correlation $P < 0.05$; see Table S1). For spatial predictors, we used edge and isolation measures. We classified each plant's location relative to the geographical centre of plants flowering in the plot that year (Fig. 1). Half of the plants closest to the centre were classified as 'core;' the rest of the plants were classified as 'edge.' To quantify individual plant isolation, we used the natural log of Euclidean distances to the kth nearest flowering neighbour in the plot ($k = 1–204$ in 2005, $k = 1–572$ in 2006, and $k = 1–529$ in 2007). Each measure captures a plant's isolation from its closest k potential mates. Most near-neighbour distances were correlated (e.g. distances to fifth and sixth nearest neighbours are correlated; $P < 0.01$), meaning plants isolated at one spatial scale tended to be similarly isolated when measured at a different scale.

We conducted univariate analyses to assess which phenological and spatial predictors to use in the full models. Among phenological measures, we found in all three years that peak flowering date, start date and end date predicted seed set, but peak flowering best predicted seed set (see Fig. S1 in Supporting Information). Duration of
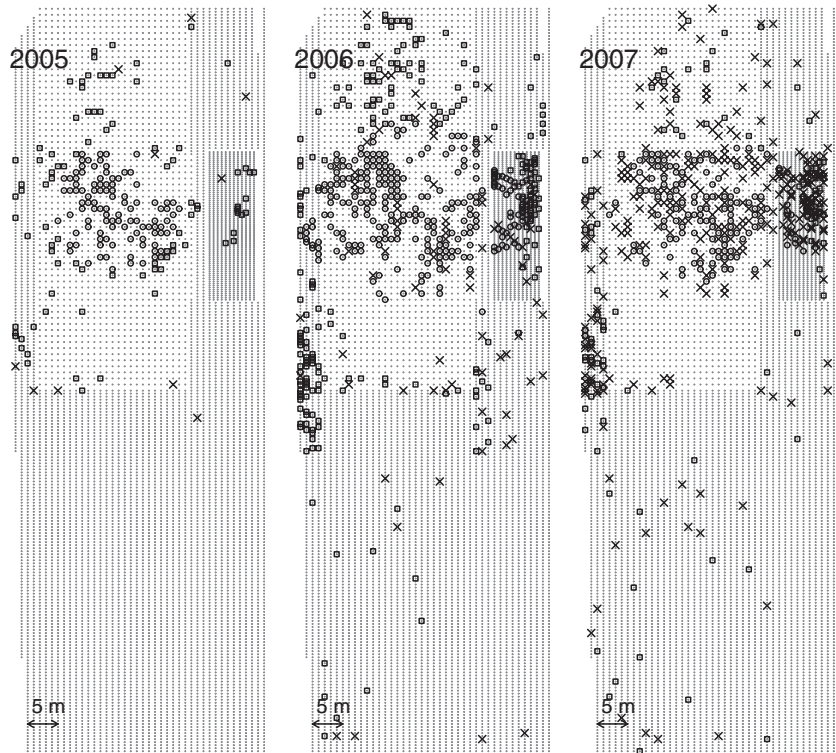
Fig. 1. Diagrams of the experimental plot from 2005 to 2007. Circles and squares indicate core and edges plants, respectively. Half of all flowering plants that were closest to the plot centre were classified as 'core'; the rest were classified as 'edge'. Crosses show flowering plants that were not used in the experiment. Small dots indicate locations where plants were planted, but in the given year, plants were either non-flowering or dead.
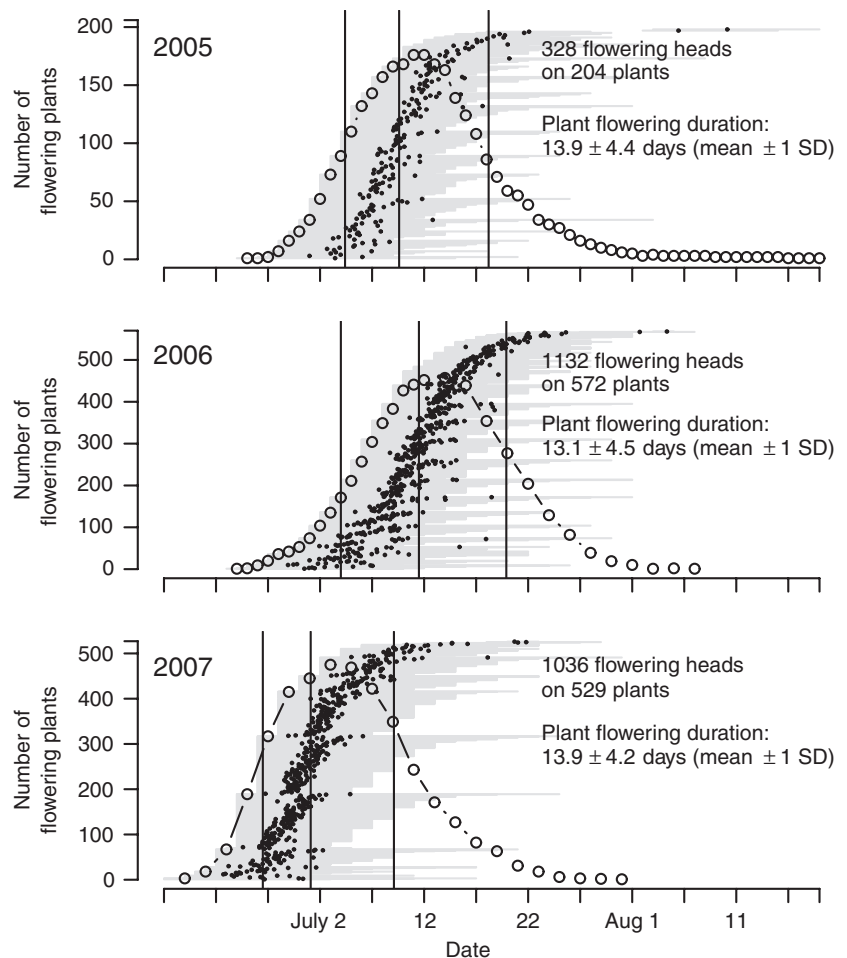

Fig. 2. Flowering schedule for plants in the experimental plot in 2005–2007. Open circles represent the total number of plants in flower for each day in 2005 and every other day in 2006 and 2007. Horizontal grey bars represent the start and end date for each flowering plant, with the peak flowering date of each plant represented by the black dot. Vertical lines indicate the dates that 5%, 50% and 95% of all plants have reached peak flowering.

flowering and synchrony did not consistently predict seed set (see Fig. S1). We used peak flowering date for further analysis. Among near-neighbour distances, we found that distances to tenth through twentieth nearest neighbours best predicted seed set (see Fig. S2). We used distance to the twelfth nearest neighbour because it captured variation in isolation experienced by plants in both the core and edge in all three years (see Fig. S3).

For all three years, we determined the minimal adequate model using stepwise backward elimination with likelihood ratio tests as described in the study by Crawley (2005) using the following predictors: peak flowering date, distance to twelfth nearest neighbour, and plant location (core or edge). Initial models included a three-way interaction between peak date, spatial isolation, and location, all two-way interactions and all main effects. The residual deviance of initial models for 2006 and 2007 exceeded the residual degrees of freedom by a factor of eight, indicating overdispersion; therefore, we used the quasibinomial family for the analysis. To improve the distribution of errors, we removed the four latest and single latest flowering plants in 2005 and 2006, respectively. These outliers had low or zero seed set and tended to be spatially isolated, consistent with overall patterns. We conducted parametric bootstrap analyses on all parametric tests where *P*-values were close to 0.05 and found trivial differences in *P*-values. We do not present bootstrap results.

Within-individual seed set, flowering time and floret position (2005)

To investigate potential effects on seed set of achene position within a head and the date its style became receptive, we constructed nested models to account for variation in seed set among all plants that flowered in 2005. Predictors included position in the head and mean date of style receptivity. To improve the distribution of errors, we removed outliers which were the three latest flowering plants. The distribution of residuals in GLMs with only fixed effects suggested heteroscedasticity; therefore, we used a mixed effect logistic regression model (GLMM) with a binomial response and both head and plant modelled as random effects (Warton & Hui 2011), as implemented in the glmer function in the lme4 package of R (Bates, Maechler & Bolker 2011). Plots of estimated random effects versus fitted values from a GLMM fit showed no evidence of heteroscedasticity. We selected the minimal adequate model using methods described in the previous paragraph. All analyses were conducted using R 2.14.1 (R Development Core Team 2011).

Results

OVERALL SEED SET

Each individual plant's seed set varied greatly over the three years of the study (0–0.94 in 2005; 0–0.96 in 2006 and 0–1.00 in 2007; see Fig. S4). Median seed set in 2005 core and edge plants was 0.60 and 0.38, respectively. In 2006, there was substantial variation in seed set, but the median proportion was around 0.60 for both core and edge plants. In 2007, median seed set was around 0.50 for both core and edge plants. Seed set and floret count are the two components of annual reproductive fitness. In 2005, median annual reproductive fitness was 80% higher in the core plants compared to the edge plants (173 and 96 seeds in core and edge plants respectively, *n* = 102, 101). This difference results from the

differences between core and edge plants in both seed set (0.60 and 0.38 medians, respectively) and floret counts (256 and 224 medians, in core and edge, respectively). We note that the distribution of floret counts is strongly skewed because the majority of plants had one head, although several had many heads (e.g. one core and two edge plants had six heads 2005). Hereafter, we report on the proportion seed set because it reflects pollination, the focus of this study.

SEED SET DEPENDS ON DISTANCE TO NEAREST-NEIGHBOURING PLANTS AND FLOWERING PHENOLOGY

The probability that a floret was fertilized decreased significantly with the plant's peak flowering date and varied with its location in the core or edge every year (all *P* < 0.05, Fig. 3, see Tables S3–S5 for results of model selection, and see Fig. S5). From beginning to end of the season, mean seed set decreased 50% in 2007 (from 0.55–0.62 to 0.27–0.30) and 46% in 2006 (from 0.73 to 0.39). In 2005, significant location (core versus edge) by peak flowering date interaction existed (*P* = 0.003, Fig. 3 and see Table S3). Seed set in 2005 decreased 70% (from 0.82 to 0.25) among core plants and only 25% (from 0.46 to 0.34) among edge plants. The effect of phenology is less variable than the effect of distance to neighbouring conspecifics. This is evident in the consistent and significant negative slope for peak flowering date predictor in linear models and GLMs in all years (all *P* < 0.001, see Tables S3–S5). In 2005, the flowering time effect differed markedly between core and edge plants, with seed set in core plants 78% higher than edge plants at the beginning of the season (0.82 vs. 0.46) and 26% lower than edge plants at the end of the season (0.25 vs. 0.34).

Seed set declined with distance to twelfth nearest neighbour in every year but declined the least in 2007 (Fig. 3a,c,e). This spatial isolation effect was independent of location and phenology in all years (*P* > 0.05). In 2005, seed set declined about 20% from the least to most isolated plants as predicted for plants flowering during mid-season (0.69–0.55, *P* = 0.009). Similarly, in 2006, seed set declined about 25% from the least to most isolated plants as predicted for plants flowering during mid-season (0.82–0.60, *P* < 0.001). In 2007, seed set's decline with distance to twelfth nearest neighbour was minimal and was not significant (*P* = 0.133, Fig. 3e).

Seed set differed between plant locations in the experimental plot (core versus edge) in every year (all *P* < 0.048), but the main effect was not consistent in direction and its magnitude was often much smaller than the phenology effect, with the largest difference between core (~0.45) and edge (~0.55) occurring in 2007 (see Fig. S5e,f).

SEED SET BASED ON A FLORET'S POSITION WITHIN A HEAD

The probability of seed set was influenced by the position of a floret within a head (head nested within plant; see Table S6). Seed set rates for the middle florets started at 0.87 and declined to 0.06. This proportion was 20–30% greater than

Fig. 3. Seed set predicted from a plant's spatial isolation (S), peak flowering date (T) and location within the plot (L) in 2005 (a,b), 2006 (c,d) and 2007 (e,f). A plant's spatial isolation was quantified as the distance (m) to its 12th nearest flowering conspecific. Peak flowering date was the date with the most florets shedding pollen. Core and edge plants are shown as circles with solid lines and squares with dashed lines, respectively. The prediction lines result from a linear model with logit-transformed seed set in 2005 and generalized linear models with quasibinomial response in 2006 and 2007. In panels a, c, and e, seed set is predicted separately for the core and edge over the range of observed spatial isolation values for hypothetical plants flowering on three dates: when 5%, 50% and 95% of all plants have reached peak flowering. In panels b, d and f, seed set is predicted separately for each location (core and edge) over all dates with observed flowering for hypothetical plants representing the mean and 95% confidence intervals of spatial isolation. To compare among years, all models used here include all three explanatory variables and a location X peak date interaction term (ANOVA results see Tables S3–S5). Graphs of predicted seed set based on best models for each year are shown in Fig. S5. Sample sizes indicate the number of flowering plants included in the model out of all flowering plants in the experimental plot.
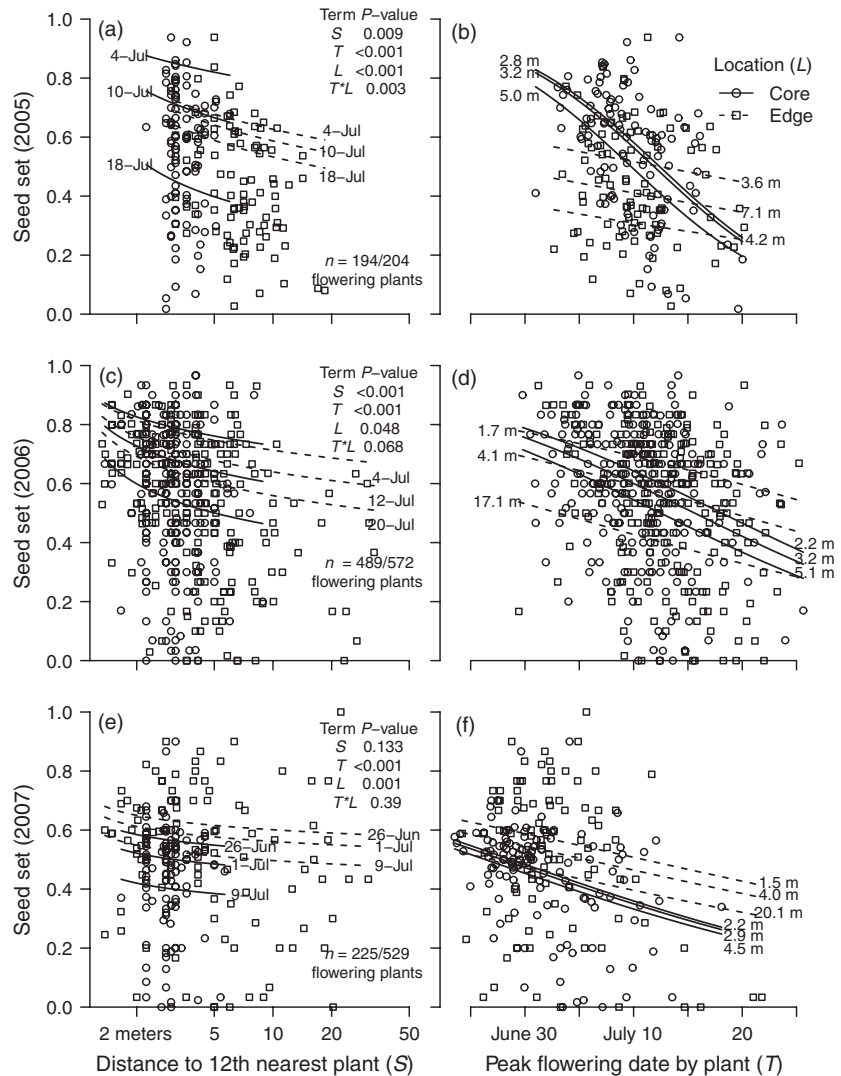
seed set in bottom florets until around 20 July at which point seed set rates for the middle and bottom florets was nearly identical (Fig. 4). Seed set in top florets was nearly identical to seed set in middle florets early in the season but declined to nearly zero by the end of the season. The difference between plants flowering early or late greatly exceeded the differences among positions within a head (Fig. 4). The seed set for the earliest florets in any position was >0.76, while the latest florets in any position had seed set less than 0.11, a sevenfold difference. Within seed heads, seed set in top florets was positively correlated with seed set in both bottom and middle florets ($P < 0.0001$, Pearson's product-moment correlation: 0.51 and 0.58, respectively, $n = 309$ heads).

Discussion

SPATIAL AND PHENOLOGICAL PATTERNS OF SEED SET

Seed set, a key determinant of annual reproductive fitness in *Echinacea*, decreased with distance to neighbours in both 2005 and 2006 (Fig. 3 and see Fig. S5). These results mirror patterns of spatial isolation observed in nearby remnant

populations of *Echinacea* where individual seed set decreased with spatial isolation, even in small isolated populations (Wagenius 2006). In a paternity study of seedlings from this experimental plot in 2005, Ison *et al.* (2014) found that pollen donors were much closer than expected with random mating; the closest 10% of flowering plants represented around 40–50% of sires. Restricted pollen movement within the plot provided a mechanistic explanation for the observed pattern in seed set. Pollinator visitation often decreases in more isolated individuals (Kunin 1993; Field, Ayre & Whelan 2005; Dauber *et al.* 2010), but it may increase if plant densities are high and individuals compete for pollinators (Steven *et al.* 2003). This may have occurred in 2007 when distance to neighbour did not significantly predict seed set ($P = 0.133$, Fig. 3 and see Tables S3–S5). Substantial interannual variation in plant fecundity is common owing to the many factors that influence fecundity (Ågren 1996; Alexandersson & Ågren 1996; Duffy, Patrick & Johnson 2013). Although the patterns of seed set differ between years in *Echinacea*, spatial isolation consistently played a key role in reproductive outcomes.

Location in the core or edge influenced seed set in a manner distinct from distance to neighbouring plants. Seed set in
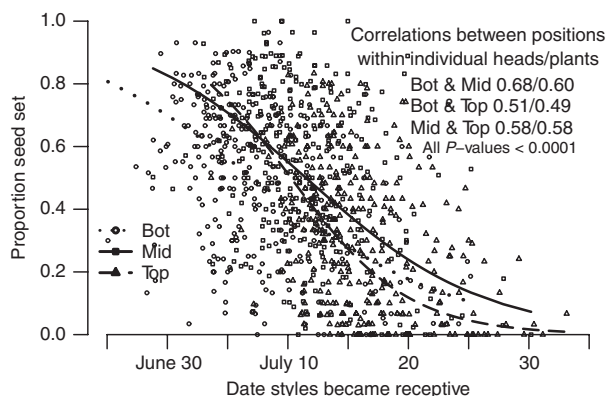
Fig. 4. The relationship between seed set and the floret's position within an inflorescence (head) and flowering time in 2005. Circles refer to the earliest 30 florets to shed pollen in a head (Bot), triangles refer to the last 30 florets to shed pollen in a head (Top), and the squares refer to a sample of 30 florets in between (Mid). Flowering time is the mean date that the florets' styles became receptive. Best-fit lines result from a GLMM with seed set modelled as a binomial response; position and date were modelled as fixed effects; and head nested within individual were modelled as random effects (see Table S5). The minimal adequate model includes the position X date interaction term ($P < 0.0001$). $N = 940$ observations from 316 heads.

core plants declined much less over the course of the 2005 and 2006 seasons compared with seed set in edge plants (Fig. 3). Even though the flowering time effect on seed set did not differ between the core and edge in 2007, location was still significant ($P = 0.001$). We emphasize that we have no evidence of habitat differing between the core and edge, although it may in fact differ (McKone *et al.* 2001). We do know that the floral resource landscape differs around core and edge plants and may affect pollinator behaviours, such as spending time collecting pollen, grooming, searching for predators or looking for the next place to visit.

Timing of flowering strongly and consistently influenced seed set but not in the same manner as distance to neighbours. Seed set was higher among plants with earlier start, end or peak flowering dates (Figs 3 and 4, and see Fig. S5). There was a pronounced difference between these 'seasonal' or date-dependent, phenological predictors compared with measures of synchrony (see Fig. S1). If asynchrony of flowering decreased seed set, then we would expect that both early and late plants would suffer from temporal isolation and reduced seed set. We found that synchrony alone was a poor predictor of seed set, consistent with patterns across species (Munguía-Rosas *et al.* 2011). These results contrast with our expectations based on the study in this plot which found that flowering synchrony between *Echinacea* individuals significantly increased the probability of those individuals mating (Ison *et al.* 2014). To interpret our results, we turn to potential mechanisms that do not hinge on mate availability.

POTENTIAL MECHANISMS FOR SEASONAL DECLINES IN SEED SET

At least five ecological mechanisms could account for the general pattern of decreasing seed set over a flowering season.

First, later flowers may experience greater rates of predation (English-Loeb & Karban 1992). However, we limited exposure to predators by harvesting seed heads immediately upon ripening and saw no obvious signs of predation.

Secondly, later flowering plants may experience greater pollinator limitation. A change in the composition or abundance of pollinators over the season likely occurs because *Echinacea* has a diverse pollinator community consisting mostly of native solitary bees (Wagenius & Lyon 2010). Pollen limitation could also increase if interspecific competition for pollinator service intensifies over the season (Gross & Werner 1983; Campbell 1985). While an early flowering plant may have the same level of conspecific synchrony as a late flowering plant, the heterospecific flowering plants competing for pollinator services might be quite different. At the beginning of *Echinacea*'s flowering season, typically only a few other species are flowering; however, by the end of its flowering, many other prairie plant species are flowering (Anderson & Schelfhout 1980). Interspecific competition typically increases with the number of coflowering species (Feldman, Morris & Wilson 2004; Mitchell *et al.* 2009).

Thirdly, later flowering plants may have reduced seed set due to rapid floral senescence at the end of the growing season (Schemske 1977; Stephenson 1981). However, in 2005, we found that styles in later flowering plants were, on average, receptive for 1.5 days longer than in earlier flowering plants, indicating that later flowering plants senesced more slowly (J. L. Ison & S. Wagenius, unpubl. data). In fact, previous research has shown that style persistence is a good proxy of pollen limitation in *Echinacea* (Wagenius 2004; Wagenius & Lyon 2010). Therefore, this finding is more consistent with pollen limitation as the primary mechanism for reduced seed set in late flowering plants.

A fourth potential mechanism is that late flowering plants may have fewer available resources to set seed. In perennials, plant size (Widén 1991; Bishop & Schemske 1998; Forrest & Thomson 2010) and flowering duration (Ollerton & Lack 1998) typically decrease with later flowering onset, perhaps because earlier flowering plants have greater maternal resources (Forrest & Thomson 2010). Pollen limitation of *Echinacea* has been well documented, and no evidence of resource limitation with respect to annual reproduction has emerged (Wagenius 2004, 2006; Wagenius & Lyon 2010). However, this is the first study in *Echinacea* to examine changes in seed set throughout the flowering season. Longer flowering duration for early flowering plants may indicate that these plants have more maternal resources than late flowering plants. We did observe a negative correlation between onset and duration of flowering in 2005 and 2006, but the relationship was not significant in 2007 (see Table S1). This indicates that maternal resources may decrease in later flowering individuals. However, it cannot account for the overall trend of decreasing seed set observed every year and likely plays only a minor role compared to individual flowering time.

Finally, a fifth potential mechanism is that pollen production per plant decreases at the end of the season, such that seed set decreases, even though pollinator visitation rate per

plant remains the same (Pauw 2013). Such a mechanism would be difficult to disentangle from changes in pollinator efficiency resulting from changing pollen availability. Also, pollen production may decrease per floret, which we did not measure. However, we did follow daily anther production within heads and gained insight into potential causes of seasonal variation in seed set.

SEED SET WITHIN FLOWERING HEADS

Comparing seed set between the bottom and top florets within a head enables us to distinguish positional effects from seasonal effects related to the pollination environment. Positional effects were small such that seed set proportion differed by only 0.05–0.10 among positions within flowering heads. In contrast, seed set proportion decreased by 0.60 over the course of the flowering season for all three positional groups (Fig. 4). The positional effects could result from resource allocation, floral architecture or pollen limitation (Medrano, Guitian & Guitian 2000). Plants often preferentially allocate resources to the early fruits or to lower fruits that are closer to nutrients (e.g. Stephenson 1981; Herrera 1991). Aside from resources, floral architecture often causes the lowest flowers in a floral display to have higher seed set (Diggle 1997). Because the earliest florets, in *Echinacea* are also the lowest florets, both resource allocation and floral architecture predict highest seed set in the bottom achenes. However, we found the greatest seed set in mid achenes (Fig. 4).

In addition, if the observed positional effects resulted from resource allocation, then we would expect that seed set in top florets would be negatively correlated with seed set in the bottom and middle florets of the same head. Instead, we observed substantial positive correlations in seed set rates between all positions within an individual flowering head which is more consistent with pollen limitation hypotheses (Fig. 4). It is unclear why pollen limitation would vary within a flowering head. One potential mechanism is that pollinator visitation often increases with display size (Setsuko *et al.* 2008). The tops of *Echinacea* heads usually produce the fewest male florets per day and could be less attractive to pollinators. Another mechanism is that differences in bee behaviour among positions may affect pollinator efficiency and thus seed set. Although there are small positional or resource effects on seed set, the weight of several lines of evidence indicates that pollen limitation drives the overall observed spatial and temporal patterns of seed set in *Echinacea*.

CONSTRAINTS TO RANDOM MATING IN NATURAL POPULATIONS

Regardless of the mechanisms, our results have broader implications for natural populations, especially in fragmented landscapes. We conducted this study in an experimental plot which eliminated the spatial genetic structure in mating compatibility that is typical of nearby natural populations (Wagenius, Lonsdorf & Neuhauser 2007). Thus, the patterns of seed set that we observed reflect the movement of pollen by

pollinators more closely than would observations in natural remnant populations. In many other respects, our experimental plot resembles the nearby remnant *Echinacea* populations, such as topography, population size, density of flowering plants, the surrounding landscape and the community of pollinators. The diversity of coflowering plants varies considerably among populations, and our experimental plot is less diverse than average. Pollination visitation rates are comparable and may be higher than in many remnants (unpublished data). Seed set rates in the remnants vary greatly because of the constraints to reproduction imposed by greater mating incompatibility and spatially structured mating incompatibility (Wagenius, Lonsdorf & Neuhauser 2007). Our results illustrate that flowering time, distance to neighbouring conspecifics and edge effects can cause reproductive failure in fragmented populations, even in the absence of spatial genetic structure.

CONSEQUENCES OF VARIATION IN SEED SET FOR POPULATION DYNAMICS

The observed declines in individual annual fitness due to flowering phenology and to spatial isolation may be considered component Allee effects (Courchamp, Berec & Gascoigne 2008). Our study complements work showing temporal Allee effects in insects in which some females go mateless by virtue of temporal isolation (Robinet *et al.* 2008; Fagen *et al.* 2010). Small insect populations greatly exacerbated temporal Allee effects which could lead to potential demographic Allee effects. Our findings also complement a study of *Echinacea* population dynamics that found a demographic Allee effect owing to a genetic contribution to the Allee effect caused by a loss of self-recognition alleles which reduces mate availability in small populations, thereby decreasing reproduction (Waites & Ågren 2004; Wagenius, Lonsdorf & Neuhauser 2007; Young & Pickup 2010). In this study, we have empirically quantified a strong temporal Allee effect and its interaction with a spatial Allee effect in a plot designed to minimize a genetic Allee effect. Our findings suggest that joint effects of flowering time and distance to conspecifics on reproduction may have consequences for the persistence of *Echinacea* and other populations in fragmented habitats. Furthermore, variation in individual reproductive fitness indicates that selection on flowering time may also play an important role in ongoing population dynamics. Our new perspective on the strength and interactions of temporal and spatial Allee effects causes us to question the mechanisms leading to previously observed spatial patterns of reproductive failure in our study system and in other studies in fragmented habitats (Steffan-Dewenter & Tscharntke 1999; Wagenius 2006). Perhaps variation in flowering phenology regularly contributes to reproductive failure in fragmented habitats.

Data accessibility

Data files and R scripts are available from the Dryad repository (Ison & Wagenius 2014).

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Supporting Information

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

Figure S1. Seed set by single phenological predictors in all three years (2005–2007).

Figure S2. Model slopes and AIC values of models examining seed set by distances to 1st–50th nearest conspecifics in all three years (2005–2007).

Figure S3. Histograms of the variation in the distance to each plant's 12th nearest conspecifics for all three years (2005–2007).

Figure S4. Histograms of seed set variation for all three years (2005–2007).

Figure S5. Best-fit models for seed set predicted by a plant's spatial isolation, peak flowering date, and location within the plot.

Table S1. Flowering synchrony equation and correlations of phenological measures for all three years (2005–2007).

Table S2. Plant totals for each of the three study years.

Table S3. ANOVA table comparing models of seed set in 2005 using stepwise model simplification via backward elimination.

Table S4. Analysis of deviance table comparing generalized linear models of seed set in 2006 using stepwise model simplification via backward elimination.

Table S5. Analysis of deviance table comparing generalized linear models of seed set in 2007 using stepwise model simplification via backward elimination.

Table S6. Analysis of deviance table comparing generalized linear mixed models of floret positions in 2005 with seed set as the response using stepwise model simplification via backward elimination.