Habitat fragmentation decouples fire-stimulated flowering from plant reproductive fitness

Jared Beck[1,3], Amy Waananen[2], and Stuart Wagenius[1]

[1]Negaunee Institute for Plant Conservation Science, Chicago Botanic Garden, 1000 Lake Cook Rd, Glencoe, IL, 60022 USA

[2]Department of Ecology, Evolution and Behavior, University of Minnesota, 1479 Gortner Avenue, St. Paul, MN, 55108 USA

[3]Email: jared.j.beck@gmail.com

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SIGNIFICANCE STATEMENT

Periodic fire maintains plant diversity in fire-dependent ecosystems worldwide. Fire effects on population dynamics and demographic rates, such as reproduction, are primarily attributed to fire's influence on the physical environment. However, our 6-yr experimental study of 6357 individual plants across 35 fragmented prairie populations reveals that fire effects on sexual reproduction depend on population size. Burns consistently boosted reproductive effort (flowering) yet fire increased reproductive outcomes (seed production) only in large populations. Fire did not consistently improve pollination or seed production in populations with <20 individuals. This decoupling of fire effects on reproductive effort from reproductive outcomes in small populations may limit reproductive benefits of fire in fragmented habitats and diminish the capacity of fire to maintain plant diversity.

ABSTRACT

Many plant species in historically fire-dependent ecosystems exhibit fire-stimulated flowering. While greater reproductive effort is expected to result in increased seed production, plant reproductive outcomes often depend on pollination, the spatial distribution of prospective mates, and the timing of their reproductive activity. Fire-stimulated flowering may thus have limited fitness benefits in small, fragmented populations where mating opportunities are restricted and pollination rates are low. We conducted a six-year study of 6357 *Echinacea angustifolia* (Asteraceae) individuals across 35 prairies in Minnesota (USA) to experimentally evaluate how fire effects on multiple components of reproduction vary with population size in a common species. Fire increased annual reproductive effort consistently across populations, doubling the proportion of plants in flower and increasing the number of flower heads 65% per plant. In contrast, fire's influence on pollination rates and reproductive outcomes differed between large and small populations, reflecting density-dependent effects of fire on spatiotemporal mating potential. In populations with fewer than 20 individuals, fire did not consistently increase pollination or seed production. Above this threshold, fire increased mating potential, leading to a 24% increase in seed set and a 71% increase in annual seed production. Our findings suggest that density-

dependent effects of fire on pollination largely determine plant reproductive outcomes and could influence population dynamics across fire-dependent systems. Failure to account for the density-dependent effects of fire on seed production may lead us to overestimate the beneficial effects of fire on plant demography and the capacity of fire to maintain plant diversity, especially in fragmented habitats.

INTRODUCTION

Fire shapes the physical structure and biological diversity of ecosystems worldwide (1–3). Across historically fire-dependent ecosystems, periodic fire maintains plant species diversity by influencing demographic rates such as survival and reproduction (2, 4–9). Fire conspicuously influences plant reproductive effort. Thousands of plant species inhabiting historically fire-dependent habitats flower profusely after fire (10–12). While fire-stimulated flowering is expected to increase seed production and thus promote plant population persistence (13), few empirical studies directly quantify the fitness consequences of fire-stimulated flowering (13–16). For many plant species, reproductive outcomes (seed production) depend on both reproductive effort and pollination rates. Annual reproductive effort largely reflects patterns of resource availability, acquisition, and storage (15, 17). Thus, fire effects on flowering rates and resource allocation to reproduction are presumed to be *density independent* – in other words, fire similarly influences plant reproductive effort in large, or high-density, populations and small, or low-density, populations. However, the potential for fire to differentially influence reproductive outcomes in large versus small populations – *density-dependent* effects of fire on reproductive outcomes – has not been examined. Even if fire increases reproductive effort in small populations, mating limitations could restrict pollination and seed production. Failure to account for such *density-dependent* fire effects on pollination rates and reproductive outcomes will yield misleading inferences about the extent to which post-fire reproduction contributes to population growth and persistence. Specifically, we may overestimate the capacity of fire to promote population growth and plant diversity in many fire-dependent communities.

Despite the prevalence of fire-stimulated flowering in historically fire-dependent habitats such as South African fynbos (10, 18), Australian shrublands (10, 19), Brazilian cerrado (11, 20), North American longleaf pine savannas (12, 21), and North American tallgrass prairie (13, 17, 22), the expected association between increased reproductive effort after fire and increased seed production has been empirically examined in only a handful of species (13, 15, 23). These include several recent studies which reveal fire-stimulated flowering can enhance pollination and improve seed production by increasing

77 spatial and temporal mating opportunities (13, 14, 16). Fire-stimulated flowering reduces the spatial
78 distance between prospective mates and thereby increasing the likelihood of pollen transfer between
79 individuals (13). Similarly, fire can increase overlap in the timing of reproductive activity between
80 individuals, leading to synchronized within-season flowering phenology and improved pollination (13,
81 14, 16). These empirical studies examining fire's influence on pollination rates and seed production have
82 focused exclusively on large populations, but the fitness consequences of fire-stimulated flowering could
83 be very different in small populations. Greater reproductive effort per individual after fire may not
84 appreciably increase mating potential – the capacity for sexual reproduction, as dictated by the location
85 and reproductive timing of prospective mates (13) – when the density of sexually mature individuals is
86 low. Consequently, fire-stimulated flowering may not improve pollination in seed production in small
87 populations. Empirical research is needed to quantify the potentially density-dependent effects of fire on
88 plant reproduction and to establish how various components of reproduction – namely reproductive effort,
89 mating opportunities, pollination rates, and seed production – vary with population size.
90 Realized fitness outcomes resulting from the presumably *density-independent* effects of fire on
91 reproductive effort and the potentially *density-dependent* effects of fire on mating opportunities and
92 pollination have particularly important implications for the sexual reproduction and demography of plants
93 within fragmented habitats. Many historically fire-dependent systems have undergone extensive habitat
94 loss and fragmentation (24–27), including North American tallgrass prairie, which has been reduced to
95 less than one percent of its historic extent across much of its range (28). In these fragmented habitats,
96 many plant species exhibit chronically low seed production. Rates of reproductive failure are highest in
97 small populations where reduced mating opportunities and pollen limitation constrain sexual reproduction
98 (29–31). Persistent reproductive failure depresses population growth rates and threatens the persistence of
99 small plant populations (32). Fire could alleviate these mate-finding Allee effects by synchronizing
100 flowering, improving pollination, and enhancing seed production (13). However, the lack of data about
101 fire effects on reproductive outcomes across a range of population sizes precludes a rigorous assessment
102 of fire's capacity to alleviate mate-finding Allee effects.

We conducted a 6-year experiment to evaluate potential density-dependent effects of fire on plant reproductive fitness in fragmented tallgrass prairies. Before European settlement, prairies burned every 1-5 years. Many prairie plant species exhibit fire-stimulated flowering. Our study focused on 35 populations of *Echinacea angustifolia* (Asteraceae) within a fragmented landscape in western Minnesota, USA (Fig. S1). We conducted prescribed burns in a stratified sample of 18 of these populations ranging in size from three to nearly 4000 sexually mature individuals (Table S1). We then quantified multiple components of annual reproductive fitness in 6357 individuals to evaluate how fire affects plant reproduction – both reproductive effort and outcomes – and whether fire effects vary with census population size, the count of sexually mature individuals. We hypothesize that fire has consistent effects on reproductive effort that do not vary with populations size, i.e., *density-independent* effects of fire on reproductive effort. In contrast, we hypothesize that fire effects on pollination and seed production vary with population size, i.e., *density-dependent* effects of fire on reproductive outcomes. To test whether the effects of fire on reproductive outcomes are mediated by mating opportunities, we quantify the relationship between spatiotemporal mating opportunities and pollination rates per population every year. Finally, we assess whether fire effects on spatiotemporal mating potential vary with population size, i.e., *density-dependent* effects of fire on mating potential.

The long-lived, iteroparous *E. angustifolia* is widely distributed across central North American grasslands and exhibits life-history traits common among herbaceous perennials. Individual plants often live for decades, reproduce only by seed, are pollinated by small bees, and cannot self-pollinate (33, 34). Each year, *E. angustifolia* plants resprout from their deep taproots. Sexually mature plants are obvious in years when they flower but in non-flowering years produce inconspicuous basal leaves. In a single, large population, fire promoted pollination and seed production in *E. angustifolia* by synchronizing reproduction among years and increasing spatial and temporal mating opportunities within years (13).

We quantified plant reproductive effort, pollination rates, and annual seed production in 210 mating scenes – the unit of replication in this study defined by unique population-year combinations. Our study included 22 burned mating scenes, each burned prior to the growing season, and 188 non-burned

scenes. We measured three aspects of plant reproductive effort. First, we censused flowering plants and calculated the proportion of sexually mature plants flowering in each year (N = 210 mating scenes). Second, we counted the number of flowering heads each reproductive individual produced each year and calculated the mean for each mating scene (N = 199 mating scenes in which at least one individual flowered). Third, we harvested randomly selected seed heads from mating scenes to quantify mean fruit count per individual (N = 152 mating scenes). Each *E. angustifolia* floret yields one fruit whether that floret receives compatible pollen or not. To quantify pollination rates, we measured mean seed set and mean style persistence. Seed set quantifies the proportion of fruits containing a fertilized embryo. Our previous work indicates seed set in *E. angustifolia* primarily reflects pollination, but seed set can also be affected by seed predation, resource allocation, and disease. Thus, we also measured style persistence (N = 171 mating scenes), which is a resource-independent index of pollen limitation, to validate that our inferences about seed set reflect pollination rather than other ecological processes (34). We then estimated total number of seeds produced per plant for each mating scene by multiplying mean head count, mean achene count, and mean seed set (N = 152 mating scenes). To gauge how much fire effects on pollination and seed production are mediated by mating potential, we precisely mapped the spatial location of every flowering plant and monitored flowering phenology to identify the first and last day each flowering individual produced pollen (N = 181 mating scenes). We then calculated mean outcross mating potential (OMP) – a metric that integrates the spatial location of an individual's prospective mates and the relative timing of their reproductive activity (32).

RESULTS

Fire has density-independent effects on plant reproductive effort. Fire consistently increased plant reproductive effort across *E. angustifolia* populations (Fig. 1; Table S2). Average proportion of plants flowering more than doubled from 23.6 percent to 47.7 percent after experimental burns (Fig. 1a; Likelihood ratio test: $\chi^2 = 45.545$, $P < 0.001$). We found no evidence that fire effects on flowering proportion depended on population size ($\chi^2 = 0.009$, $P = 0.924$) or that population size influenced

155 variation in flowering among populations (χ^2 = 2.422, P = 0.120). Similarly, mean head counts per
156 flowering plant increased from 1.15 to 1.83 after fire (Fig. 1b; χ^2 = 47.330, P < 0.001) and there was no
157 evidence these effects depended on population size (χ^2 = 0.254, P = 0.614) or that population size
158 influenced mean head count (χ^2 = 0.0.019, P = 0.890). We found no evidence that fire influenced the
159 mean number of fruits per flowering head (χ^2 = 0.001, P = 0.976), though the mean number of fruits
160 decreased with census population size (Fig. 1c; χ^2 = 6.134, P = 0.0.013).
161
162 **Fire has density-dependent effects on reproductive outcomes.** In contrast to the density-independent
163 response of plant reproductive effort to fire, fire effects on seed set and total seed production are strongly
164 dependent on census population size (Fig. 2; Table S3). Mean seed set increased non-linearly with census
165 population size, and fire effects differed considerably between small and large populations, though
166 inferences about fire effects were sensitive to an influential point from a small, burned population (see
167 asterisk in Fig. 2a). Exclusion of this data point improved the distribution of residuals (see Discussion for
168 description of this outlier and relevant biological considerations). With the outlier excluded, fire
169 influenced both the rate at which mean seed set increased with population size (estimates from 3-
170 parameter logistic model [95% confidence interval]: 0.414 [0.036, 0.793] burned versus 1.133 [0.085,
171 2.182] unburned) as well as asymptotic estimates in large populations (0.558 [0.484, 0.633] burned versus
172 0.449 [0.364, 0.535]). We predict burns have the greatest effect on seed set in populations with 97
173 sexually mature plants where fire is predicted to increase seed set by 44 percent (Fig. S2a; 0.534 versus
174 0.372). In small populations (<20 sexually mature plants), mean seed set was low for both burned and
175 unburned mating scenes.
176 Mean annual fecundity increased non-linearly with census population size and fire had different
177 effects in small versus large populations (Fig. 2b), though our inferences about fire effects were again
178 complicated by the highly influential point from the same small population after our experimental burn
179 shown with an asterisk in Fig. 3c. Inclusion of this point prevented model convergence. With the outlier
180 excluded, fire increased asymptotic estimates of mean annual fecundity by 71 percent (125.849 [83.945,

188.426] seeds per plant versus 73.814 [52.091, 104.531] seeds per plant) as well as the rate at which fecundity increased with census population size (0.559 [0.144, 0.975] burned versus 1.059 [0.294, 1.824] unburned). Our model predicts fire has the greatest effect on mean annual fecundity in populations with 74 sexually mature plants (Fig. S2b). Here fire is predicted to more than double mean annual fecundity (116.8 versus 52.6 seeds produced per flowering plant in burned versus unburned).

As expected, mean seed set and mean style persistence were negatively correlated (Pearson's correlation: $r = -0.59$, $N = 152$, $P < 0.001$). Style persistence declined with census population size (Fig. 2c; $\chi^2 = 93.206$, $P < 0.001$) and style persistence was lower in burned populations ($\chi^2 = 8.360$, $P = 0.004$). Nevertheless, we found no evidence that fire effects on style persistence differed between large and small populations ($\chi^2 = 1.098$, $P = 0.295$).

Fire effects on pollination and seed production reflect spatiotemporal mating potential. Both measures of pollination (seed set and style persistence) closely tracked variation in spatiotemporal mating potential. Mean seed set was positively associated with mean OMP (Fig. 3a; $r^2 = 0.33$, $N = 152$, $P < 0.001$). Mean style persistence was also strongly associated with mean OMP (Fig. 3b; $r^2 = 0.43$, $N = 181$, $P < 0.001$). Fire effects on reproductive outcomes reflected the density-dependent effects of fire on mating opportunities and pollination (Fig. 4). Fire influenced the rate at which mean OMP increased with census population size (0.347 [0.107, 0.587] burned versus 0.917 [0.520, 1.313] unburned). It also tended to increase asymptotic estimates of mean OMP though confidence intervals overlapped (0.755 [0.685, 0.825] burned versus 0.668 [0.583, 0.753] unburned). We predict fire has the greatest effect on mean mating potential in populations with 41 sexually mature plants (Fig. S2c). Here fire increased mean OMP by 93 percent (0.687 versus 0.356). Mean OMP primarily reflected variation in spatial mating opportunities. Mean OMP was strongly correlated with harmonic mean distance to nearby neighbors (all $r < -0.61$ and $P < 0.001$ for distance to $k = 1$-7 nearest neighbors, $N = 181$) but less strongly correlated with mean pairwise synchrony ($r = 0.25$, $N = 181$, $P = <0.001$; see Table S4).

DISCUSSION

Our six-year experimental study of fire effects on plant reproduction in fragmented tallgrass prairie demonstrated that plant reproductive effort responded consistently to burns across a wide range of population sizes (Fig. 1), but fire effects on pollination rates and seed production varied with population size (Fig. 2). Pollination rates were closely associated with spatiotemporal mating potential (Fig. 3) implying that differential effects of fire on reproductive outcomes in small versus large populations reflect variation in spatial and temporal mating opportunities. In large populations, fire consistently increased mating potential by reducing distance to prospective mates and increasing the temporal synchrony of flowering leading to improved pollination and greater annual seed production the growing season after fire (Fig. 4). However, the fitness benefits of synchronized post-fire reproduction declined rapidly in populations with fewer than ~20 sexually mature individuals. In these small, fragmented populations pollen limitation often constrained seed production despite greater reproductive effort after fire. These findings offer new and fundamental insights into the effects of fire on plant reproduction.

Previous investigations of fire effects on various plant vital rates and population growth rates have largely focused on density-independent processes related to how fire affects the physical environment. For instance, the benefits of fire for seedling recruitment and plant survival at multiple life stages have been attributed to increased resource availability and reduced competition for light (4, 7, 8, 35, 36). Similarly, previous studies of fire effects on plant reproduction have almost exclusively focused on reproductive effort and how flowering responses to fire depend on environmental conditions and resource availability (17, 37), fire seasonality (12, 38–40), and plant characteristics that influence allocation to reproduction such as ontogeny and variation in stored resources (15, 39, 41). Our findings elucidate the previously overlooked role of density-dependent processes (namely pollination) in determining post-fire plant reproductive fitness. Previous research in large plant populations demonstrates how fire effects on spatial and temporal mating potential can influence population-level reproductive outcomes (13) as well as variation in reproductive fitness among individuals of several obligately outcrossing plant species (14, 16). Our work reveals these pollination and fitness benefits of synchronized

post-fire flowering vary among populations, reflecting the density-dependent effects of fire on spatiotemporal mating potential. Fire did improve pollination and seed production in one small population with five sexually mature individuals that exhibited high flowering rates, many heads per individual plant, and relatively high seed set after our experimental burn in spring 2021 (though seed set ranged from 3 to 86 percent for the four individuals in this mating scene). It remains unclear whether the anomalously high pollination rates were driven by pollen import from the nearest remnant populations which are >1 km away, an industrious bee or group of bees moving pollen among individuals with the 0.03 ha remnant, or cross-pollination with non-native *E. purpurea* (which can hybridize with *E. angustifolia*) planted in a prairie restoration >100 m away. This example highlights the pronounced role that chance pollination events can play in determining reproductive responses in small populations.

This study contributes new and fundamental insights into the fitness benefits of synchronized reproduction and plant demography. Synchronized interannual reproductive variation (e.g., masting) is prevalent among long-lived iteroparous plants (10, 11, 19, 42, 43). To explain the potential adaptive significance of this reproductive synchrony, researchers often point to density-dependent processes such as predator satiation and pollination efficiency (19, 44). Yet, few empirical studies of synchronized reproduction employ the stratified sampling necessary to identify critical population thresholds in the efficacy of such density-dependent processes. Our results elucidate minimum population thresholds necessary for synchronized reproductive effort to consistently increase pollination efficiency and seed production. We also empirically validate theoretical predictions about the relationship between population density and pollination benefits of synchronized reproduction in wind-pollinated masting species (45). Future comparisons among species will be instructive and reveal how such thresholds and the strength of density dependence vary among species with different mating systems, pollination syndromes, reproductive responses to fire, and other relevant life history traits.

In addition to these new insights into the context-dependent fitness benefits of synchronized reproduction, our findings highlight a potentially important gap in our understanding of the demography of perennial plants in fire-dependent ecosystems. Most mechanistic explanations for fire's beneficial

effects on population dynamics in fire-dependent ecosystems rates focus on vital rates associated such as plant survival and recruitment (4, 7–9, 36). Although population growth rates in long-lived, iteroparous species are less sensitive to variation in annual reproduction than survival and recruitment, persistent reproductive failure can slow population growth and heighten risk of local extinction (32, 46), as well as hinder adaptation to changing environmental conditions. Without accounting for density-dependent effects of fire on seed production, demographic models may provide incomplete and potentially misleading inferences about population growth rates. Specifically, models that do not explicitly account for density-dependent reproductive responses to fire may underestimate the extent to which reproduction contributes to population growth and overestimate the beneficial effects of fire for population growth in small populations. There is a need for rigorous demographic analyses incorporating density-dependent fire effects on multiple vital rates. Explicit consideration of density-dependent processes will also elucidate how the relative influence of recruitment, survival, and seed production on population growth rates may vary across populations.

Our findings have important implications for plant conservation in historically fire-dependent ecosystems worldwide. Many fire-dependent ecosystems have experienced extensive habitat loss and fragmentation. North American Longleaf pine systems (24) and tallgrass prairie (28) have both been reduced to less than 3 percent of their historic extent. Accelerating rates of land conversion and habitat loss threaten fire-dependent biodiversity hotspots such as South African fynbos (26) and Brazilian cerrado (25). Increasingly, conservation practitioners advocate for the use of prescribed fire in remaining patches of fire-dependent biomes to maintain habitat structure and promote species diversity. Moreover, conservation and restoration efforts are often limited by seed availability and prescribed fire can be an effective tool for increasing seed yields (47, 48). This study reveals that habitat loss and fragmentation may constrain the beneficial effects of fire in some plant populations. We predict small populations of obligately outcrossing plant species will suffer high rates of reproductive failure and declining population growth even when managed with prescribed fire. Nevertheless, reproductive benefits can accrue to even relatively small populations of 30-100 sexually mature individuals.

Fire plays a central role in the maintenance of biological diversity across ecosystems worldwide. While previous research has focused on density-independent processes associated with fire's influence on the physical environment to explain fire effects on plant demographic rates, our experimental study of *E. angustifolia* elucidates how plant reproductive responses to fire vary with population size. These findings both enrich our understanding of fire's role in plant reproduction and highlight gaps in our knowledge of plant demography in fire-dependent systems. Failure to account for the density-dependent effects of fire on seed production may lead us to overestimate the beneficial effects of fire on plant demography and the capacity of fire to maintain plant diversity, especially in fragmented habitats.

MATERIALS AND METHODS

Study system

Our experimental study of fire effects on plant reproduction focused on 35 natural populations of *E. angustifolia* (Asteraceae) within prairie remnants distributed across a ~6400 ha block of rural western Minnesota, USA (centered near 45 49' N, 95 43' W). The focal populations included in this study were located within patches of remnant tallgrass prairie ranging from 0.01 ha to 18.5 ha (Table S1). Previous studies of focal *E. angustifolia* populations in our study area found high rates of reproductive failure among the fragmented patches of remnant prairie. Reproductive failure in small populations reflected limited mating opportunities (29, 34) rather than a paucity of pollinators or low rates of pollinator visitation (33).

Experimental design

We investigated fire effects on reproduction within 35 patches of remnant tallgrass prairie where we have studied *E. angustifolia* reproduction and demography continuously since 1996 (Fig. S1). This study focuses on reproductive data collected during six growing seasons: 2014-2016 and 2020-2022. In 2021 and 2022, during the spring prior to each growing season, we experimentally burned a stratified sample of 15 populations. Seven additional burns were conducted for management purposes during the

same season as our experimental burns and under similar conditions (i.e., similar fuel conditions, time of year, weather conditions, fire behavior, etc.). By including replication of burn treatments in both space and time, our study design reveals burn effects while accounting for interannual variation in reproduction and systematic variation in reproduction among sites (see Supplemental Information).

Reproductive effort

During each growing season (June – August), we searched for and mapped the location of all flowering *E. angustifolia* within the study populations. Flowering plants were mapped using survey grade GPS units (TOPCON GRS-1) that yield <10 cm precision. We estimated census population size (number of sexually mature individuals) using longitudinal records of flowering plants between 2012 and 2022. High precision spatial data and permanent markers allow us to track the annual reproductive effort of 6357 individual *E. angustifolia* over the six-year study (see Supplemental Information for details). We quantified three measures of plant reproductive effort that could be influenced by fire. First, we estimated the proportion of sexually mature plants that flowered in each year within patches by dividing the count of flowering plants in each mating scene – unique population-year combinations – by census population size. Second, we calculated the mean number of heads that produced pollen per flowering plant for each mating scene. Third, we determined the average number of fruits produced per head using a random sample of heads from each mating scene (estimates based on 1452 heads sampled during the study, see SI for details). Flowering *E. angustifolia* plants produce an average of ~150 florets per flowering head. Each floret yields one fruit regardless of whether a floret receives compatible pollen.

Mating potential

To quantify variation in temporal mating potential, we monitored the daily flowering status of reproductive *E. angustifolia* individuals to identify the first and last day each plant produced pollen (see SI for detailed description of our protocol for monitoring flowering phenology). We monitored the flowering phenology of all reproductive plants across every population in 2014-2016 and 2020-2021. In

2022, exceptionally high flowering rates led us to track phenology in a subset of individuals within large populations (see SI for description of subsampling). We quantified mating potential within each mating scene using outcross mating potential (OMP), a metric that integrates spatial and temporal mating potential (32). This metric utilizes spatially explicit information about the daily flowering phenology of each flowering plant to calculate the distance from each focal individual to its k^{th} nearest reproductively active neighbor on each day the focal individual is flowering (see Supplemental Information for detailed description of OMP and its calculation). An OMP value of 0 reflects a focal plant whose nearest prospective mates are distant or have little overlap in flowering phenology. An OMP value of 1 corresponds to a focal plant whose prospective mates are nearby and flowering at the same time. We chose to use $k = 7$ because previous studies of *E. angustifolia* found strong associations at this scale with pollination rates (13, 29) as well as mating events (49). We estimated mean OMP within 181 mating scenes using high-resolution maps and daily flowering phenology from 6446 instances of flowering.

Pollination rates and reproductive outcomes

We quantified pollination rates using two distinct metrics: mean seed set and mean style persistence. Seed set quantifies the proportion of fruits from harvested heads that contain a fertilized embryo. To estimate mean seed set, we X-rayed a random sample of fruits from each harvested head, scored X-ray images to quantify the proportion of fruits containing embryos, and averaged across individuals within each mating scene (mean seed set estimates derived from 1452 heads sampled during the study). Although seed set appears to primarily reflect mating opportunities (13, 29, 34), embryos may fail to develop for reasons other than pollen limitation (e.g., disease, predation, or seed abortion can all cause embryo loss after pollination). Thus, we also quantified mean style persistence – a resource-independent index of pollen limitation. Style persistence quantifies the average number of days styles are receptive to pollen on a flowering head (34). *Echinacea angustifolia* styles shrivel after receiving compatible pollen. Longer style persistence indicates failure to receive compatible pollen, though some styles may disappear due to herbivory or other physical damage (Wagenius 2004). We averaged style

persistence across individuals within each mating scene (estimates of mean style persistence derived from 5368 individual-level observations). Finally, to evaluate fire effects on reproductive outcomes we estimated mean annual fecundity (total annual seed production) by multiplying mean achene count, mean seed set, and mean head count. Annual fecundity thus reflects both reproductive effort and pollination rates.

Data analysis

We analyzed fire effects on six components of plant reproductive fitness. To evaluate fire effects on measures of plant reproductive effort (flowering proportion, mean head count, and mean fruit count), we fit linear mixed-effects models that included predictors for burn treatment ("Burned" vs. "Unburned"), census population size (log-transformed), and an interaction term between burn treatment and population size (log-transformed). These models included a random effect for site (to account for potential non-independence among individuals within sites) as well as a random effect for year (to account for variation among years). We used likelihood ratio tests to evaluate the significance of fixed predictors. For mean seed set, mean annual fecundity, and mean OMP, we fit non-linear models to capture the sigmoidal relationships between measured responses and census population size. Our non-linear models were based on three-parameter logistic function allowing the upper asymptote, the rate of increase, and the inflection point of the logistic function to differ between burned and unburned samples (see Supplemental Information).

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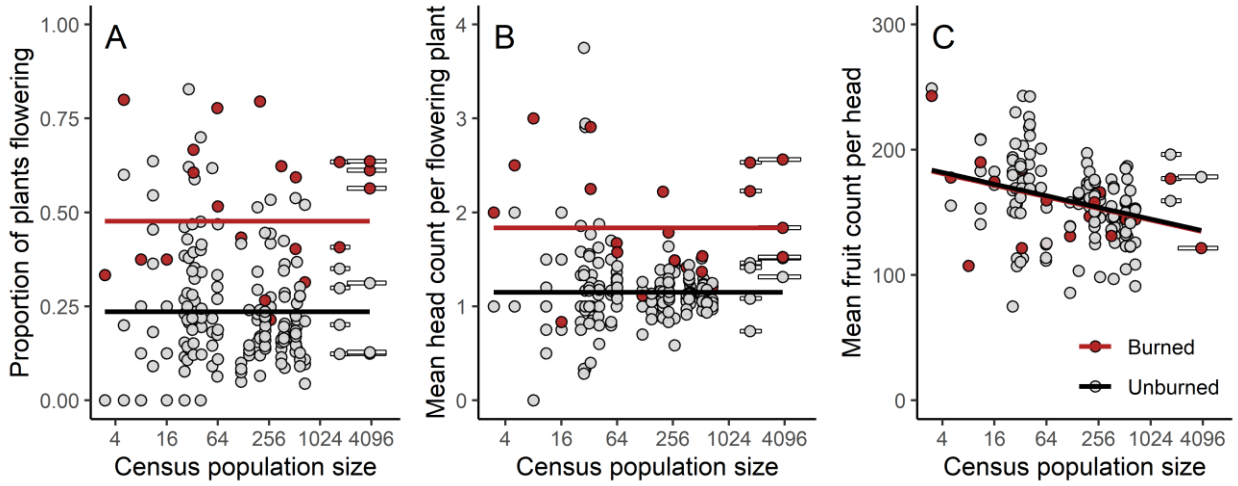


Fig. 1. Fire has density independent effects on three components of reproductive effort over six years and across 35 fragmented populations of *Echinacea angustifolia*. Fire increases the mean annual proportion of plants that flower (A) from 24% to 48%. This effect of fire does not vary with populations size (interaction effect, P = 0.924, N = 210 mating scenes), in other words, the influence of fire is density independent. Similarly, fire increases the mean number of heads that a plant produced (B) from 1.15 to 1.83, but this effect of fire does not vary with population size (interaction effect, P = 0.614, N = 199 mating scenes). Although the mean number of fruits per head (C) decreases with population size, no evidence exists of a main or interacting fire effect (interaction effect, P = 0.976, N = 152 mating scenes). Solid lines represent predicted estimates from statistical model (see Materials and Methods for details). Rectangular boxes depict the upper and lower bounds for estimated census population size in the two largest populations (see details in Supplemental Information).

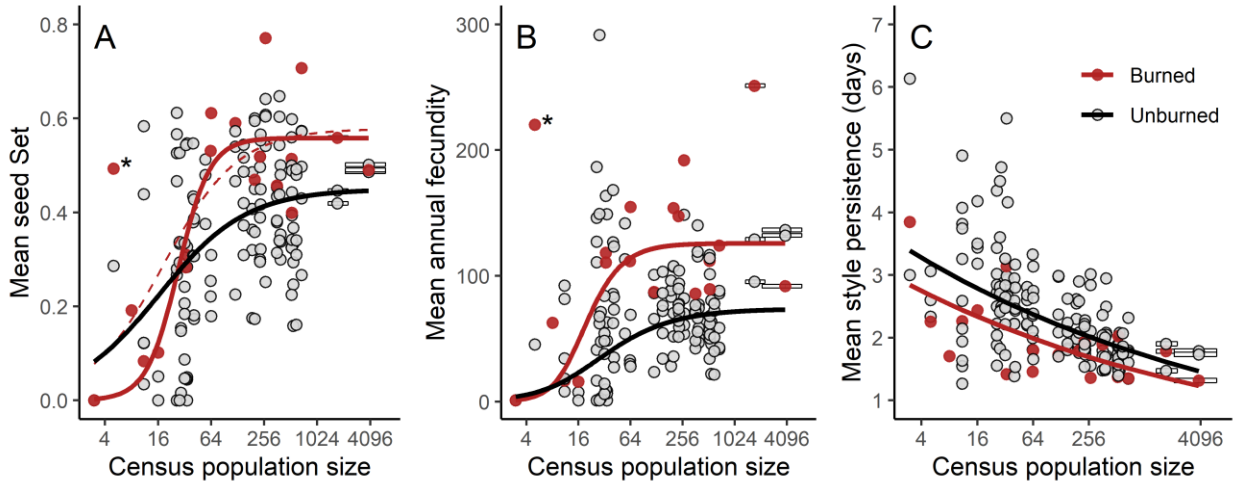


Fig. 2. Fire effects on reproductive outcomes depend on census population size in fragmented *E. angustifolia* mating scenes but evidence for such density dependent effects on pollination is mixed. Mean seed set, the proportion of fruits containing a fertilized embryo (A), increases with population size. Fire effects strongly and consistently enhance seed set in large populations but the effects sharply diminish becoming negative and inconsistent in the smallest populations (N = 152 mating scenes). Mean annual fecundity, the total number of seeds produced per plant (B) increases with population size. Fire effects are strong and consistent in large population but they sharply decline in the smallest populations (N = 152 mating scenes). Mean style persistence, a measure of pollen limitation (C), decreases with population size. There is no evidence (N = 171 mating scenes) that the effect of fire in ameliorating pollen limitation varies with population size. Solid lines represent predicted estimates from statistical model (see Materials and Methods for details). The dashed line in panel (a) illustrates model predictions when outlier point (marked with asterisk) is included. Rectangular boxes depict upper and lower bounds for estimated census population size in our two largest populations (see details in Supplemental Information).

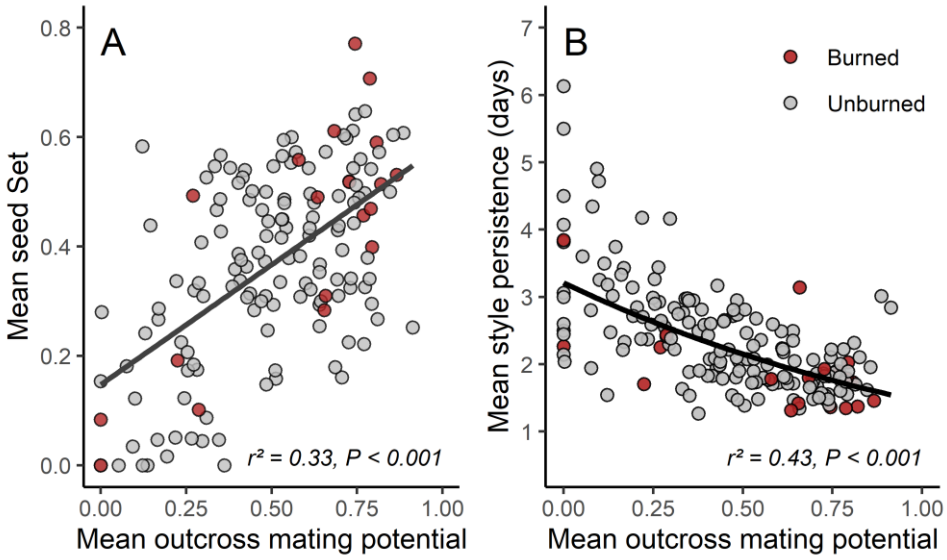
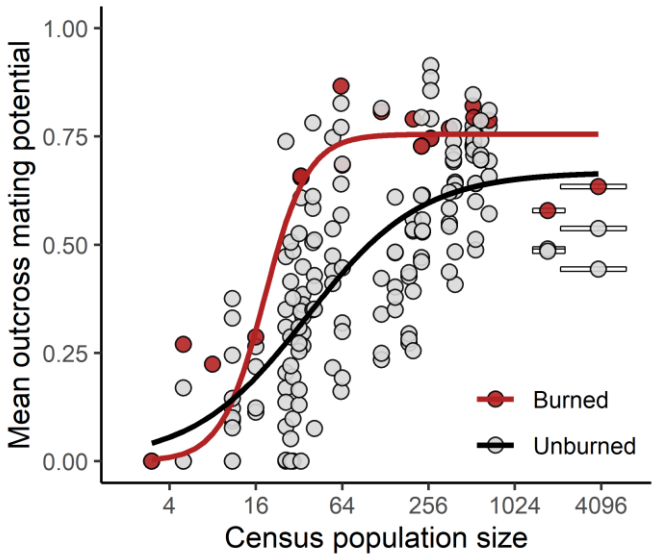


Fig. 3. Seed set and pollination in fragmented *Echinacea angustifolia* mating scenes reflects spatiotemporal mating opportunities. Mean seed set per mating scene increases three-fold from the scene with the worst to best mating opportunities and this relationship is not influenced by the burn status of the scenes (N = 152 mating scenes). Mean style persistence, a measure of pollen limitation, decreases with mean outcross mating potential and this relationship does not depend on burn status (N = 171 scenes). Mean outcross mating potential quantifies average spatiotemporal mating opportunities within a scene on a scale from zero, in which prospective mates are distant or flowering asynchronously, to 1, in which prospective mates are both nearby and flowering synchronously.



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543 **Fig. 4.** Fire effects on spatiotemporal mating opportunities vary with population size, i.e., they are
544 density-dependent effects, in 35 *Echinacea angustifolia* populations over six years (N = 181 mating
545 scenes). Fire strongly and consistently increases mating potential in intermediate-sized population with
546 smaller and less consistent effects in the largest and smallest populations. Mean outcross mating potential
547 quantifies average spatiotemporal mating potential within a mating scene. A mean value of 0 indicates
548 scenes where prospective mates are distant or have little overlap in flowering phenology. A mean value of
549 1 corresponds with scenes where prospective mates are nearby and flowering at the same time. Lines
550 represent predicted values from non-linear models (see Materials and Methods for details).

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