



Habitat fragmentation decouples fire-stimulated flowering from plant reproductive fitness

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Many plant species in historically fire-dependent ecosystems exhibit fire-stimulated flowering. While greater reproductive effort after fire is expected to result in increased reproductive outcomes, seed production often depends 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, isolated populations where mating opportunities are restricted and pollination rates are low. We conducted a 6-y study of 6,357 *Echinacea angustifolia* (Asteraceae) individuals across 35 remnant 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 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 reproductive outcomes differed between large and small populations, reflecting the density-dependent effects of fire on spatiotemporal mating potential and pollination. In populations with fewer than 20 individuals, fire did not consistently increase pollination or annual 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.

prairie | fire | Allee effect | phenology | density dependence

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 vital 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 in the months after fire (10–12). While fire-stimulated flowering is often assumed to increase annual seed production, 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. Thus, even if fire increases reproductive effort among plants in small populations, limited mating opportunities could restrict pollination and seed production. The potential for fire-stimulated flowering to differentially influence reproductive outcomes in large versus small populations has not been previously examined. Failure to account for these potential density-dependent effects of fire-stimulated flowering on pollination rates and reproductive outcomes may yield misleading inferences about the extent to which synchronized postfire reproduction contributes to population growth and persistence. Specifically, we may overestimate the capacity of fire to promote population growth and plant diversity in historically fire-dependent habitats.

Despite the prevalence of fire-stimulated flowering in historically fire-dependent habitats such as South African fynbos (10, 17), Australian shrublands (10, 18), Brazilian cerrado (11, 19), North American longleaf pine savannas (12, 20), and North American tallgrass prairie (13, 21, 22), the expected association between increased reproductive effort after fire and increased seed production has been empirically examined in only a handful of species and almost exclusively within large populations (13, 15, 23). Annual reproductive effort in perennial plants largely reflects patterns of resource availability, acquisition, and storage (15, 21). As a result, fire is expected to influence plant reproductive effort similarly regardless of population size or density. In contrast, postfire reproductive outcomes depend not only on reproductive effort but also spatiotemporal mating opportunities and pollination (13, 14, 16), which often vary with population size. In large populations, fire-stimulated flowering reduces the spatial distance between prospective mates, which increases the likelihood that pollen is transferred between individuals (13). Similarly, fire

Significance

Periodic fire maintains plant diversity in fire-dependent ecosystems worldwide. Fire effects on population dynamics and vital rates, such as reproduction, are primarily attributed to fire's influence on the physical environment. However, our 6-y experimental study of 6,357 individual plants across 35 fragmented prairie populations reveals that fire effects on sexual reproduction depend on population size. Burns consistently boosted annual reproductive effort, 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 the reproductive benefits of fire in fragmented habitats and diminish the capacity of fire to maintain plant diversity.

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can increase overlap in the timing of reproductive activity among individuals, leading to synchronized within-season flowering phenology and improved pollination (13, 14, 16). However, at low population densities, greater reproductive effort after fire may not appreciably increase mating potential—the capacity for sexual reproduction, as dictated by the location and reproductive timing of prospective mates (13). Consequently, fire-stimulated flowering may not improve pollination or seed production in small populations. Empirical research is needed to quantify these potentially density-dependent effects of fire on plant reproduction and to establish how the influence of fire on various components of reproduction—namely reproductive effort, mating opportunities, pollination rates, and seed production—depends on population size.

Realized fitness outcomes resulting from the presumably density-independent effects of fire on reproductive effort and the potentially density-dependent effects of fire on mating opportunities and pollination have particularly important implications for plants within fragmented habitats. Many historically fire-dependent systems have undergone extensive habitat loss and fragmentation (24–27), including North American tallgrass prairie, which has been reduced to less than one percent of its historic extent across much of its range (28). In these fragmented habitats, many plant species exhibit chronically low seed production. Rates of reproductive failure are highest in small populations where reduced mating opportunities and pollen limitation constrain sexual reproduction (29–31). Persistent reproductive failure can depress population growth rates and threaten the persistence of small plant populations (32). Fire could alleviate these mate-finding Allee effects by synchronizing flowering, improving pollination, and enhancing seed production (13). However, the lack of data about fire effects on reproductive outcomes across a range of population sizes precludes a rigorous assessment of fire's capacity to alleviate mate-finding Allee effects.

We conducted a 6-y experiment to evaluate potential density-dependent effects of fire on plant reproductive fitness in fragmented tallgrass prairies. Before European settlement, prairies burned every 1 to 5 y. Many prairie plant species flower vigorously during the growing season after fire. Our study focused on 35 populations of *Echinacea angustifolia* (Asteraceae) within a fragmented landscape in western Minnesota, USA (*SI Appendix, Fig. S1*). We conducted prescribed burns in a stratified sample of 18 populations ranging in size from three to nearly 4,000 sexually mature individuals (*SI Appendix, Table S1*). We then quantified multiple components of annual reproductive fitness in 6,357 individuals to evaluate how fire affects plant reproduction—both reproductive effort and outcomes—and whether fire effects vary with census population size. We define census population size as the count of sexually mature individuals. We hypothesize that fire has consistent effects on reproductive effort that do not vary with population 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 spatiotemporal mating potential within each mating scene—the unit of replication in this study defined by unique population-year combinations—and assess how the influence of fire on spatiotemporal mating potential depends on population size. Finally, we evaluate the relationship between spatiotemporal mating opportunities and pollination rates to evaluate the contribution of pollination to postfire reproductive outcomes.

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 nonflowering years produce inconspicuous basal leaves. Flowering plants produce one to many heads, each composed of many florets. Pollen limitation contributes to reproductive failure in small *E. angustifolia* populations but seed production increases in dense populations and when prospective mates are nearby (29, 33). A previous study of *E. angustifolia* within a single, large population revealed that 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 *E. angustifolia* plant reproductive effort, pollination rates, and annual seed production in 210 mating scenes. Our study included 22 burned mating scenes which were burned 1 to 2 mo before the growing season and 188 unburned mating scenes which were not burned for at least 12 mo before the growing season. We quantified three aspects of plant reproductive effort. First, we censused flowering plants across all mating scenes and calculated the proportion of sexually mature plants flowering within each mating scene ($N = 210$ mating scenes). Second, we quantified mean head count per flowering plant within each mating scene ($N = 199$ mating scenes in which at least one individual flowered). Third, we quantified the mean fruit count per seed head using seed heads harvested from a random sample of flowering individuals within mating scenes and counting individual fruits ($N = 152$ mating scenes). Each *E. angustifolia* floret produces one dry fruit whether or not the floret is successfully pollinated. Thus, fruit count does not depend on pollination.

To quantify pollination rates, we measured mean seed set and mean style persistence. Seed set quantifies the proportion of fruits containing a fertilized embryo. We quantified individual-level seed set using randomly sampled fruits from each harvested head and averaged these values across individuals within each mating scene to calculate mean seed set per plant. Our previous work indicates seed set in *E. angustifolia* primarily reflects pollination, but seed set can also potentially be influenced 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 assessed before seeds ripen, to validate that our inferences about seed set reflect pollination rather than other ecological processes (34). We then estimated mean annual fecundity per scene (average total number of seeds produced per plant) by multiplying mean head count, mean achene count, and mean seed set for each mating scene ($N = 152$ 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) based on the seven nearest prospective mates—a metric that integrates the spatial location of an individual's prospective mates and the relative timing of their reproductive activity during the growing season (32). This metric quantifies spatiotemporal mating potential for each individual on a scale from 0—when the seven nearest prospective mates are distant or there is no overlap of the days when individuals are blooming—to 1—when the seven nearest prospective mates are close and there is complete overlap of the days when individuals are blooming (see *Materials and Methods* for details).

We averaged OMP values for individuals within each mating scene to calculate mean OMP.

Results

Fire Has Density-Independent Effects on Plant Reproductive Effort.

Fire consistently increased plant reproductive effort across *E. angustifolia* mating scenes (Fig. 1 and *SI Appendix, Table S2*). The average proportion of sexually mature plants flowering per scene doubled from 23.6 percent to 47.7 percent in scenes immediately following experimental burns compared to all other scenes (Fig. 1A; Likelihood ratio test: $\chi^2 = 45.545$, $P < 0.001$). We found no evidence that fire effects on flowering rates per scene depended on population size ($\chi^2 = 0.009$, $P = 0.924$) or that population size influenced variation in the proportion of plants flowering among populations ($\chi^2 = 2.422$, $P = 0.120$). Similarly, the mean head count per flowering plant per scene increased 58% from 1.15 to 1.83 during the growing season after fire (Fig. 1B; $\chi^2 = 47.330$, $P < 0.001$) and there was no evidence that these

effects depended on population size of the scene ($\chi^2 = 0.254$, $P = 0.614$) or that population size influenced mean head count per scene ($\chi^2 = 0.019$, $P = 0.890$). We found no evidence that fire influenced the mean number of fruits per flowering head per scene ($\chi^2 = 0.001$, $P = 0.976$), though the mean number of fruits per head decreased with census population size of the scene (Fig. 1C; $\chi^2 = 6.134$, $P = 0.013$).

Fire Has Density-Dependent Effects on Reproductive Outcomes.

In contrast to the density-independent response of plant reproductive effort to fire, fire effects on seed set and total seed production are strongly dependent on census population size of mating scenes (Fig. 1 and *SI Appendix, Table S3*). Mean annual fecundity per scene increased nonlinearly with census population size and fire had different effects in small versus large populations (Fig. 1D), though our inferences about fire effects were complicated by the highly influential point from one scene of a small population (see asterisk in Fig. 1D). Inclusion of this point prevented model convergence. With the outlier excluded, fire increased asymptotic

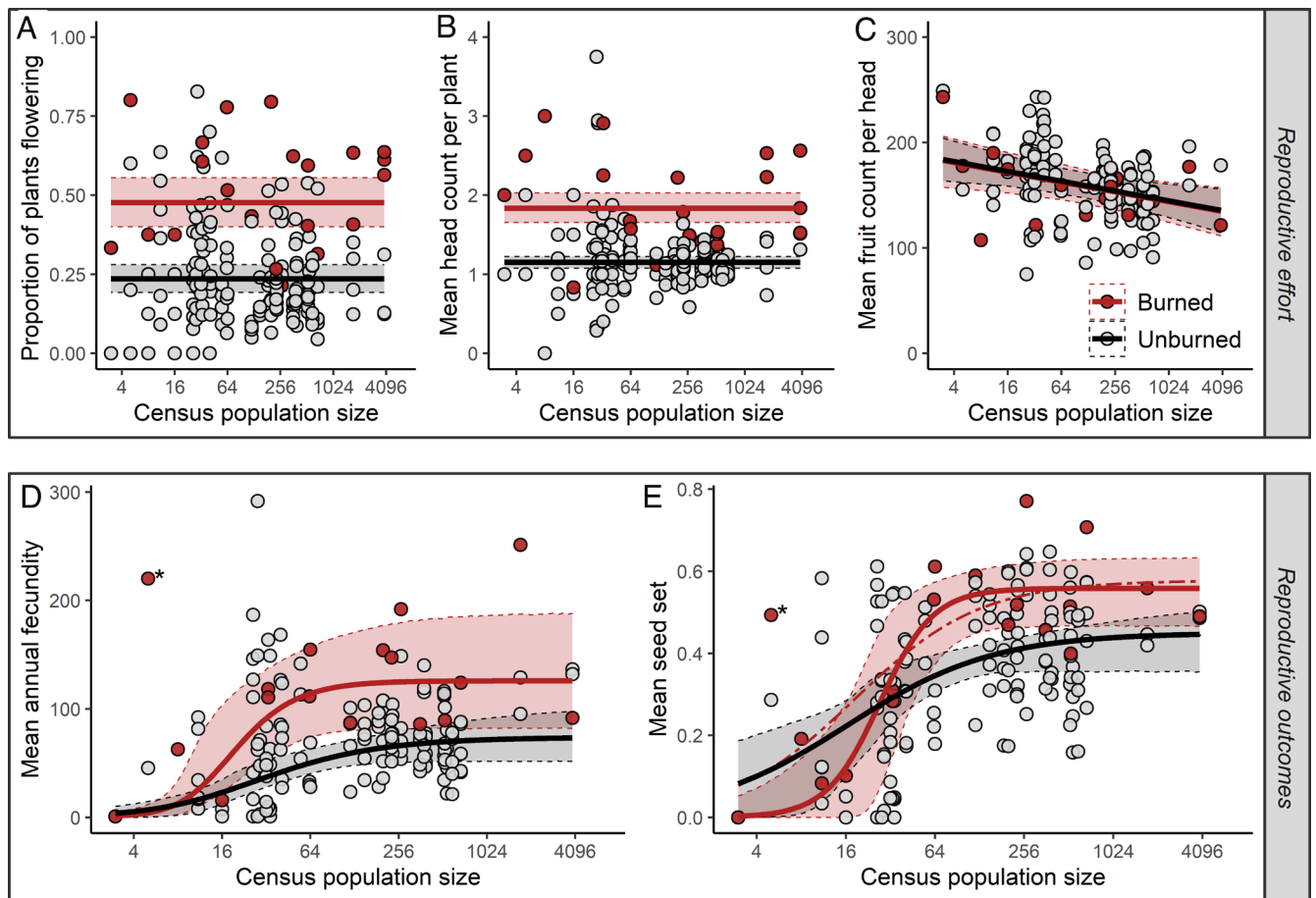


Fig. 1. Fire has consistent effects on plant reproductive effort across mating scenes of 35 fragmented populations of *Echinacea angustifolia* while the influence of fire on reproductive outcomes depends on population size. Over a 6-y experiment, fire increased the mean annual proportion of plants that flower per mating scene (A) from 24 to 48%. This effect of fire did not vary with population size (interaction effect, $P = 0.924$, $N = 210$ mating scenes); in other words, the influence of fire is density independent. Similarly, fire increased the mean number of heads that a plant produced per mating scene (B) from 1.2 to 1.8, but this effect of fire did not vary with population size (interaction effect, $P = 0.614$, $N = 199$ mating scenes). Although the mean number of fruits per head per mating scene (C) decreased with population size, no evidence exists of a main or interacting fire effect (interaction effect, $P = 0.976$, $N = 152$ mating scenes). Mean annual fecundity, the total number of seeds produced per plant per mating scene (D), increased nonlinearly with population size. Fire consistently increased fecundity in mating scenes of large populations but fire did not consistently increase fecundity in the smallest populations ($N = 152$ mating scenes). These density-dependent effects of fire on mean annual fecundity were driven by variation in seed set. Mean seed set, the proportion of fruits containing a fertilized embryo per mating scene (E), increased with population size. Fire consistently increased seed set in mating scenes of large populations but the effects of fire sharply diminished in the smallest populations where there was no consistent effect of fire on mean seed set per mating scene ($N = 152$ mating scenes). Solid lines represent predicted estimates from statistical models (see *Materials and Methods* for details). Shaded areas and dotted lines depict bootstrapped 95% CI for model predictions. The dashed line in panel E illustrates model predictions when including one outlier (marked with asterisk). Statistical models of mean annual fecundity failed to converge when this outlier was included so we only present model predictions excluding this outlier in panel D.

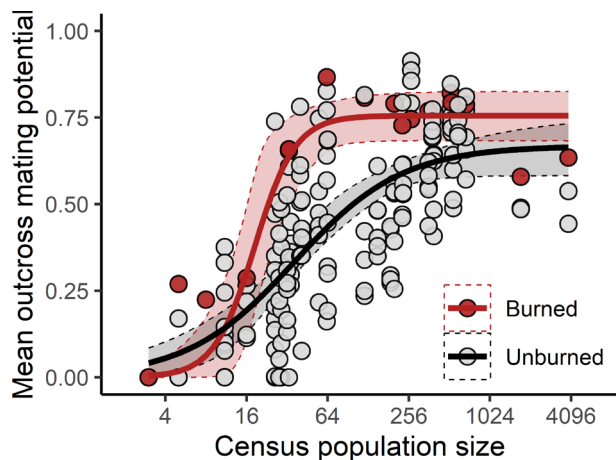


Fig. 2. The influence of fire on spatiotemporal mating opportunities depends on population size across 35 *Echinacea angustifolia* populations over 6 y (N = 181 mating scenes). Fire consistently increased mating potential of mating scenes in intermediate-sized populations and had smaller and less consistent effects in the largest and smallest populations. Mean OMP quantifies average spatiotemporal mating potential of all individual plants flowering within a mating scene. A mean OMP value of 0 indicates scenes where prospective mates are distant or have little overlap in flowering phenology. A mean OMP value of 1 corresponds with scenes where prospective mates are nearby and flowering at the same time. Solid lines represent predicted values from nonlinear models (see *Materials and Methods* for details). Shaded areas and dotted lines represented bootstrapped 95% CI for model predictions.

estimates of mean annual fecundity per scene by 71 percent (125.8 [83.9, 188.4] seeds per plant versus 73.8 [52.1, 104.5] 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 scenes). Our model predicts that fire has the greatest effect on mean annual fecundity in populations with 74 sexually mature plants (*SI Appendix, 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 scenes).

Mean seed set per scene increased nonlinearly with census population size and fire effects differed considerably between small and large populations, though inferences about fire effects were sensitive to an influential point from the same small, burned scene

(see asterisk in Fig. 1E). Exclusion of this data point improved the distribution of residuals though general qualitative inferences about fire effects were similar whether the point was excluded or not (see *Discussion* for description of this outlier and relevant biological considerations). With the outlier excluded, fire influenced both the rate at which mean seed set per scene increased with population size (estimates from three-parameter logistic model [95% CI]: 0.41 [0.04, 0.79] burned versus 1.13 [0.09, 2.18] unburned scenes) as well as asymptotic estimates in large populations (0.56 [0.48, 0.63] burned versus 0.45 [0.36, 0.54] unburned scenes). We predict that burns have the greatest effect on seed set in populations with 97 sexually mature plants where fire is predicted to increase seed set per scene by 44 percent (*SI Appendix, Fig. S2A*; 0.53 versus 0.37). In populations with fewer than 20 sexually mature plants, mean seed set was low for both burned and unburned mating scenes.

Fire Effects on Seed Production Reflect Spatiotemporal Mating Potential and Pollination.

The density-dependent effects of fire on plant reproductive outcomes mirrored the differential effects of fire on spatiotemporal mating opportunities in large versus small populations (Fig. 2). OMP increased nonlinearly with census population size (0.35 [0.11, 0.59] burned versus 0.92 [0.52, 1.31] unburned scenes). Fire also increased asymptotic estimates of mean OMP (0.76 [0.69, 0.83] burned versus 0.67 [0.58, 0.75] unburned scenes). We predict that fire has the greatest effect on mean mating potential in populations with 41 sexually mature plants (*SI Appendix, Fig. S2C*). Here, fire increased mean OMP by 93 percent (0.69 versus 0.36). Mean OMP per scene primarily reflected variation in spatial mating opportunities. Mean OMP was strongly correlated with harmonic mean distance to nearby neighbors (Pearson's correlation: all $r < -0.61$ and $P < 0.001$ for distance to $k = 1$ to 7 nearest neighbors, N = 181 scenes) and was more weakly associated with mean pairwise synchrony ($r = 0.25$, N = 181, $P = <0.001$; see *SI Appendix, Table S4*).

Variation in mean seed set per scene closely tracked mean OMP (Fig. 3A; $r^2 = 0.33$, N scenes = 152, $P < 0.001$). Mean seed set was predicted to increase from 15 percent at the minimum mean OMP value of 0 to 59 percent when mean OMP reached its maximum value of 1. Mean seed set was also strongly associated with mean style persistence (Fig. 3B; $r^2 = 0.35$, N = 152, $P < 0.001$), though

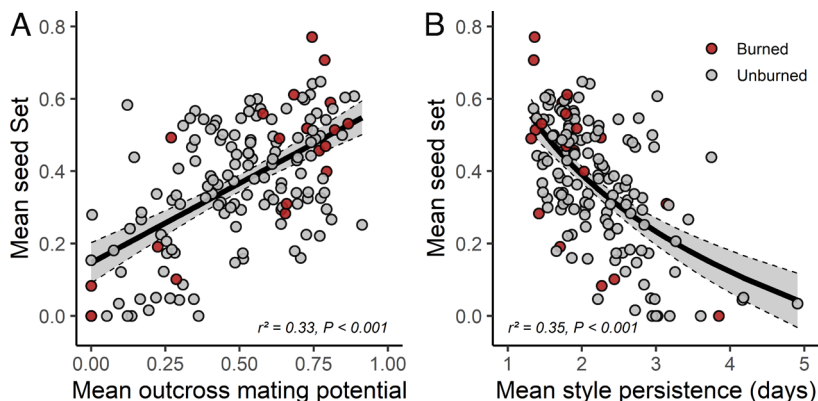


Fig. 3. Variation in mean seed set reflects differences in spatiotemporal mating opportunities and pollination in mating scenes of 35 *Echinacea angustifolia* populations over 6 y. Mean seed set (A), the proportion of dry fruits containing fertilized embryos, per mating scene increases threefold from the scene with the worst to the best mating opportunities and this relationship is not influenced by the burn status of the scenes (N = 152 mating scenes). Mean OMP 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. Mean seed set per scene declines with increasing mean style persistence (B), a measure of pollination that does not depend on resource availability, implying differences in seed set reflect variation in pollen receipt rather than resource availability. Solid lines represent predicted estimates from statistical model while shaded areas and dotted lines depict 95% CI for model predictions.

the association between mean style persistence, fire, and census population size differed somewhat from mean seed set in small (*SI Appendix*, Fig. S4). Mean seed set was greatest in mating scenes where styles shriveled quickly. Mean style persistence was strongly associated with mean OMP (*SI Appendix*, Fig. S5; $r^2 = 0.43$, $N = 181$, $P < 0.001$).

Discussion

Our 6-y 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 A–C). In contrast, the influence of fire on plant reproductive outcomes, namely seed set and annual seed production, depended on the scene's population size (Fig. 1 D and E). In large populations, fire consistently increased spatiotemporal mating potential (Fig. 2), leading to improved pollination and greater seed production during the growing season after a burn. However, the fitness benefits of synchronized post-fire reproduction declined rapidly in populations with fewer than approximately 20 sexually mature individuals. Seed set was closely associated with spatiotemporal mating potential and pollen receipt (Fig. 3), indicating that differential effects of fire on reproductive outcomes in small versus large populations reflect variation in spatiotemporal mating opportunities. In small, isolated populations, pollen limitation often constrained seed production despite greater reproductive effort after fire. These findings offer unique and fundamental insights into the effects of fire on plant reproduction.

Previous investigations of fire effects on various plant vital 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, 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 (21, 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 reveal the previously overlooked role of density-dependent processes (namely pollination) in determining post-fire plant reproductive fitness. Recent 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 a high flowering rate, many heads per individual plant, and relatively high mean seed set after our experimental burn in spring 2021 (though seed set ranged from 3 to 86 percent among the four flowering individuals in this mating scene). We observed this relatively high mean seed set despite low mean spatiotemporal mating potential. It remains unclear whether the anomalously high pollination rates in this mating scene 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 nonnative *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 outcomes within small, chronically pollen-limited populations.

This study contributes fundamental insights into the fitness benefits of synchronized reproduction. Synchronized interannual reproductive variation (e.g., masting) is prevalent among long-lived iteroparous plants (10, 11, 18, 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 (18, 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 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 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 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 emphasize vital rates 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 that incorporate density-dependent fire effects on multiple vital rates, including the potential for negative density-dependent mortality at multiple life stages to offset the positive density-dependent seed production we observe. Future studies that explicitly consider density dependence throughout the life cycle, such as life table response experiments, will reveal how different life stages contribute to plant demographic responses to fire. These studies will also provide insights into whether the influence of recruitment, survival, and seed production on population growth rates varies 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 that 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 to 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 vital 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 patches of remnant tallgrass prairie distributed across a ~6,400 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 (*SI Appendix, Table S1*). We have investigated ecological and genetic processes of *Echinacea angustifolia* within these fragmented patches of tallgrass prairie continuously since 1996, including two studies about fire effects on nonreproductive life-history stages (8, 35). Our extensive work on reproductive biology has revealed that annual reproductive fitness is variable among individuals, sites, and years (29). Much of this variation in reproductive fitness across the landscape can be attributed to the availability of potential mates—the spatial proximity of individuals to potential mates, the relative timing of their reproductive activity, and the genetic compatibility of prospective mates (29, 32, 34, 49)—rather than rates of pollinator visitation (33). In one long-term study on a large prairie preserve which has been managed with regular fires, we learned that fires consistently improve reproductive outcomes by increasing mating opportunities (13). This study provided important insights into how fire influences plant reproductive outcomes. Recent studies on two other species corroborated findings about the role pollination plays in post-fire reproductive outcomes (14, 16). However, all our previous work investigating the influence of fire on plant reproduction relied on opportunistic studies after prescribed fires within a single large prairie preserve, which precluded more general inferences about how fire effects on plant reproduction varies with population size. Consequently, we could only speculate about how fires might affect reproduction across the range of smaller populations on the landscape.

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 (*SI Appendix, Fig. S1*). This study focuses on reproductive data collected during six growing seasons: 2014 to 2016 and 2020 to 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 (*SI Appendix*).

Reproductive Effort. During each growing season (June to 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 HiPer SR) 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 tags allow us to track the annual reproductive effort of 6,357 individual *E. angustifolia* over the 6-y study (see *SI Appendix* 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 all mating scenes by dividing the count of flowering plants in each mating scene—unique population-year combinations—by census population size ($N = 210$ mating scenes). Second, we calculated the mean number of heads that produced pollen per flowering plant for each mating scene using observed head counts for all individuals within every mating scene (mean head count estimated from 9,504 instances of flowering across $N = 199$ mating scenes). Third, we determined the average number of fruits produced per seed head by sampling seed heads from randomly selected individuals within each mating scene (estimates based on random sample of 1,452 heads across $N = 152$ mating scenes, see *SI Appendix* 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.

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 random sample of 1,452 heads across $N = 152$ mating scenes). 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 (34). We averaged style persistence across individuals within each mating scene (estimates of mean style persistence derived from 5,368 individual-level observations across $N = 171$ mating scenes). 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.

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 Appendix* for detailed description of our protocol for monitoring flowering phenology). We monitored the flowering phenology of all reproductive plants across every population in 2014 to 2016 and 2020 to 2021. In 2022, exceptionally high flowering rates led us to track phenology in a subset of individuals within large populations (see *SI Appendix* for description of subsampling). We quantified mating potential within each mating scene using 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 *SI Appendix* 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 of OMP with both pollination rates (13, 29) and mating events (50). We estimated mean OMP within 181 mating scenes using high-resolution maps and daily flowering phenology from 6,446 instances of flowering.

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" versus "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 annual fecundity, mean seed set, and mean OMP, we fit nonlinear models to capture the sigmoidal relationships between measured responses and census population size. Our nonlinear models were based on three-parameter logistic functions that allowed the upper asymptote, the rate of increase, and the inflection point of the logistic function to differ between burned and unburned samples (*SI Appendix*).

Data, Materials, and Software Availability. Data and code necessary to replicate analyses and figures data have been deposited in Figshare (51).

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