



Fire synchronizes flowering and boosts reproduction in a widespread but declining prairie species

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Edited by Susan Harrison, University of California, Davis, CA, and approved December 18, 2019 (received for review April 30, 2019)

Fire is an important determinant of habitat structure and biodiversity across ecosystems worldwide. In fire-dependent communities, similar to the North American prairie, fire suppression contributes to local plant extinctions. Yet the demographic mechanisms responsible for species loss have not been directly investigated. We conducted a 21-y longitudinal study of 778 individual plants of *Echinacea angustifolia*, a widespread perennial species with chronically limited mating opportunities, to explore how fire affects reproduction. In a large preserve, with management units on different burn schedules, we investigated *Echinacea* mating scenes, which quantify isolation from potential mates and overlap in the timing of flowering, to determine the extent to which fire influences the potential for sexual reproduction. We demonstrate that fire consistently increased mating opportunities by synchronizing reproductive effort. Each fire occurred during fall or spring and stimulated flowering in the subsequent summer, thus synchronizing reproduction among years and increasing the proximity of potential mates after a fire. Greater within-season flowering synchrony in postfire mating scenes further increased mating potential. The improved postfire mating scene enhanced reproduction by increasing pollination efficiency. Seed set in scenes postfire exceeded other scenes by 55%, and annual fecundity nearly doubled (88% increase). We predict the reproductive benefits of synchronized flowering after fire can alleviate mate-finding Allee effects, promote population growth, and forestall local extirpation in small populations of *Echinacea* and many other prairie species. Furthermore, the synchronization of flowering by burning may improve mating opportunities, reproduction, and the likelihood of persistence for many other plant species in fire-dependent habitats.

fire | Allee effect | phenology | mating | synchrony

Fire plays a critical role in shaping the structure, dynamics, and diversity of ecosystems worldwide (1). However, active fire suppression and loss of connectivity have drastically reduced the frequency of fires in many ecosystems during the last 150 y, including tallgrass prairie, where the historic fire return interval ranged from 1 to 5 y (2, 3). Tallgrass prairie is now among the most imperiled habitats worldwide. More than 95% of tallgrass prairie habitat has been destroyed, and little of what remains is protected (4, 5). Most remaining prairies are small, isolated, and threatened by ongoing habitat loss. These remnants also exhibit alarmingly high, and accelerating, rates of local plant extinction because of fire suppression (3, 6). Yet the mechanism explaining why fire suppression drives these local extinctions remains unclear. The leading hypothesis is that woody plants and large herbaceous species outcompete short plants in unburned prairies, but empirical support for this hypothesis comes from patterns of community change, not direct evidence of competitive exclusion (2, 3, 7). Here we investigate the consequences of fire for plant reproduction, a potentially important but previously unstudied mechanism by which fire may promote the persistence of threatened prairie plant populations and maintain species diversity.

Fire stimulates flowering in many prairie plants, but the extent to which enhanced flowering effort affects reproductive success has

not been investigated (2, 3, 8, 9). Vigorous flowering after fire has potentially important implications for the reproduction and population dynamics of prairie plant populations. As a consequence of small population size and limited mating opportunities, many plants in fragmented prairies receive insufficient pollen and fail to produce viable seed (10, 11). Persistent mate-limitation and reproductive failure can contribute to a mate-finding Allee effect, in which small populations decline and experience a heightened risk for local extinction (12–17). Is it possible that periodic fires release mate-limited populations from these persistent constraints by synchronizing reproductive effort and enhancing mating opportunities? Here we develop a quantitative framework for assessing mating opportunities and apply this framework to a model plant system to investigate the influence of fire on mating opportunities and reproduction in the prairie.

Mating opportunities decrease when prospective mates are distant (11, 14), reproductively active at different times (14, 19), or genetically incompatible (12, 20, 21). In self-incompatible plant species, individuals reject their own pollen and pollen from other individuals that share the same genetic recognition allele (22). To integrate the influence of space, time, and compatibility on reproduction, we develop the concepts of the mating scene and mating potential (Fig. 1). Mating potential is the capacity for sexual reproduction based on the location and reproductive timing of prospective mates (16). Mating potential can be

Significance

We address a critical conservation concern: the loss of native plant species in fire-dependent ecosystems. Reduced fire frequency in ecosystems such as the North American prairie contributes to local extinctions. The leading hypothesis is that woody and large herbaceous species outcompete other species in the absence of fire. However, alternative mechanisms have not been investigated. In our 21-y study of a model prairie plant, *Echinacea angustifolia*, we demonstrate that fires synchronize reproduction, leading to increased mating opportunities and improved reproduction. Fire synchronizes flowering both among and within years, nearly doubling seed production. These findings demonstrate a potentially widespread mechanism by which fire can enhance plant reproduction, promote population growth, and maintain plant diversity in fire-dependent ecosystems worldwide.

Author contributions: S.W. designed research; S.W., J.B., and G.K. performed research; S.W. and J.B. analyzed data; and S.W., J.B., and G.K. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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Data deposition: All data and code necessary to replicate the results in this paper are available for download from the Echinacea Project, <http://echinaceaproject.org/datasets/fire-fitness-spp/>.

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This article contains supporting information online at <https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1907320117/-DCSupplemental>.

First published January 27, 2020.

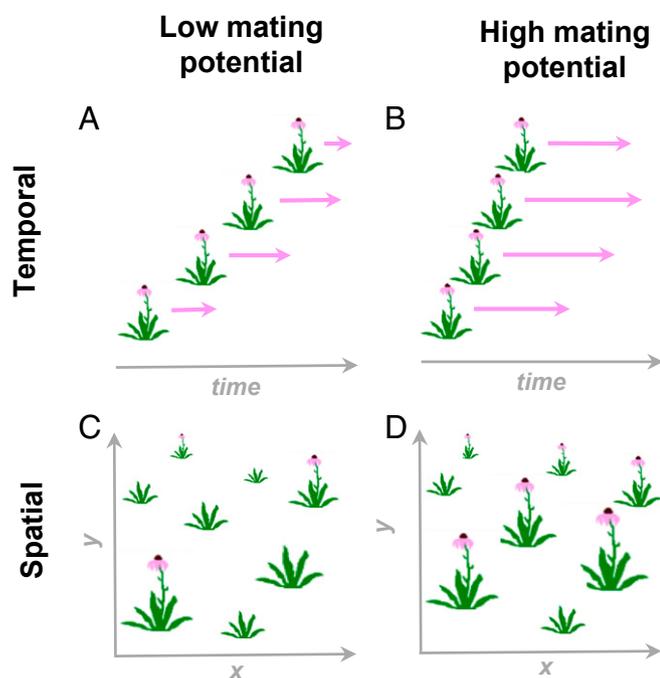


Fig. 1. Conceptual diagram illustrating spatial and temporal dimensions of mating potential. Temporal mating potential increases with the overlap in reproductive activity. Low temporal mating potential results from plants flowering less synchronously in mating scene A compared with mating scene B. Length of a purple arrow illustrates an individual's flowering duration, and timing is shown by the position along the time axis. Meanwhile, spatial mating potential increases with reduced isolation from prospective mates. Low spatial mating potential in mating scene C results from plants flowering at greater distances from mates compared with in mating scene D.

quantified for a pair of individuals based on the distance between them, the timing of reproductive activity, and their compatibility. Similarly, mating potential can be defined for an individual within the context of a mating scene, which we define as a season of reproductive activity in which coordinates of all individuals are known in spatial and temporal dimensions. In this article, we focus on entire mating scenes, each characterized by mean values of isolation (distance to nearest flowering neighbors) and synchrony (proportion of days that flowering overlaps between two plants). We hypothesize that fire increases the mating potential of mating scenes in the summer immediately after a dormant-season fire. These scenes we call “burned”; all others are “unburned.” The increase in mating potential may result from plants flowering in closer proximity and with greater synchrony.

In this study, we focus on a widespread purple coneflower (*Echinacea angustifolia* [Asteraceae], hereafter *Echinacea*) native to grasslands west of the Mississippi River. This species was chosen as a model organism to represent prairie plants, and this study system is ideal for quantifying mating scenes. Individuals can live for decades, are pollinated by native bees, reproduce only by seed (not clonally), and exhibit genetic self-incompatibility, which precludes self-pollination (23–25). An *Echinacea* plant sprouts each year from its deep, perennial taproot, regardless of fire. Adult plants are obvious in years when they flower; in other years, they produce only leaves that are shorter than surrounding vegetation. Fires promote seedling emergence and juvenile survival (26). We conducted our 21-y study on a 40-ha preserve where *Echinacea* are abundant ($n > 10,000$ adult plants) with 2,000 to 9,000 plants flowering each year. We characterized the mating scene by quantifying mating potential of all adult *Echinacea* plants, 778 individuals, in a 1.5 ha transect (~10 m × 300 m). We counted flowering

heads of all plants in every year from 1996 to 2016, and monitored their reproductive timing in 10 of those years. This long-term study allowed us to observe individual plants over many years, and examine reproductive patterns relevant to the persistence of perennial plant species. The East and West burn management units are separated by a 40-m buffer (SI Appendix, Fig. S1). Most of the remnant *Echinacea* populations near our study site have not burned in the past 20 y, but managers have conducted nine prescribed burns at the preserve since 1996, burning only a single unit per year (Materials and Methods and SI Appendix, Table S1).

A major challenge when quantifying effects of fire on reproduction is distinguishing reproductive patterns related to mating opportunities from those patterns associated with resource availability and seed predation. To disentangle these different components of reproductive success, we measured several aspects of *Echinacea* reproduction (SI Appendix, Table S2). We counted the number of flowering heads per plant in every year, and the number of fruits produced per plant in 9 y. Both of these flowering traits are determinate, meaning they are set before pollination begins. Thus, these components of fitness depend on resource availability and allocation before the flowering season. In 9 y of the study, we estimated seed set in random samples of plants from both management units within the transect. Seed set, the proportion of fruits that contains a seed, is a component of reproduction that is largely limited by receipt of compatible pollen. We also estimated the duration of style persistence in 8 y; styles shrivel after receipt of compatible pollen, so longer style persistence reflects greater pollen limitation (23). In the predominantly agricultural landscape of western Minnesota, fragmented *Echinacea* populations experience limited and failed reproduction resulting from a paucity of compatible mates rather than a shortage of pollinators (11, 24).

We hypothesize that an increase in mating potential, resulting from plants flowering with greater synchrony and less isolation, improves pollination and annual reproductive success in prairie plant populations. We also hypothesize that fire increases the mating potential of burned mating scenes. In this study, we quantify synchrony as the mean pairwise overlap in flowering phenology among all individuals within a mating scene or synchrony of individuals with their four nearest neighbors. We calculate isolation as the harmonic mean of distances between each flowering plant and its k^{th} nearest flowering neighbor within a scene (isolation calculated separately for $k = 1, 2, 3,$ and 4 ; Fig. 1). Results of this investigation elucidate a mechanism by which fire promotes the persistence of threatened prairie plant populations: fire synchronizes flowering both among and within years, leading to increased mating opportunities and improved reproduction.

Results

Consistent with our first hypothesis that mating potential would largely determine reproductive success in *Echinacea*, reproduction flourished in mating scenes where potential mates were in close proximity and flowering synchronously (Fig. 2A and B). Average seed set increased with temporal overlap in flowering phenology scene-wide (linear regression: $r^2 = 0.57$; $P < 0.001$) and among nearby plants ($r^2 = 0.42$; $P = 0.003$); decreased with isolation, quantified as distances to a plant's first through fourth nearest flowering neighbors ($r^2 = 0.43$ – 0.56 ; all $P < 0.002$); and increased with flowering plant density ($r^2 = 0.38$; $P = 0.004$; SI Appendix, Table S3 and Figs. S2 and S3). The strong, positive relationship between average pairwise flowering synchrony and average seed set remained significant whether or not we included the anomalous 1997 unburned mating scene, in which flowering plants were so distant from prospective mates that high flowering synchrony did not increase seed set. Style persistence, a direct measure of pollen limitation, and seed set were negatively correlated (Fig. 2C; $r^2 = 0.56$; $P = 0.001$; $n = 16$), which indicates that differences in seed set among mating scenes reflect mating opportunities and

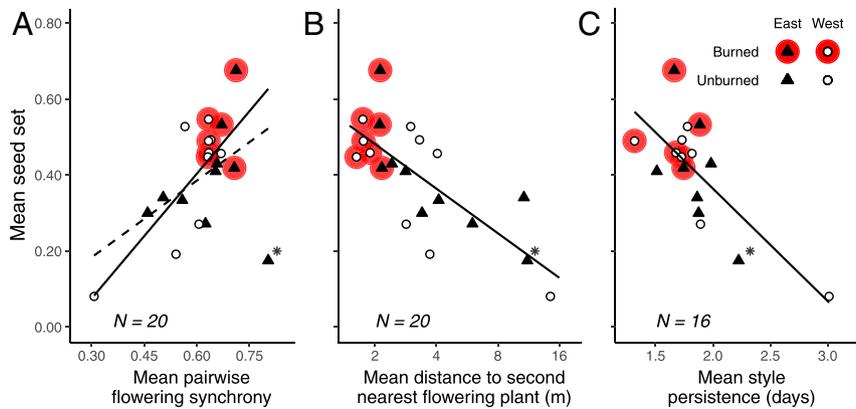


Fig. 2. Reproduction in *Echinacea* improves with mating potential and in burned mating scenes. The mating scenes in two management units at Staffanson Prairie Preserve are characterized by mating potential in time and space. Mean seed set, the proportion of florets that produces a seed, (A) increased with flowering synchrony and (B) decreased with average spatial isolation (harmonic mean of distance to second nearest flowering neighbor). The relationship between flowering synchrony and reproduction was consistent across two measures of synchrony: scene-wide synchrony of individuals with all other individuals, shown here, and local synchrony of individuals with their four nearest neighbors (*SI Appendix*, Fig. S3 and Table S3). The relationship between isolation and reproductive success was consistent across distances to a plant's first through fourth nearest flowering neighbors and flowering plant density (*SI Appendix*, Fig. S2 and Table S3). Mean style persistence, a direct measure of pollen limitation that is not influenced by resource limitation, strongly predicted mean seed set (C), indicating that reproduction is limited primarily by mate finding rather than pre-mating-scene resource availability or postpollination seed failure. A style persistence value of 1 indicates no evidence of pollen limitation. All slopes differ significantly from zero (*SI Appendix*, Table S3), and these strong relationships held, regardless of whether an outlier mating scene with high synchrony but low seed set was included in the analysis (point with asterisk in Figs. 2 and 3). Note that although the plants in this outlier scene had high flowering synchrony, plants were also distant from prospective mates. This may explain why synchrony and seed set were decoupled. Details in *SI Appendix*.

pollination efficiency to a great extent. These style persistence results are also consistent with previous work demonstrating that seed set in *Echinacea* is primarily limited by the receipt of compatible pollen, rather than resources or postpollination seed failure (*Materials and Methods* and refs. 11, 23, and 24).

Our results also support the second hypothesis that fire restructures the temporal and spatial dimensions of the mating scene. Flowering was more synchronous within burned scenes compared with unburned scenes (Fig. 3A; $W = 82$ [$P = 0.039$] with outlier; $W = 82$ [$P = 0.012$] excluding outlier). On average, pairwise flowering overlapped from 63% to 71% of days in burned scenes and from 31% to 67% (apart from outlier with 80%) in unburned scenes (*SI Appendix*, Fig. S4). Flowering synchrony among nearby plants showed similar patterns (*SI Appendix*, Table S4 and Fig. S5). These synchrony measures are independent of sample size and depend on the synchrony of flowering start dates and the duration of plants' flowering (*Materials and Methods*). We monitored both the start and duration of flowering of individuals in nine seasons. Although the synchrony of flowering start dates contributes to mean synchrony of mating opportunities within mating scenes, scant evidence exists for a consistent effect of fire on variation in start dates (Fig. 3B; $W = 35$; $P = 0.237$). In contrast, the mean duration of flowering per plant was consistently longer in burned scenes (17.2 vs. 14.8 d on average), although negligibly so in some years (Fig. 3C; $W = 82$; $P = 0.039$). Longer flowering contributed to greater overall synchrony despite considerable variation among years: from mean durations less than 12 d to more than 21 d (Fig. 3). Fire also restructured spatial aspects of the mating scene. Flowering *Echinacea* plants in burned scenes were less isolated from their first through fourth nearest potential mates (Fig. 4A; $W = 11-42$; all $P < 0.001$; *SI Appendix*, Table S4 and Fig. S6). This increased proximity resulted from highly synchronized flowering of plants among years. The proportion of adult plants that flowered was higher in burned scenes (41 to 72%) compared with unburned scenes (5 to 40%; $W = 297$; $P < 0.001$; Fig. 4B).

Apart from experiencing greater seed set, flowering plants in burned scenes consistently produced more heads than those in unburned scenes (1.49–3.07 vs. 0.75–1.65 heads; $W = 287$; $P <$

0.001; Fig. 4C). The combination of increased seed set and the production of more flowering heads resulted in average fecundity (the total number of seeds produced per flowering plant) nearly doubling in burned scenes (192.0 vs. 101.7; $W = 52$; $P = 0.031$). The total number of fruits produced per head, both fertilized and unfertilized, did not differ between burned and unburned scenes ($W = 24$; $P = 0.47$).

Discussion

Our 21-y demographic study demonstrates how fire increases mating potential and improves reproductive outcomes in this *Echinacea* population. Fire synchronized reproductive effort both among years, by stimulating flowering and thereby increasing proximity to potential mates, and among days within years, by increasing the temporal overlap of flowering phenology. Higher mating potential yielded greater pollination efficiency, which resulted in 55% greater seed set, on average, in burned mating scenes compared with unburned scenes, and an 88% increase in total seed production per flowering plant. In previous studies of natural and experimental *Echinacea* populations, we found that both synchrony and isolation influence the likelihood of successful pollination (11, 16, 18, 27). Here, the overall findings match those of previous studies, and they are robust over time, across both measures of synchrony (local and scene-wide), and across all of the measures of isolation (*SI Appendix*).

Many prairie plants, including *Echinacea*, are known to exhibit fire-stimulated flowering. In this study, we demonstrate that a major portion of the reproductive benefits of flowering in response to fire result from increased mating potential and pollination efficiency. Previous studies attribute fire-stimulated flowering to increased postfire resource availability (7). While resources play an important role in plant reproduction, not all prairie species flower in response to fire, suggesting that a resource pulse alone cannot explain general patterns of fire-stimulated flowering (2, 8, 9, 28, 29). We hypothesize that fire-stimulated flowering in *Echinacea* reflects a more complex reproductive strategy, in which fire acts simply as a proximate cue that synchronizes flowering among years (30). In empirical studies of another pollen-limited perennial plant, researchers demonstrate that patterns of variable but

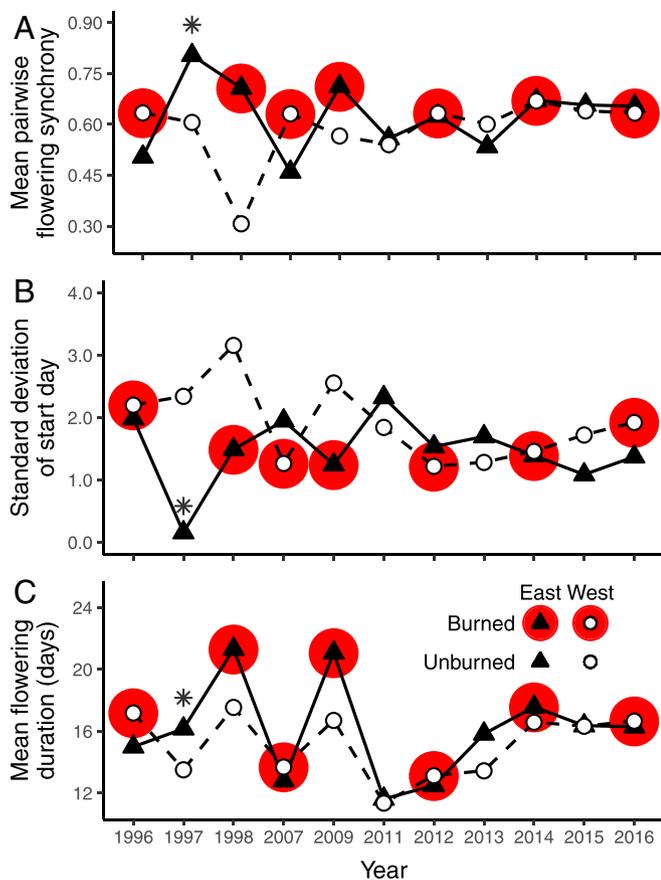


Fig. 3. *Echinacea* plants tend to flower synchronously within the summer after a fire. (A) Mean pairwise flowering synchrony was higher in six of seven burned scenes compared with in the unburned scene in the 9 y of the study when we measured synchrony, for scene-wide synchrony shown here (SI Appendix, Table S4) and for local synchrony with four nearest neighbors (SI Appendix, Fig. S5). Two aspects of flowering phenology in individuals promote synchrony, and thus mating potential, of the mating scene: (B) reduced variation in plant start dates, characterized by the standard deviation and (C) longer mean duration of flowering per plant (SI Appendix, Table S4). Asterisk denotes the mating scene with high flowering synchrony but low seed set in Fig. 2. Details in SI Appendix.

synchronized interannual flowering depend on the acquisition, storage, and allocation of resources to reproduction over several years (31). Given that successful reproduction in *Echinacea* depends primarily on receiving compatible pollen, any cue that serves to synchronize reproductive effort among years would be advantageous, independent of annual resource availability. The intermittent bouts of synchronized reproduction we observed in *Echinacea* resemble well-documented patterns of masting in nut trees, conifers, and some herbaceous perennials (32). Masting is hypothesized to improve reproductive success primarily by either reducing rates of per capita seed predation or improving pollination efficiency (31–33). Although theory predicts predator satiation drives masting in animal-pollinated plants (32), seed predation is rare in this *Echinacea* population; we observed rodent predation of heads in only one mating scene during this study (and it was confined to the densest area of flowering plants). Instead, our findings provide strong evidence that synchronized reproduction, both among years and within years, improves reproductive outcomes by enhancing pollination efficiency.

We predict that the reproductive benefits of synchronized flowering after fire help promote population growth and avert local extinctions in *Echinacea* and many other prairie plants.

Species that experience pollen-limited reproduction (10, 11) and flower vigorously after fire (2, 7) may benefit from synchronized flowering among years, a well-known fire-related phenomenon; from synchronized flowering within years, which has been little investigated; or from both. Evidence from experimental *Echinacea* populations suggests that flowering synchrony among years influences mating opportunities more than flowering synchrony within years (19). In our study, *Echinacea* reproduction was relatively robust in unburned units at the preserve, but this population is unusually large and has mating compatibility rates in excess of 80% (16). The difference in reproductive success between burned and unburned mating scenes will be amplified in small populations in which mating opportunities are severely constrained by both numbers and proportions of compatible mates, which range as low as 30% (11, 16). We expect self-incompatible species, which are prevalent worldwide (~50% of species) (22), to gain the most from fire because their reproduction is most likely mate-limited (21). Consistent with this expectation, flowering in some self-compatible prairie species does not respond to fire (9). Although the population dynamics of long-lived perennials are less sensitive

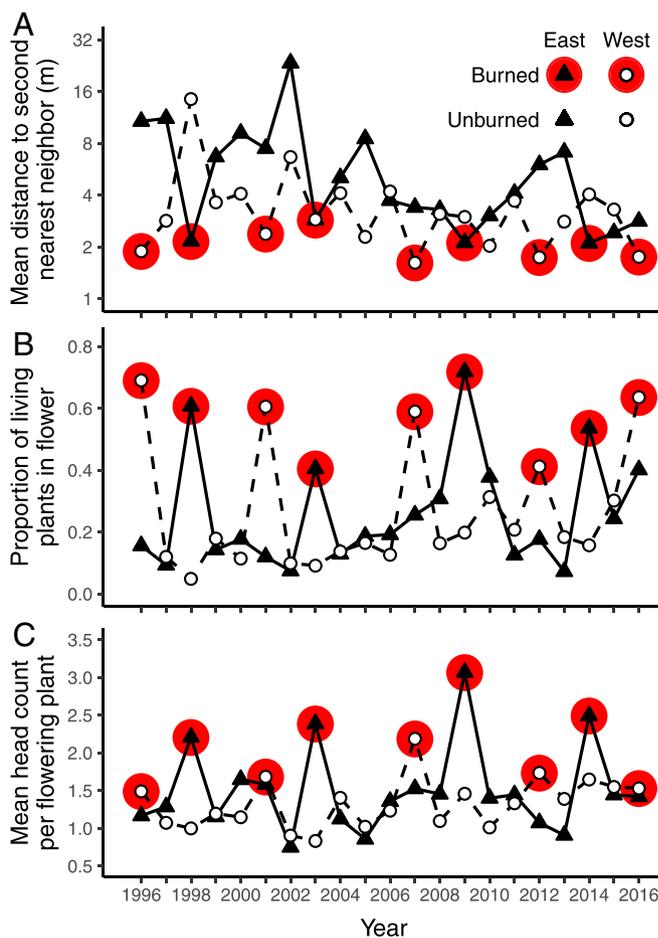


Fig. 4. Mating potential increases in burned mating scenes because plants synchronize flowering effort among years. (A) Plants are less isolated from potential mates, as quantified by the harmonic mean of distance to second nearest flowering neighbor in burned mating scenes compared with unburned scenes. Distances to first, third, and fourth nearest neighbors decrease in burned scenes as a result of the higher density of flowering plants (SI Appendix, Table S4 and Fig. S6). This pattern results from synchronized flowering effort of the long-lived *Echinacea* plants, characterized by (B), the proportion of adult plants flowering in each scene and (C), the mean head count per flowering plant.

to reproductive failure than shorter-lived species, empirical studies and models provide strong evidence that persistent mate-finding Allee effects, similar to the one described here, influence population dynamics and increase the risk for local extinction, even in long-lived perennials (15–17, 34). Populations of both *E. angustifolia* and *Echinacea pallida*, a close relative of *E. angustifolia*, have declined in fire-suppressed prairie remnants (35, 36). We hypothesize that a demographic mate-finding Allee effect contributes to the observed declines of *Echinacea* and other plant species in fire-suppressed prairies. These predictions offer insights into the reproduction and population dynamics of prairie plants (2, 3).

Reduced fire frequency threatens the persistence of plant populations in fire-dependent ecosystems throughout the world (1, 3, 6, 37, 38). Our 21-y study of *Echinacea* reproduction illustrates an important but previously unexplored mechanism by which fire improves plant reproduction and maintains plant diversity. The reproductive benefits of synchronized flowering in response to fire are distinct from, and complementary to, previously described mechanisms expected to contribute to the persistence of plant populations, such as reducing competition for light and other resources, preventing the establishment of fire-intolerant species, and maintaining habitat structure. The global prevalence of fire-stimulated flowering suggests the mechanism we describe here could influence plant population dynamics in many fire-prone ecosystems (39–41). Future studies investigating mechanisms responsible for the loss of plant species in fire-dependent ecosystems must consider plant reproduction and population dynamics. Such research efforts will help improve management strategies for maintaining plant diversity. We strongly encourage increasing the study of, and expanding the use of, prescribed fires in fragmented prairies and other habitats with a history of fire suppression. Rekindling fires may have widespread benefits for plant reproduction and diversity in fire-dependent ecosystems worldwide.

Materials and Methods

Study Site. We conducted our longitudinal study of *Echinacea* reproduction at Staffanson Prairie Preserve, a 40-ha prairie remnant located in Douglas County, Minnesota. Historically, the site was part of a contiguous expanse of tallgrass prairie that burned frequently, probably every 1 to 5 y, before European settlement (2), but is currently surrounded by agriculture (*SI Appendix, Fig. S1*). The majority of the preserve has never been plowed, but the property was hayed annually starting as early as the 1930s until 1980. Since acquiring the property in 1972, The Nature Conservancy has conducted prescribed burns regularly. The preserve is divided into an East and a West burn unit (management unit), and each unit is burned approximately once every 5 y (*SI Appendix, Table S1*). Native bees, which are declining in many places, are prevalent in the surrounding landscape and on the Staffanson preserve. Many generalist native bee species visit *Echinacea*. Bee visitation rates were not associated with limited reproduction of isolated *Echinacea* plants in a study conducted in and around the preserve in 2004 and 2005 (24).

Demographic Census. Each summer, beginning in 1996, we searched for and mapped the location of all flowering *Echinacea* plants along a belt transect spanning the burn units. For this study, we excluded plants growing within a 40-m buffer separating the East and West units to account for the varying location of the mowed burn break, to minimize potential edge effects, and to distance them sufficiently to consider them distinct mating scenes (*SI Appendix, Fig. S1*). The transect encompassed ~1.5 ha (0.449 ha in the West unit and 0.997 ha in the East unit) and included 778 individual plants. Each management unit was burned during the dormant season at least four times between 1996 and 2014 (*SI Appendix, Table S1*). In addition to mapping flowering *Echinacea* plants, we collected demographic data for all flowering plants every year, and vegetative plants in some years. These demographic metrics included whether individuals were alive, whether plants were vegetative or reproductive, and the number of flowering heads produced by each reproductive plant. High-resolution mapping (<10 cm precision) and permanent identification markers allowed us to track the demography of individual plants during the 21-y study period. Detailed methods are in *SI Appendix*.

The Mating Scene and Mating Potential. Mating potential is quantified for a pair of individuals based on the distance between them and the timing of

their mating behavior. Mating potential increases with spatial proximity and with the synchrony of flowering. For an individual, mating potential is defined as a summary of pairwise potentials of that individual with all other individuals. Previous work (16) quantified mating potential based solely on isolation and assumed complete overlap in flowering period. Here, we limit our consideration to flowering individuals in the same management unit. Previous studies indicate that a 40-m buffer would effectively separate the East and West units into distinct mating scenes (11, 16, 24, 27). We defined each group of potential mates, with their coordinates measurable in time and space, as a mating scene. Mating scenes varied each year in the identity of individuals that participate, their locations, and the timing of their flowering. We refer to a mating scene in the summer after a fall or spring prescribed fire as a burned scene; all other scenes are unburned. To characterize the potential of an entire mating scene, we quantified the mean values of spatial isolation for all participating individuals and mean flowering synchrony for all participating pairs. Viewed this way, mating potential and scenes are analogous to electric potential and fields in classical physics (42).

Spatial Mating Potential. We calculated several measures of mating potential based on the spatial isolation of flowering plants. Each measure captures a different aspect of mean mating potential of a mating scene. We calculated harmonic means of distances from each plant to its k^{th} nearest flowering neighbor within the transect for $k = 1, 2, 3,$ and 4 . While both the harmonic mean and arithmetic mean provide qualitatively similar results, we present the harmonic mean because it is less biased toward flowering individuals that are very distant from flowering neighbors. Previous studies in our study site have shown that the relationship between spatial isolation and pollination efficiency peaked at intermediate ranks of flowering neighbors ($k = 2$ – 18) and that the distances that strongly influence pollinator visitation, seed set, style persistence, pollen dispersal are comparable to the width of our transect, 10 m (11, 16, 24, 27). We also calculated the density of flowering plants (count of flowering plants per unit area). Log transformations of isolation and density improved the distribution of residuals in regression models (Fig. 2 and *SI Appendix, Fig. S2* and *Table S3*). See *SI Appendix* for further details.

Temporal Mating Potential. In 10 y (7 y in which one of the units was burned), we monitored reproductive plants to determine the first day and last day of flowering for all flowering heads. Each head comprises many florets, each with one ovule. We define flowering as a day when at least one floret shed pollen. We then calculated mean pairwise synchrony to quantify overall synchrony for a mating scene. To quantify pairwise synchrony for each pair in a mating scene, we divided the number of days that both individuals flowered by the total number of days that either of those plants flowered (43). We also calculated a measure of synchrony among nearby individuals (see details in the *SI Appendix*). These synchrony measures are appropriate for comparing mating scenes with varying numbers of flowering plants because the measures are independent of sample size. Mean flowering duration varied considerably among years and depends on summer weather, other environmental factors, and plant traits. In particular, duration increases with the number and size of heads, indicating a role for resources and heritable traits to influence the mating scene. We calculated the standard deviation of start dates among individuals to gauge the extent to which variation in initiation of flowering influences synchrony. We note that the average annual start date of mating scenes varied considerably, from 27 June to 18 July, but the variation around the annual mean is what influences synchrony of the mating scene. See *SI Appendix* for further details.

Reproductive Outcomes. We measured five aspects of annual reproduction in flowering plants (*SI Appendix, Table S2*). First, in all 21 y of the study, we counted the number of heads every plant produced, which influences the number of fruits per plant and total seed production. Second, we randomly sampled flowering plants to estimate the total number of fruits produced per plant in 8 y (7 y in which half of the preserve, one scene, burned). This count represents the maximum potential number of seeds that a plant could produce that year. Third, in 10 y, we randomly sampled fruits to estimate seed set, the proportion of fruits containing a seed. Each *Echinacea* floret produces a fruit, but only florets that have received compatible pollen produce fruits containing a seed. Thus, seed set, the proportion of fruits containing a seed, is a measure of reproduction influenced by the mating scene and mate limitation. Fourth, we also assessed style persistence of all plants in 8 y (6 y in which a scene burned), following standard protocol (23). Style persistence quantifies mean longevity of styles (in days) before they shrivel due to receiving compatible pollen or to age. Thus, style persistence directly measures

pollen limitation at the time of pollination and is independent of resource limitation. This measure is valuable because embryos may fail to develop into seeds for reasons other than pollen availability. Finally, we estimated the total number of seeds produced per plant per year, fecundity, as the product of seed set, fruit count, and head count per plant per year. Fecundity is the best measure for projecting future population dynamics. Whereas head count and floret count are components of both male and female reproductive fitness, seed set, style persistence, and fecundity measure aspects of female reproductive fitness. Detailed methods are described in *SI Appendix* (11, 23).

Data Analysis. Throughout, we present analyses of mating potential and reproductive outcomes that are averaged across plants within a given mating scene. These plants all experience the same burn “treatment,” and therefore cannot be considered independent. We analyzed data using several techniques that accounted for differences in sample size and variance among mating scenes, but overall results did not depend on the statistical technique used, so we report only the simplest analyses here. We analyzed the relationship between mating potential and annual reproductive success, using linear models. All statistical models were robust to potential outliers and influential data points unless otherwise noted. These and all other analyses were conducted using R 3.5.1 (44). All data and scripts used in this study are archived and available at <http://echinaceaproject.org/datasets/fire-fitness-spp/>. We used Wilcoxon rank-sum tests to test for differences in mating potential and

reproductive success between burned and unburned scenes. These non-parametric tests offer a more conservative method for assessing differences between burned and unburned scenes than parametric or resampling tests but yield qualitatively similar results. Although we lack truly independent replicates when comparing population means over time, we found no evidence of temporal autocorrelation in reproductive effort. Furthermore, the strength and consistency of fire effects in this long-term study suggest it is exceedingly unlikely that some unmeasured environmental variable unrelated to fire drives the observed differences between burned and unburned scenes. See *SI Appendix* for further details.

ACKNOWLEDGMENTS. We thank the many interns, students, and volunteers who contributed to field and laboratory efforts, especially J. Nicol, A. Zahler, K. Kapsar, S. Baker, and A. Dykstra. We thank B. Winter of The Nature Conservancy for burning and D. Wagenius and J. Wagenius for support. We thank R. G. Shaw, M. McKone, E. Menges, J. Ison, and many colleagues at the Chicago Botanic Garden for valuable comments and discussion. The editor and two anonymous reviewers helped improve this paper. This research has been supported by the National Science Foundation (awards 1355187, 1052165, 1051791, and others), National Geographic Society, and early on, intramural University of Minnesota awards supporting dissertation research of S.W. During manuscript preparation, J.B. was supported by a National Science Foundation Graduate Research Fellowship (DGE-1747503).

- W. J. Bond, J. E. Keeley, Fire as a global ‘herbivore’: The ecology and evolution of flammable ecosystems. *Trends Ecol. Evol. (Amst.)* **20**, 387–394 (2005).
- S. L. Collins, L. L. Wallace, Eds., *Fire in North American Tallgrass Prairies* (University of Oklahoma Press, Norman, OK, 1990).
- M. K. Leach, T. J. Givnish, Ecological determinants of species loss in remnant prairies. *Science* **273**, 1555–1558 (1996).
- F. B. Samson, F. L. Knopf, Prairie conservation in North America. *Bioscience* **44**, 418–421 (1994).
- J. M. Hoekstra, T. M. Boucher, T. H. Ricketts, C. Roberts, Confronting a biome crisis: Global disparities of habitat loss and protection. *Ecol. Lett.* **8**, 23–29 (2005).
- A. O. Alstad *et al.*, The pace of plant community change is accelerating in remnant prairies. *Sci. Adv.* **2**, e1500975 (2016).
- L. C. Hulbert, Causes of fire effects in tallgrass prairie. *Ecology* **69**, 46–58 (1988).
- D. C. Hartnett, Effects of fire in tallgrass prairie on growth and reproduction of prairie coneflower (*Ratibida columnifera*: Asteraceae). *Am. J. Bot.* **78**, 429–435 (1991).
- E. S. Menges, R. W. Dolan, Demographic viability of populations of *Silene regia* in midwestern prairies: Relationships with fire management, genetic variation, geographic location, population size, and isolation. *J. Ecol.* **86**, 63–78 (1998).
- S. D. Hendrix, J. F. Kyhl, Population size and reproduction in *Phlox pilosa*. *Conserv. Biol.* **14**, 304–313 (2000).
- S. Wagenius, Scale dependence of reproductive failure in fragmented *Echinacea* populations. *Ecology* **87**, 931–941 (2006).
- J. Ågren, Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* **77**, 1779–1790 (1996).
- M. J. Groom, Allee effects limit population viability of an annual plant. *Am. Nat.* **151**, 487–496 (1998).
- J. M. Calabrese, W. F. Fagan, Lost in time, lonely, and single: Reproductive asynchrony and the Allee effect. *Am. Nat.* **164**, 25–37 (2004).
- H. G. Davis, C. M. Taylor, J. G. Lambrinos, D. R. Strong, Pollen limitation causes an Allee effect in a wind-pollinated invasive grass (*Spartina alterniflora*). *Proc. Natl. Acad. Sci. U.S.A.* **101**, 13804–13807 (2004).
- S. Wagenius, E. Lonsdorf, C. Neuhauser, Patch aging and the S-allee effect: Breeding system effects on the demographic response of plants to habitat fragmentation. *Am. Nat.* **169**, 383–397 (2007).
- J. Gascoigne, L. Berec, S. Gregory, F. Courchamp, Dangerously few liaisons: A review of mate-finding Allee effects. *Popul. Ecol.* **51**, 355–372 (2009).
- J. L. Ison, S. Wagenius, Both flowering time and distance to conspecific plants affect reproduction in *Echinacea angustifolia*, a common prairie perennial. *J. Ecol.* **102**, 920–929 (2014).
- A. Waananen, G. Kiefer, J. L. Ison, S. Wagenius, Mating opportunity increases with synchrony of flowering among years more than synchrony within years in a non-masting perennial. *Am. Nat.* **192**, 379–388 (2018).
- M. M. DeMauro, Relationship of breeding system to rarity in the lakeside daisy (*Hymenoxys acaulis* var. *glabra*). *Conserv. Biol.* **7**, 542–550 (1993).
- M. A. Aizen, L. D. Harder, Expanding the limits of the pollen-limitation concept: Effects of pollen quantity and quality. *Ecology* **88**, 271–281 (2007).
- D. De Nettancourt, Incompatibility in angiosperms. *Sex. Plant Reprod.* **10**, 185–199 (1997).
- S. Wagenius, Style persistence, pollen limitation, and seed set in the common prairie plant *Echinacea angustifolia* (Asteraceae). *Int. J. Plant Sci.* **165**, 595–603 (2004).
- S. Wagenius, S. P. Lyon, Reproduction of *Echinacea angustifolia* in fragmented prairie is pollen-limited but not pollinator-limited. *Ecology* **91**, 733–742 (2010).
- S. Wagenius, H. H. Hangelbroek, C. E. Ridley, R. G. Shaw, Biparental inbreeding and interremnant mating in a perennial prairie plant: Fitness consequences for progeny in their first eight years. *Evolution* **64**, 761–771 (2010).
- S. Wagenius, A. B. Dykstra, C. E. Ridley, R. G. Shaw, Seedling recruitment in the long-lived perennial, *Echinacea angustifolia*: A 10-year experiment. *Restor. Ecol.* **20**, 352–359 (2012).
- J. L. Ison, S. Wagenius, D. Reitz, M. V. Ashley, Mating between *Echinacea angustifolia* (Asteraceae) individuals increases with their flowering synchrony and spatial proximity. *Am. J. Bot.* **101**, 180–189 (2014).
- D. L. Lovell, R. A. Henderson, E. A. Howell, “The response of forb species to seasonal timing of prescribed burns in remnant Wisconsin prairies” in *Proceedings of the Eighth North American Prairie Conference*, R. Brewer, Ed. (Western Michigan University Press, Kalamazoo, MI, 1982), pp. 11–15.
- R. H. Pemble, G. L. Van Amburg, L. Mattson, “Intraspecific variation in flowering activity following a spring burn on a northwestern Minnesota prairie” in *The prairie peninsula, in the “shadow” of Transeau: proceedings of the 6th North American Prairie Conference, 12-17 August, 1978*, R. Stuckey, K. Reese, Eds. (The Ohio State University, Columbus, Ohio, 1981), pp. 235–240.
- J. S. Brewer, W. J. Platt, Effects of fire season and herbivory on reproductive success in a clonal forb, *Pityopsis graminifolia*. *J. Ecol.* **82**, 665–675 (1994).
- E. E. Crone, E. Miller, A. Sala, How do plants know when other plants are flowering? Resource depletion, pollen limitation and mast-seeding in a perennial wildflower. *Ecol. Lett.* **12**, 1119–1126 (2009).
- D. Kelly, V. L. Sork, Mast seeding in perennial plants: Why, how, where? *Annu. Rev. Ecol. Syst.* **33**, 427–447 (2002).
- P. D. Vickery, Effects of the size of prescribed fire on insect predation of northern blazing star, a rare grassland perennial. *Conserv. Biol.* **16**, 413–421 (2002).
- S. H. Anderson, D. Kelly, J. J. Ladley, S. Molloy, J. Terry, Cascading effects of bird functional extinction reduce pollination and plant density. *Science* **331**, 1068–1071 (2011).
- M. L. Bowles, M. D. Jones, J. L. McBride, Twenty-year changes in burned and unburned sand prairie remnants in Northwestern Illinois and implications for management. *Am. Midl. Nat.* **149**, 35–45 (2003).
- A. B. Dykstra, “Seedling recruitment in fragmented populations of *Echinacea angustifolia*” PhD dissertation, University of Minnesota, St. Paul, MN (2013).
- D. A. Rogers, T. P. Rooney, D. Olson, D. M. Waller, Shifts in southern Wisconsin forest canopy and understory richness, composition, and heterogeneity. *Ecology* **89**, 2482–2492 (2008).
- D. Li, D. Waller, Drivers of observed biotic homogenization in pine barrens of central Wisconsin. *Ecology* **96**, 1030–1041 (2015).
- B. B. Lamont, K. S. Downes, Fire-stimulated flowering among resprouters and geophytes in Australia and South Africa. *Plant Ecol.* **212**, 2111–2125 (2011).
- G. H. Pyke, Fire-stimulated flowering: A review and look to the future. *Crit. Rev. Plant Sci.* **36**, 179–189 (2017).
- N. A. L. Pilon, W. A. Hoffmann, R. C. R. Abreu, G. Durigan, Quantifying the short-term flowering after fire in some plant communities of a cerrado grassland. *Plant Ecol. Divers.* **11**, 259–266 (2018).
- E. M. Purcell, *Electricity and Magnetism* (McGraw Hill Book Company, New York, USA, ed. 2, 1985).
- S. Wagenius, D. Hanson, A. Waananen, Mateable: Tools to Assess Mating Potential in Space and Time. R Package (Version 0.3.1, 2016). <https://CRAN.R-project.org/package=mateable>. Accessed 17 April 2018.
- R Core Team, R: A Language and Environment for Statistical Computing (Version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria, 2019).