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Effects of wildfire on floral display size and pollinator community reduce outcrossing rate in a plant with a mixed mating system

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PREMISE OF THE STUDY: Wildfire changes the demography, morphology, and behavior of plants, and may alter the pollinator community. Such trait changes may drastically alter the outcome of pollination mutualisms on plants; however, the direct role of fire on these mutualisms is poorly known.

METHODS: Following a pair of fires in the northern California coast range chaparral, we censused floral visitor communities of *Trichostema laxum* (Lamiaceae), quantified visiting bee behavior, and estimated outcrossing rates using a widespread Mendelian recessive floral polymorphism across a matrix of populations in burned and unburned sites. We also compared pre- and postfire floral visitation in two populations.

RESULTS: Outcrossing rates were significantly lower in burned areas; however, our data suggest that the much larger size of plants in burned areas, not burn status itself, drove this pattern. Large-bodied bees dominated floral visitor communities after fire, likely recruiting to the abundant postfire floral resources. These bees visited more flowers per plant than did the smaller bees prevalent before fire and in unburned areas, likely increasing selfing through geitonogamy (within-plant pollination), an effect made possible by the far larger size of plants in burned areas.

CONCLUSIONS: Outcrossing rates dropped substantially after wildfires because of changes in the pollinators, plant display size, and their interactions. Reductions in outcrossing following fire may have important implications for population resilience and evolution in a changing climate with more frequent fires.

KEY WORDS Bee assemblages; wildfire; disturbance; pollen transfer; geitonogamy; mutualisms; autogamy; Lamiaceae; McLaughlin Reserve.

Wildfires are a regular occurrence in many ecosystems worldwide, which can shape community structure and evolutionary trajectories of organisms within them (e.g., Keeley et al., 2011; Burkle et al., 2015). Community-level changes following fire depend on characteristics of the fire as well as differing responses of individual species, which in turn affect the structure and strength of interactions (e.g., Platt et al., 1988; Safford and Harrison, 2004; Moretti et al., 2009; Dafni et al., 2012; Burkle et al., 2015; Ponisio et al., 2016; Brown et al., 2017). Such differing responses may have consequences for reproduction, dispersal, life history, and population structure of many species. Of particular interest are those species requiring a mutualist for one of these stages (e.g., reproduction or dispersal) (Dafni et al., 2012). Almost 90% of plants utilize animals for pollination (Ollerton et al., 2011), and pollination represents one primary stage where fire-induced changes to interactions can greatly influence plant reproductive success and demography.

The period from one to several years after wildfire is usually characterized by markedly greater abundance and diversity of flowering forbs (Keeley et al., 1981; Safford and Harrison, 2004). The mechanisms accounting for increased germination, growth, and flowering after fires differ by species, but the pattern is broadly consistent across ecosystems and biogeographic regions (e.g., Keeley and Keeley, 1982; Wrobleski and Kauffman, 2003; Lybbert et al., 2017). Some forb species are “fire-followers,” a loose group of seed-banking forbs and short-lived woody plants that germinate in response to fire cues and are normally present for one to several seasons postfire (Keeley and Keeley, 1987). Many nonfire-following forbs also have massive recruitment, attain abnormally large sizes, and produce far greater reproductive output following fire (Stone, 1951; Wrobleski and Kauffman, 2003; Lybbert et al., 2017) though the ecological effects of these changes are less studied.

For plants, a pulse of greatly increased reproductive effort after wildfire (e.g., Wrobleski and Kauffman, 2003; Lybbert et al., 2017; Mola and Williams, 2018) has the potential to create a vast seed bank and a plethora of novel genotypes, which may be important in adapting to changing climate and altered fire regimes. However, the reliance of many plant species on animal pollinators means a postfire reproductive benefit is not guaranteed. Pollinators and the plants they pollinate may differ in the magnitude or direction of their responses to fire; with effects ranging from total pollination failure to greatly increased pollination success. Changes in pollinator identity may also alter the scale of pollen movement—certain pollinator species move pollen over greater distances than others (Thompson, 2001; Brunet and Sweet, 2006; Whelan et al., 2009; Gomez et al., 2010).

Mating systems in plants span a continuum from complete self-pollination to obligate outcrossing. The consequences of fire for reproduction differ dramatically, and possibly predictably, across this spectrum. Autogamous species experience no pollinator mismatch, and any postfire reproductive consequences would be dictated solely by the plant, such as the plant's response to increased nutrients (the evolution of selfing in fire-adapted lineages is discussed in Carpenter and Recher [1979]). Alternatively, the fitness of nonautogamous species may be strongly impacted by the response of their pollinators to fire, direct effects of fire, and the interaction between these two changes. Many species, estimated at ~40% of all plants (Goodwillie et al., 2005), fall somewhere in between complete autonomous selfing and obligate outcrossing. Whether fire changes selfing rates on a short time-scale, or affects fitness tradeoffs among mating strategies is a little-explored topic and may be especially important in plants with mixed-mating systems.

In mixed-mating species, self-pollination usually occurs in two ways, within a flower without a pollinator moving the pollen (autogamy or autogamous selfing), or with a pollinator moving pollen between flowers on the same plant (geitonogamy). In mixed-mating systems, autonomous selfing may serve as a sort of reproductive assurance such that flowers self if their ovules are not completely fertilized through vectored pollen from animal visitors (but certainly not always, reviewed in Eckert et al., 2006). A mixed-mating species reliant on pollinators (zoophilous) could experience increased outcrossing rates following fire if: (1) more effective pollinators increase in absolute or relative abundance due to any number of factors stemming from the fire, resulting in greater between-plant movement of pollen; (2) massively increased floral resources increase overall pollinator abundance (either through enhanced reproduction or recruitment to the site) resulting in greater overall visitation rates per flower; or (3) increased plant resources (nutrients, water, and light) following fire make a given species of plants more attractive and change pollinator behavior, increasing pollination success or pollinator efficiency on that plant. Alternatively, the same postfire changes could result in decreased outcrossing rates if (1) less-effective pollinators increase disproportionately following fire, (2) pollinator abundance decreases overall reducing pollen removal and delivery or the fewer pollinators move to higher quality floral resources, (3) massively increased floral resources depress floral visitation rates per flower, decreasing pollen removal and increasing autonomous selfing, or (4) increased plant resources (nutrients, water, light) and the resulting increases in floral display on the per-plant basis increases within-plant pollen movement and self-pollination facilitated by pollinators (geitonogamy). Several of these proposed mechanisms could occur simultaneously, with positive and/or negative effects on outcrossing rates, making strong a priori predictions difficult.

Changes in the pollinator/floral visitor community following fire are well documented (Ne'eman et al., 2000; Potts et al., 2001, 2003; Moretti et al., 2009; Lillie, 2011), although their effect on plant reproduction is less well known. These community changes are partially predictable and dictated by the life histories of each species. Some bees may survive fire if heat exposure is brief enough or nests are protected, e.g., deep enough underground (Cane and Neff, 2011). Alternatively, these insects may recruit into burned areas to access abundant resources; the potential for such recruitment is filtered by dispersal ability (Ne'eman et al., 2000; Moretti et al., 2009). Abundance and diversity of visitors probably increase in the years immediately following fire; although this pattern has usually been inferred indirectly by a decrease in these metrics with increasing time since fire (Potts et al., 2003; Moretti et al., 2009; Van Nuland et al., 2013; but see Potts et al., 2001) or comparisons in burned and unburned areas several years after fire (e.g., Ne'eman et al., 2000; Lillie, 2011).

Few studies have addressed the reproductive consequences of these shifts in pollinator communities or extrinsic factors (i.e., pulses of nutrients, water, light) immediately following a fire for plant species that have postfire reproductive pulses. Ne'eman et al., (2000) examined areas 5–7 years after fire and found solitary bees were more common in unburned areas than in burned areas. This change likely drove the higher seed set of several self-incompatible plants in those areas. In burned areas, solitary bee abundance was lower, and increased visitation by bumblebees did not increase pollination of self-incompatible plants because they visited many flowers per plant visit (because geitonogamy is impossible in these plants). Potts et al. (2001) recorded decreased visitor abundance and diversity in burned areas, but found that in both burned and unburned habitats pollination services were sufficient to prevent pollen limitation. Geerts et al., (2012) found that despite increased flowering of two *Chasmanthe* (Iridaceae) species following fire, nectivorous bird abundance decreased, which resulted in decreased floral visitation. However, pollen limitation or total seed set were not quantified. None of these studies directly address the effects of fire on the mating system of a mixed-mating species.

Here we investigated the consequences of wildfire on the reproductive success of a bee-pollinated annual mint, *Trichostema laxum* Gray (Lamiaceae), with a mixed-mating system (Spira, 1978). We used data from the season prior to 2015 wildfires (2014) and comparisons of burned and unburned populations in the summer of 2016 to examine the consequences of fire on the display size, floral visitor community, and outcrossing success of this species. We also determined whether selfing increased or decreased following fire.

MATERIALS AND METHODS

Study populations and natural history

At our study site, the University of California-Davis (UC-Davis) McLaughlin Reserve in the Northern California coast range, *T. laxum* grows on serpentine soils in small washes, where it flowers from May to October. It is one of few plants flowering during dry Mediterranean summers and as such, it receives considerable attention from summer-flying pollinators. A pair of wildfires (the "Rocky" and "Jerusalem" fires) burned much of our study site in August 2015.

To study outcrossing in natural populations, we made use of a naturally occurring Mendelian recessive pink-flowered floral morph. During the summers of 2014–2016, we characterized the reproductive biology of populations of *T. laxum* at the reserve and adjacent public lands (coordinates and site specific information in Appendix S1, see the Supplemental Data with this article). Purple flowered morphs made up >99% of almost every population; however, the pink morph occurred rarely (usually <1:10,000), but widely, across populations (Fig. 1). Over half of the populations on and around McLaughlin Reserve contained one or more pink individuals in them. This polymorphism is not regionally confined, it occurs over much of the range of *T. laxum*, as well as in the closely related *T. simulatum* and *T. lanceolatum* (LoPresti and Miller: personal observation). Plant size was largely dictated by microenvironment, and pink individuals did not differ in size or any other measured characteristic from their nearest purple neighbors in any season of study (2014–2017: LoPresti and Miller, unpublished data), thus they served as reasonable proxies for randomly chosen individuals in the populations in which they occur. Additionally, we took data on all pink plants found from May to October, making it less likely to have of seasonal bias in plant size or any other phenotype.

Outcrossing rates

To determine outcrossing rates, we monitored and collected seeds from pink-flowered focal plants, with the assumption that pink offspring would be selfed and purple would be outcrossed (as these phenotypically pink individuals have two recessive alleles). For this assumption to hold, pink morphs had to be rare in our populations, because we did not want outcrossed offspring to also show the pink phenotype. From May to September, we examined every population of *T. laxum* in the area for pink flowers. In populations that had pink-flowered individuals, we estimated population sizes to ascertain the approximate allele frequency of this morph. Populations with <1:1000 pink:purple-flowered plants were used in this study, to minimize the frequency of pink alleles being present in non-selfed seeds. At these extremely low levels, we do not believe potential pollinator constancy on the pink morph to be an issue moving pink pollen between individuals. Two populations (1 burned, 1 unburned) had excessive numbers of pink morphs (>1:100) and thus were not used for outcrossing estimates. For each focal plant, we measured stem diameter as a proxy for plant size, because it correlates strongly with reproductive output (LoPresti and Miller, unpublished data). For most plants, we also counted buds, flowers, and fruits, although this was infeasible for the largest plants, which have >5000 total reproductive structures. These measurements and counts were all made by one person (EL) for consistency.

FIGURE 1. Floral morphs of *Trichostema laxum*. Left: purple morph, >99% of plants in most populations, note dark anthers and green pollen; Right: pink morph, Mendelian recessive to purple, <1% in most populations, note pink-red anthers and bright yellow pollen. Some pink morphs have darker speckling on the lower lip, some lack it entirely.

The seeds of each plant were collected throughout the summer and fall as they ripened. These seeds were then germinated and plants grown in growth chambers at UC-Davis and a glasshouse at Duke University to determine flower color morph. Specific growing procedures are outlined in Appendix S1.

Single-plant visitation observations

To estimate floral visitation rates, we conducted 10 min observations of individual plants (1) in two populations (first and second stream) during summer 2014, for unrelated experiments, and (2) in both burned (n = 8) and unburned (n = 4) populations in 2016 (including the same two surveyed in 2014, both of which burned). For the latter, 20 focal plants were chosen haphazardly from within each population and a single observer counted all visits to all visible receptive flowers. We determined the total number and identity of visitors, as well as the number of flowers visited by each visitor. The number of open observable flowers per plant was also recorded, allowing us to calculate visitation rate relative to floral display.

Floral visitor community censuses

We surveyed bees in each focal population twice during peak bloom between July and August 2016. Population size, on a log scale, was estimated for each surveyed population. Surveys were carried out on days with full sun, temperatures above 15°C, and wind less than 2 m/s (as measured 1.5 m above the ground). All plots were surveyed by one researcher (JVW) to avoid observer bias. A 30 min observation was conducted by continually moving throughout the population while observing visitors to *T. laxum* flowers. Individual plants were observed for a maximum of 15 s each, and any one patch was observed for a maximum of 2 min to avoid recounting of individual insects and over-representing sections of the population. Each population had one census between 8:00–11:00 AM and the other between 2:00–5:00 PM. We recorded individuals to the lowest obvious taxonomic resolution to which they could be reliably distinguished in the field, using a voucher collection made by EL and determined by JVW (and retained by the authors for continuing studies).

Analysis: outcrossing rates

We analyzed outcrossing rates with generalized linear mixed models using R package 'glmm'. As each individual offspring from the focal plants was either outcrossed (phenotype: purple) or selfed (phenotype: pink), we used a binomial error distribution, with stem diameter of the maternal plant and burned status as fixed effects, and population and maternal plant ID as random effects.

Analysis: floral visitation and visitor communities

To parse out trends in floral visitor abundance and visitation behavior, we used three response variables (all rates, per 10 min observation) from the single plant visitation observations:

(1). Total visits per plant: the sum of all flowers visited by all visitors (note: may be higher than floral display).

(2). Visits per flower: total visits per plant divided by the total number of flowers observed.

(3). Mean flowers visited per visitor: the total number of visits divided by the number of unique visitors.

As we conducted these observations at discrete sites, we used "site" as a random variable for all analyses, all of which were conducted in R (R Development Core Team, 2013). Before analyzing the effect of burn status, we needed to know whether floral morph had an effect on pollination rates and whether predictive variables were strongly collinear with burned status. We first tested for an effect of floral morph (pink vs. purple) on visitation rate, using a linear mixed model (package 'lme4'; Bates et al., 2015). Because we found no differences between morphs (as with other floral morphs; LoPresti, unpublished data), we initially fit a full model of total visits per plant as a function of burn status and floral display, but tests for multicollinearity revealed a strong correlation between these two independent variables (variance inflation factor > 6, *vif* function in the 'car' package: Fox and Weisberg, 2011), so we fit reduced models, using the display size for most analyses, as detailed in results and figure legends (Graham, 2003). (This collinearity was noticeable in the field, is very biologically relevant, and probably drove all the observed patterns; see discussion section below). Therefore, to determine if plants within burned and unburned sites differed in floral display, we fit a model for number of flowers as a function of burn status. Next, to determine the effect of burn status on visitation, we fit models for number of individual visits, visits per flower, and flowers visited per visitor as a function of number of flowers. Total visits per observation bout were analyzed using the 'glmmadmb' package, which allowed us to fit a zero-inflated Poisson error distribution with site as a random intercept (Skaug et al., 2012). Other continuous data were analyzed using the 'lme4' package.

To test the effect of fire on two sites where we observed focal plants in both 2014 (prefire) and 2016 (postfire) we fit linear models with a normal distribution at two sites looking at visitation rate as a function of year. The *P*-values were obtained by the result of one-way ANOVAs.

We compared abundance and species richness of the floral visitor communities to *T. laxum* between burned and unburned populations using Welch two sample *t*-tests. To analyze community compositional differences among sites, we applied nonmetric multidimensional scaling (NMDS) ordination techniques (Legendre and Legendre, 1998; Vázquez and Simberloff, 2003). Bray-Curtis dissimilarity (Bray and Curtis, 1957) was selected as the distance measure, because it is a relative measure that emphasizes species differences between sites regardless of absolute magnitude. We tested for differences among visitor communities as a function of burn status and population size on community using PerMANOVA ('Adonis' package; Anderson, 2001). Genus centroids were overlain on the NMDS ordination to visualize differences in community composition between sites. All community analyses were conducted in R, using the 'vegan' package (Oksanen et al., 2013) and adonis function.

RESULTS

Outcrossing rates

Outcrossing rates in burned areas (overall 63%; n = 546; mean of six populations: 73%) were substantially lower than in unburned areas (overall 84%; n = 90, mean of three populations: 81%). This comparison came from 636 plants grown to flowering from 42 wild-collected pink focal plant families (n = 28 burned; n = 14 unburned). The smaller sample size of unburned plants was unavoidable; there were fewer unburned populations in the vicinity of McLaughlin Reserve and the small size of focal plants in unburned areas resulted in very few seeds collected from these fewer plants. However, we believe our estimates of their outcrossing rates are likely accurate, because we grew out a high proportion of their offspring.

The difference between outcrossing rates in burned and unburned areas is highly significant in a simple binomial model (coefficient of burn = 0.88 ± 0.28, z = 3.20, p = <0.01), which fit better than a null model (Likelihood ratio test, $p < 0.001$). However, this fire effect was not detectable when plant size was included in the model. A binomial model, including burned status and plant size either additively or interactively, fit no better than one without burned status (Likelihood ratio tests, respectively, p = 0.87 and 0.91). Maternal plants from unburned areas had average stem diameters of 0.72 mm ± 0.07 SD (n = 15) and those from burned areas 1.77 mm ± 0.28 SE (n = 28). Outcrossing rate negatively correlated with stem diameter (Fig. 2B; 0.37 ± 0.07, z = 5.46, $p < 0.001$). Burn status and plant size were thus highly collinear and necessarily confounded—indeed, many plants in the burned area were larger than we observed in any unburned populations in years of studying them, and this larger size of plants drove the pattern of decreased outcrossing in burned areas.

Floral visitor observations and community censuses

Single-plant observations reveal, in part, the mechanisms by which plant size contributed to changes in outcrossing rate. Larger plants, which occurred more commonly in burned areas, received more total visits per plant (Fig. 3). Pink and purple floral morphs did not differ in the number of total number of visits received (Tukey's posthoc comparison of means; z = 0.96, p = 0.34), and this model fit no better than a null one without flower color included (χ^2 = 1.63, df =1, P = 0.20). Therefore, floral colors were combined for all subsequent analyses. Plants in burned areas had significantly more flowers than those in unburned sites (Gaussian with log-link error distribution; Tukey's posthoc comparison of treatment means z = −2.64, $p < 0.01$). In a simple model, floral display size was positively correlated with total visits per plant (zero-inflated Poisson model, coefficient for floral number = 0.032 ± 0.002, z = 18.29, $p < 0.0001$), and this model fit significantly better than one without display size

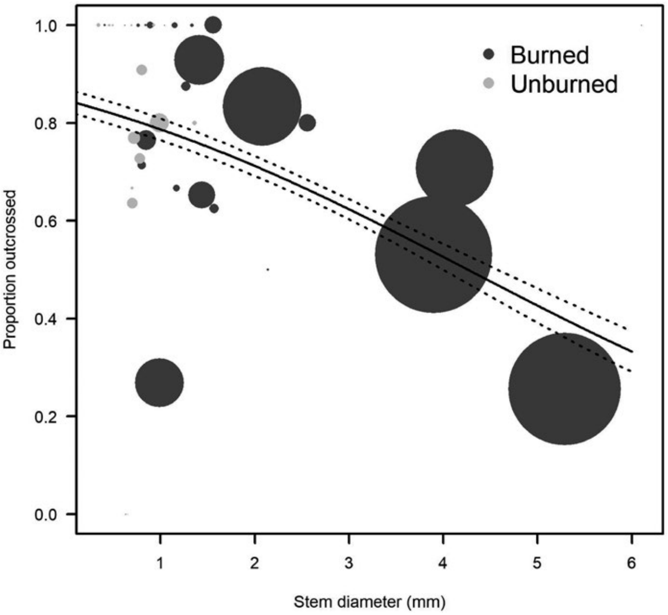

FIGURE 2. Proportion of offspring outcrossed (purple) from the field seed growouts. Circle size indicates number of offspring grown out from each family, light gray dots represent focal plants from unburned areas, dark from burned areas. The line of best fit in the best-fitting binomial model (with only stem diameter as a fixed effect) is plotted; dotted lines represent predicted 95% CI of the predicted mean.

($\chi^2 = 144.49$, df = 1, $p < 0.0001$). This correlation caused visits per flower (Fig. 4) to increase with increasing floral display, but this model fit no better than one without a floral display effect, i.e., intercept only: ($\chi^2 = 2.5637$, df = 1, $P = 0.1093$). Single visit data are summarized by floral visitor species in Table 1.

To examine whether geitonogamy (by intraplant pollen movement) may have increased with increased size and burn status, we analyzed the effect of burn on mean flowers visited per visitor (Harder and Barrett, 1996). Flowers visited per floral visitor significantly increased with increasing floral display size (Fig. 5; compared to null model: ($\chi^2 = 129.74$, df = 1, $P < 0.001$).

Comparing 2014 (prefire) to 2016 (postfire), fire reduced visitation rates per flower at the two sites observed in 2014 and 2016. The difference was marginally significant at "First Stream" site (F = 3.1223, df = 1, 187, $P = 0.07886$), but was highly significant at "Second Stream" (F = 23.152, df = 1, 123, $P < 0.001$, Fig. 6).

Our community censuses of 13 sites totaled 2121 floral visitors (639 in unburned sites, 1482 in burned sites); the most common genera and site totals are shown in Table 2. These floral visitor communities differed significantly between burned and unburned sites following the fires (adonis: F = 2.51, $p = 0.023$; Fig. 7). This significant difference is driven by a compositional shift in dominance of the bees between the burned and unburned sites. The burned areas were dominated by large-bodied bees (illustrated in bold text), primarily *Bombus vosnesenskii* and *Anthophora urbana*. While the unburned ares were dominated by small-bodied bees (plain text) primarily from the genus *Lasioglossum* (Fig. 7, lower panel). The NMDS ordination resulted in a two-dimensional solution with a final stress of 0.157. See Appendix S2 for richness, abundance, evenness, and diversity comparisons.

DISCUSSION

We found *Trichostema laxum* had reduced outcrossing rates in burned areas compared to nearby unburned areas the season after a wildfire. Two interacting mechanisms likely drove this shift: (1) Plants in burned areas were far larger and had more flowers than those in unburned areas, a common result for forbs after fire due to increased light, nutrients, and water (e.g., Stone, 1951; Wrobleski and Kauffman, 2003). (2) Floral visitor communities shifted from small-bodied bee genera (e.g., *Lasioglossum*), to larger-bodied species (e.g., *Bombus vosnesenskii* Radoszkowski*, Anthophora urbana* Cresson), whose behavior at plants differed. This pollinator trait shift occurred both across the burned/unburned landscape matrix, as well as between preburn and postburn observations in the same *T. laxum* populations.

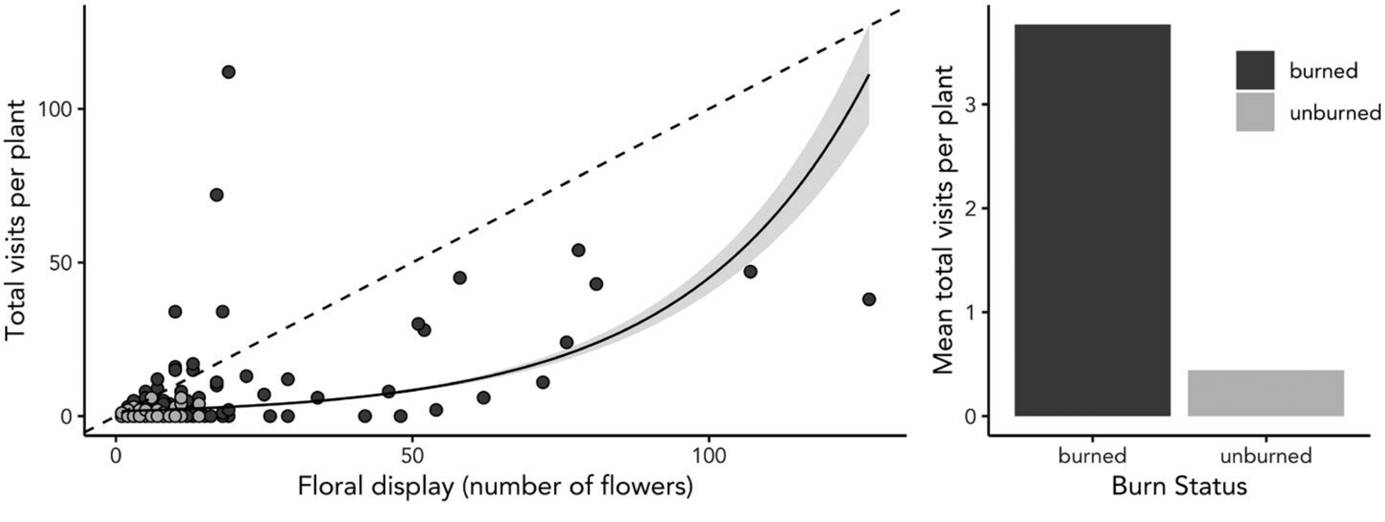

FIGURE 3. Regression of total number of visits, the sum of all flowers visited by all visitors on floral display (number of flowers per plant). Dark shaded dots are plants in burned areas, lighter dots in unburned areas. Gray areas around lines are 95% confidence interval of the mean. Bar chart shows mean of all flowers; statistics not performed on this exact comparison (given nonindependence of plants within a site).

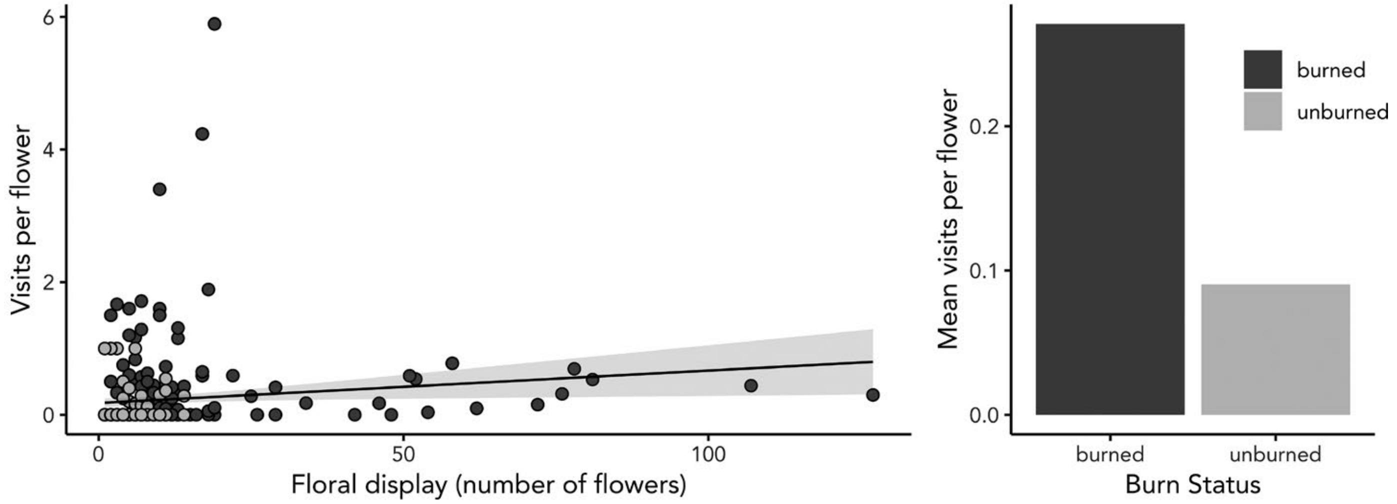

FIGURE 4. Regression of mean visits per flower on total floral display. Dark shaded dots are plants in burned areas, lighter dots in unburned areas. Gray areas around lines are 95% confidence interval of the mean. Bar chart shows mean of all flowers; statistics not performed on this exact comparison (given nonindependence of plants within a site).

TABLE 1. Flowers visited per foraging bout in the 10 min pollinator observation rounds. Species in bold are "large-bodied" (>3 mm intertegular span). Preburn data is from first and second stream sites in 2014; all burned and unburned sites are respectively pooled. All bees were determined by JVW from collected vouchers, and observations were done by JVW, JMM, and EL.

	Burned			Unburned			Preburn		
Species	mean	sd	N	mean	sd	n	mean	sd	n
Bombus vosnesenskii	5.76	5.83	33	2.33	1.53	3	5.36	4.62	14
Anthophora urbana	3.86	6.55	97	2.38	2.26	8	-	-	-
Ceratina sp.	3.33	2.08	3	-	-	-	-	-	-
Perdita sp.	2.60	1.14	5	-	-	-	2.00	-	1
Ashmaediella sp.	2.40	1.34	5	-	-	-	1.18	0.53	17
Halictus tripartitus	2.25	1.91	12	-	-	-	1.61	1.02	84
Lasioglossum subgenus *dialictus*	2.11	1.47	135	1.14	0.36	14	1.89	1.41	459
Apis mellifera	2.00	-	1	-	-	-	-	-	-
Unknown bees	-	-	-	-	-	-	2.00	1.67	6
***Melissodes* sp.**	2.00	1.41	2	-	-	-	-	-	-
Unknown	-	-	-	-	-	-	1.58	0.99	12
Syrphidae spp.	1.47	1.06	15	1.50	0.71	2	1.52	0.85	31
Coleoptera	-	-	-	-	-	-	1.00	-	1
Other Diptera	-	-	-	-	-	-	1.00	-	4
***Osmia* sp.**	-	-	-	-	-	-	1.00	-	2

The observed decreased outcrossing rate in burned areas was driven by the pronounced increase in focal plant size in burned areas; adding an independent variable for plant size to our model took away the effect of burn status completely. Larger display size of plants in burned areas could have decreased outcrossing through increased geitogamy or autonomous selfing. Geitogamy increases with floral display size because larger displays promote increased numbers of flower visits by individual pollinators during single visits to the plant; thus there is a higher probability to deposit pollen removed from the anthers of one flower to other flowers on the same plant. Our observed increase in mean flowers visited per visitor in burned sites and in larger plants strongly suggests this mechanism was the primary driver of the observed pattern; this measure for geitonogamy was suggested in Harder and Barrett (1996).

If autonomous selfing drove the decreased outcrossing rate, we would predict a lower per flower visitation rate in larger plants (and burned areas), resulting in more pollen remaining on anthers to passively contact the stigma. Per flower visitation rates did not decrease (nor significantly increase) with increasing floral display (and thus, burn status) across sites making it unlikely that increased autonomous selfing led to decreased outcrossing. Most *T. laxum* individuals are partially autogamous in the absence of pollinators (e.g., in the pollinator-free laboratory almost all plants set seed), and seed set in the lab is comparable to that in the field (~2–3 seeds/fruit; EL, unpublished data). Although autonomous selfing likely did not contribute to the outcrossing reduction in larger plants and in burned areas; the drastic reduction in visitation rate following fire (2014 vs. 2016) does suggest that this form of autogamy may have increased overall in 2016 compared to prefire conditions, and it is reasonable to assume lower outcrossing postfire in the populations as a result. We have no preburn data against which to compare 2016 outcrossing measurements to,

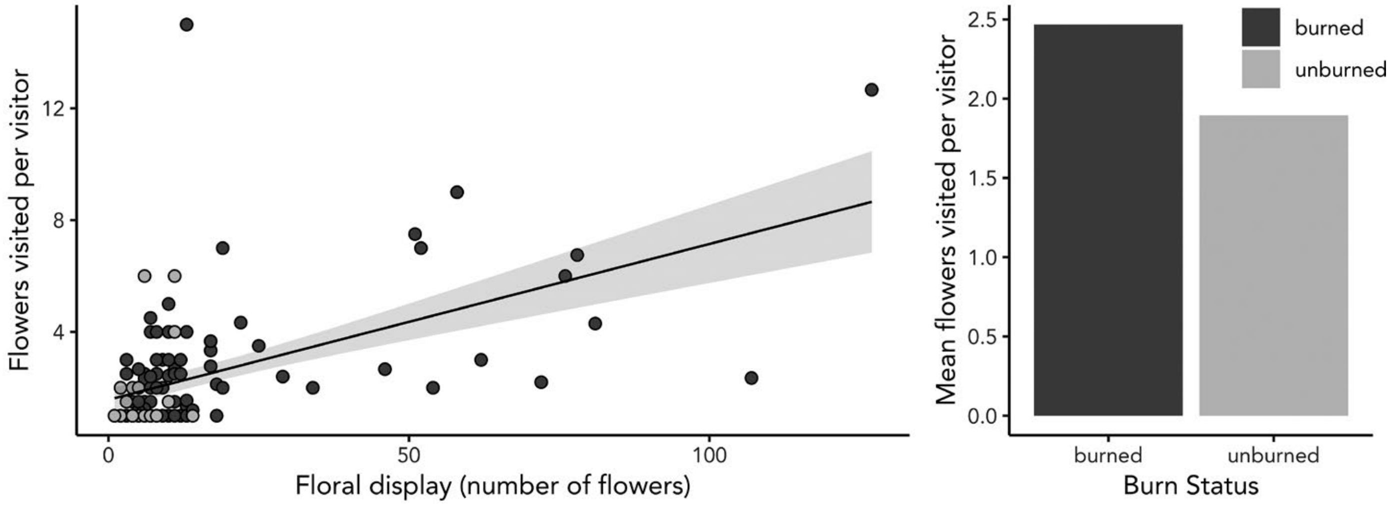

FIGURE 5. Regression of mean flowers visited per floral visitor on total floral display. These values are means per plant per observation bout; any observation periods with no visitors were necessary excluded (therefore, the lowest possible value is 1; for one visitor to one flower or two visitors to one flower each, etc.). Dark shaded dots are plants in burned areas, lighter dots in unburned areas. Gray areas around lines are 95% confidence interval of the mean. Bar chart shows mean of all flowers; statistics not performed on this exact comparison (given nonindependence of plants within a site).

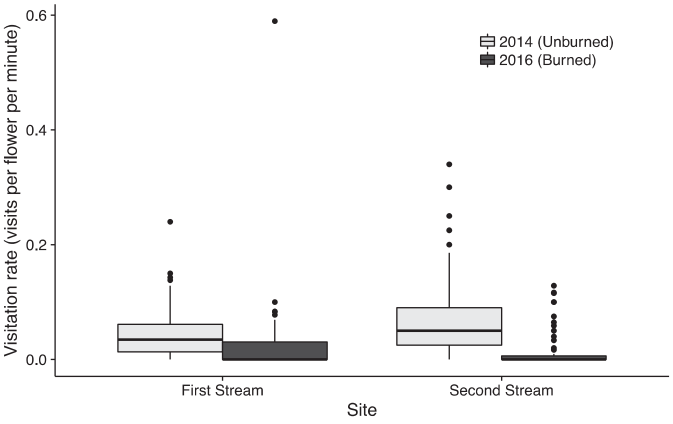

FIGURE 6. Boxplots showing visitation rates before (2014) and after (2016) fire at two focal sites, "First Stream" and "Second Stream." Differences in visitation rates following fire were found at the Second Stream population ($p < 0.001$), but were only marginally significant at First Stream population ($p = 0.07886$).

though we plan to follow outcrossing for several years in these populations.

Changes in the visitor community that we observed are also consistent with an increase in geitonogamy in burned areas and on larger plants. Certain potential pollinators, especially the larger *Bombus vosnesenskii* and *Anthophora urbana* usually visited multiple flowers per plant in each foraging bout. These bee species may have been more effective at exploiting the post-fire resource pulse because of their likely higher dispersal ability (Ne'eman et al., 2000; Greenleaf et al., 2007). It is hard to overstate the effect the fire had on plant size; only one plant—growing on a pocket gopher mound—observed in three field seasons prior to the fires was even close to as large as many of the postfire plants. Furthermore, geitonogamy is not even a possibility for many *T. laxum* individuals in normal years, because these plants often have a single flower open at a time. Ascribing the observed decrease in outcrossing to a specific pollinator-driven mechanism is not possible, though we believe our data and our observations suggest that greatly increased geitonogamy caused by plant morphology and pollinator changes was the primary driver and increased autonomous selfing caused by lack of pollen removal in burned areas also may have contributed to the decrease in outcrossing. However, we cannot conclusively distinguish between the two pollinator-driven mechanisms and both likely occurred, especially given the strong reductions in visitation between 2014 and 2016.

The functional traits of pollinator communities shift in many ways after fire (Moretti et al., 2009). The observed shift from small-bodied to larger-bodied pollinators on *T. laxum* may be a common result following fire (e.g., Ne'eman et al., 2000; but such shifts were not observed in chronosequences, Moretti et al., 2009). Besides body size, other functional traits of the floral visitor community shifted. Wood-nesting bees noticeably decreased following fire: the genus *Ceratina* comprised 2.66% of visitors in censuses at unburned sites, but only 1.1% at burned sites; and the formerly common *Xylocopa californica* Cresson disappeared as nectar robbers of columbine (*Aquilegia eximia* Planch.) following the same fire in nearby patches of the same reserve (LoPresti, 2017). Interestingly, Moretti et al., (2009) found that both *Ceratina* and *Xylocopa* were increased in sites burned <17 years before the study-compared sites >20 years postburn. The conflicting results with our study are likely due to differences in time scale, because it is almost assured that dead-wood–nesting bees in those areas experienced the same temporary reduction as we observed, due to burning of their nesting substrate.

Bumblebees forage far longer distances than many other bee genera and may recruit more quickly into areas with resource flushes (Greenleaf et al., 2007; Jha and Kremen, 2013; Pope and Jha, 2017). Additionally, an increase in population in response to resources can occur more quickly in colonial species (e.g., bumblebees) compared to solitary species (all others in our study); within a season in the former case, but only after a year in the latter) (Crone, 2013). Community analyses of floral visitor shifts following fire have largely focused on species composition (e.g., Moretti et al.,

TABLE 2. Most common floral visitors in the 30 min community censuses at each population (both censuses pooled). Taxa unique to burned sites in these censuses were *Agapostemon, Bombus, Epeolus, Nomada, Osmia, Perdita*, and "Wasp"; no taxa occurred only in unburned areas. Large-bodied taxa (>3 mm intertegular span) are bolded.

Burned?	Site	Genus	(morpho)species	Count	Site Total
Unburned	Anu's Valley	*Lasioglossum*	subgenus *dialictus*	26	109
		Anthophora	***urbana***	39	
		Syrphid	spp	29	
	Cypress Hell	***Anthidium***	***manicatum***	8	35
		Anthophora	***urbana***	7	
		Syrphid	spp	7	
	Davis Creek Res.	***Melissodes***	**"sp.2"**	51	109
		Anthophora	***urbana***	34	
		Lasioglossum	subgenus *dialictus*	10	
	Dunnigan Hill Rd.	*Lasioglossum*	subgenus *dialictus*	94	170
		Ashmaediella	"small"	33	
		Syrphid	spp	12	
	Reiff Rock	***Anthophora***	***urbana***	113	216
		Syrphid	spp	20	
		Melissodes	**"sp.2"**	16	
Burned	Bear Meadow	***Anthophora***	***urbana***	100	241
		Bombus	***vosnesenski***	79	
		Melissodes	**"sp.2"**	30	
	Camel	***Anthophora***	***urbana***	90	136
		Syrphid	spp	11	
		Lasioglossum	subgenus *dialictus*	9	
	County Line Seep	***Anthophora***	***urbana***	44	90
		Melissodes	**"sp.2"**	10	
		Bombus	***vosnesenski***	7	
	First Stream	***Bombus***	***vosnesenski***	95	338
		Anthophora	**urbana**	112	
		Melissodes	**"sp.1"**	58	
	Hunting Creek Camp	***Anthophora***	***urbana***	45	95
		Anthidium	***manicatum***	15	
		Lasioglossum	subgenus *dialictus*	15	
	Knoxville	***Anthophora***	***urbana***	67	197
		Lasioglossum	subgenus *dialictus*	47	
		Syrphid	spp	29	
	Little Pond	***Anthidium***	***manicatum***	71	181
		Anthophora	***urbana***	55	
		Lasioglossum	subgenus *dialictus*	16	
	Second Stream	***Anthophora***	***urbana***	58	204
		Bombus	***vosnesenski***	58	
		Lasioglossum	subgenus *dialictus*	48	

2009), but shifts in broad functional traits (including size and behavior) may be most important in dictating the reproductive consequences for plants—a result we feel is supported by the data and observations in this study.

We do not know the demographic or population genetic consequences of fire and the reduction in outcrossing for *T. laxum* because we do not know whether seed predation, survival, germination, and eventual reproduction are density-dependent. Furthermore, while we know a seed bank exists (pers. obs.), we do not know the longevity or size of it, but it may dampen any effects of years of low outcrossing. While outcrossing rates decreased overall, fire may have positive consequences for population genetic diversity. It is likely that the overall production of outcrossed progeny was higher in burned areas; plants in these areas were far larger, and produced *proportionally fewer*, but *absolutely more* outcrossed progeny. The increase in large, vagile pollinators we observed increases the probability of rare, long-distance dispersal of pollen, resulting in novel combinations of genotypes (Greenleaf et al., 2007). We have not investigated dispersal or patch dynamics in *T. laxum*, but novel combinations of genotypes may be important for long-term species survival and evolution, especially in variable environments such as the California chaparral.

Additionally, we do not know the potential costs of inbreeding in this species, therefore we caution against any speculation on the fitness effects of outcrossing. All *Trichostema* species are self-compatible, though they differ markedly in their reliance on pollinators (Spira, 1978). The clade containing *T. laxum* includes both mostly small-flowered selfing species (e.g., *T. micranthum*) and large-flowered outcrossing ones (e.g., *T. lanceolatum*), suggesting that reproductive strategy is very labile in this group (Spira, 1980; Huang et al., 2008). However, many mixed-mating annuals show at least some inbreeding depression (Groom and Peruninger, 2000; Goodwillie and Knight, 2006; also reviewed in Goodwillie et al., 2005; Winn et al., 2011), and we have observed rare deformities in field and lab, including increased and reduced petal number, as well as complete lack of petioles, though whether these are genetic or

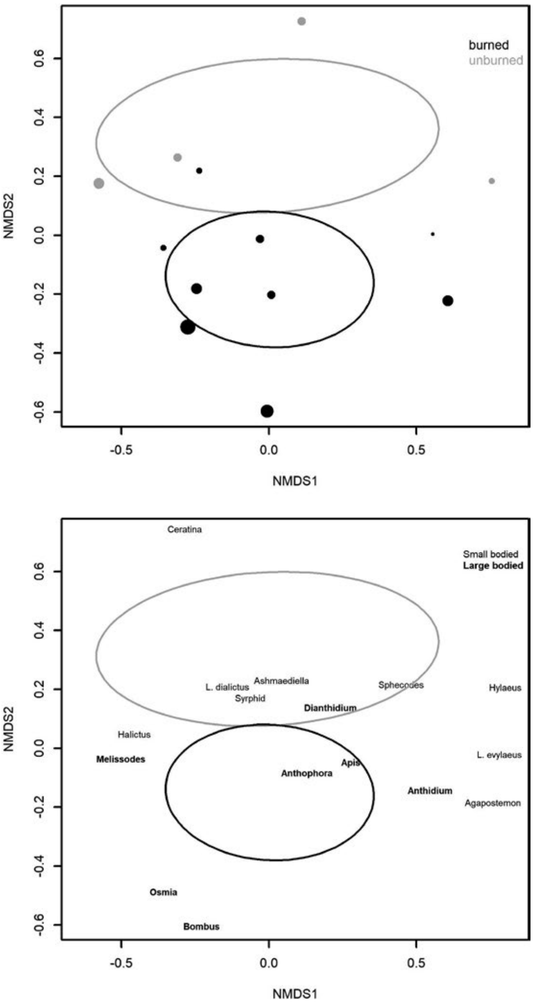

FIGURE 7. Site (upper) and species (lower) scores from NMDS analysis of flower visitor communities. Genera placement was determined by weighted averaging of species scores. Bold text taxon labels indicate large-bodied bees, plain text labels indicate small bodied bees.

environmental is unknown. Future work will examine the costs of inbreeding in this mixed-mating forb.

Whether or not fire leads to decreased outcrossing generally across plant taxa is largely an open question. Both Geerts et al. (2012) and Ne'eman et al. (2000) found similar results (lower visitation and/or seed set, depending on plant species). Other studies have found no effect or the opposite. Lewis (2000) noted that fire may promote flushes of chasmogamy in usually cleistogamous *Clitoria fragrans* Small, allowing this usually autogamous species its only chance of outcrossing. Similarly, Bourg et al. (2015) showed that fire promoted flowering and increased seed set of *Xerophyllum asphodeloides* Nutt., although they did not measure any aspect of pollination directly. Stone (1951) experimentally demonstrated that reduced light competition following fire promoted flowering of a lily in the California chaparral, allowing outcrossing. Van Nuland et al. (2013) found that burned sites had increased pollinator visitation to stands of *Verbenisia alterniflora* L., and experimentally demonstrated that a plant response to fire (increasing density) drove this pattern. However, while per patch visits increased, per plant visitation rates decreased with increasing plant density, possibly increasing geitonogamy, though outcrossing rate was not measured. (See also the review on woody perennials and fire effects on genetic diversity by Steinitz et al. [2012], which similarly concludes mixed effects of fire.)

Finally, specific fire characteristics (season, intensity, severity) may also determine plant responses and account for some variation between these studies. Vast stand-replacing California chaparral fires must affect pollinator communities fundamentally differently than smaller or low-intensity understory fires in less xeric eastern North American ecosystems (i.e., Van Nuland et al., 2013; Bourg et al., 2015). Accurate predictions for the effect of fire on reproduction of rare taxa are important given the controversial reimplementation of fire regimes. Some of this debate about ecological risks and benefits of fire almost certainly stems from the idiosyncratic responses of different taxa and emergent effects on interactions (e.g., Bradshaw et al., 2011; Keeley et al., 2011). While we and others have documented clear shifts in pollinator communities and outcrossing success in the immediate aftermath of a fire, further work is needed to inform a more accurate picture of the direct and indirect effects of fire on plant and pollinator fitness, as well as population dynamics. Furthermore, systems such as this can serve as a model for understanding how a large-scale community perturbation can affect mutualistic interactions.

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DATA ACCESSIBILITY

Data are included in three supplementary online appendices: Appendix S3 contains the outcrossing data, Appendix S4 contains the individual plant pollinator observation data, and Appendix S5 contains the pollinator community censuses.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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