

# Pathways for effects of small-scale disturbances on a rare plant: How *Mimulus angustatus* benefits from gopher mounds

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**Abstract.** Small-scale soil disturbances such as soil mounds produced by gophers are known to influence local plant communities. A variety of mechanisms might account for the influence of gopher disturbances on individual plant success, but understanding of these mechanisms is not well developed. Disturbances are often assumed to affect plants through changing competition or the abiotic environment, but disturbances might also influence plant size, which in turn influences other biotic interactions, including pollination. In this study, we tested for effects of soil disturbance on the density, flower size, and reproduction of an annual plant, *Mimulus angustatus*. We used soil disturbance manipulations, observational data, and a pollination experiment to examine two possible pathways for effects of disturbance on *M. angustatus*: competition and pollination. We also considered how effects of gopher mounds change with years since initial disturbance. We found that disturbance strongly increased the local density and flower size of *M. angustatus*, but the strength of these effects decays quickly. We found no support for these effects being mediated by competition with other plant species. We also found that *M. angustatus* with larger flowers receive more natural pollination. Our pollination experiment suggests that *M. angustatus* benefits from pollination (seed set increases with hand pollination or access to pollinators), and suggests that pollen limitation may occur. Taken together, our results indicate that increased pollination and seed set is an alternative response to disturbance that should receive further consideration. Regardless of the exact mechanism, it appears that in the absence of frequent small-scale soil disturbance, *M. angustatus* would not persist at our field site, as a large majority of plant recruitment and seed set occurs on recent disturbances.

**Key words:** disturbance; local density; mating system; plant size; pollen limitation; pollination.

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## INTRODUCTION

It is well known that soil disturbance can strongly influence the success of individual plant species; some plants cannot tolerate disturbance, whereas others can only recruit and grow in disturbed areas (Hobbs et al. 2007). There is also a wealth of work demonstrating that the frequency and extent of both larger-scale disturbances, such as the plowing of fields, and smaller-scale disturbances, such as those created by animals, are key factors determining the composition and

productivity of plant assemblages (e.g., Connell 1978, Hobbs and Huenneke 1992). Gopher mounds in particular (the piles of earth produced by gopher tunneling) are common in grasslands and known to influence plant communities (Reichman and Seabloom 2002), tending to favor forbs and annuals over perennial grasses (e.g., Hobbs et al. 1988, 2007, Stromberg and Griffin 1997, Seabloom et al. 2005). A few studies have demonstrated effects of these small-scale soil disturbances on the population dynamics of individual plant species; for example, Suding

and Goldberg (2001), Williams et al. (2010), and Sletvold and Rydgren (2007) used a combination of experimental disturbances and model fitting to demonstrate effects of small-scale disturbances on plant population dynamics. It is thus clear that gopher mounds can strongly influence plant populations and communities, but a better understanding of the mechanisms by which these disturbances affect plant performance and how these effects change over time would improve our ability to predict long-term plant dynamics as disturbance frequency changes.

A variety of mechanisms might account for the influence of gopher and other fossorial mammal disturbances on individual plant success. Most studies finding differential plant performance on and off mounds (e.g., Davis et al. 1995, Stromberg and Griffin 1997, Forbis et al. 2004, Williams et al. 2010) attribute these effects to reduction in competition from other plants for light, nutrients, or water, due to open space on mounds (Huntly and Inouye 1988, Suding and Goldberg 2001), and purely abiotic changes in soil characteristics. Abiotic soil traits, such as nutrient content and water retention, can differ between undisturbed areas and mounds where soil has been brought up from lower horizons; these traits can directly influence plant nutrient availability (Huntly and Inouye 1988, Reichman and Seabloom 2002, Eviner and Chapin 2005). However, gopher disturbances could also affect plants by influencing interactions with consumers or mutualists (pollinators, disease organisms, or herbivores other than gophers). This seems likely for several reasons. First, gopher mounds are known to affect abiotic conditions that could influence these other organisms. For example, the open, loose soil of mounds can be used as basking and oviposition sites for grasshoppers, thus possibly increasing insect herbivory in the vicinity (Huntly and Inouye 1988). Although one study has shown that gophers and insect herbivores influence each other's feeding on plants (Ostrow et al. 2002), we know of no tests of the effects of gopher disturbance per se on herbivore impacts on plants. One study (Eviner and Chapin 2003) has also documented that seedlings of an invasive grass that survive burial by gopher mounds are "cured" of a fungal infection that affects seed germination. Second, gopher mounds might influence interactions with

pollinators, herbivores, or pathogens by altering the apparency, densities, or composition of plants in the immediate area; both plant density and composition have been shown to influence pollinator and herbivore foraging (reviewed in Underwood et al. 2014). Finally, if plants grow to different sizes on gopher mounds (Davis et al. 1991, Suding and Goldberg 2001) because of different competitive or abiotic conditions, this could influence their attractiveness to herbivores or pollinators. Pollinators are known to respond to plant size and flower number (e.g., Thompson 2001). Although gopher disturbances could thus influence plants indirectly, through interactions with consumers or mutualists, these potential mechanisms have received little attention.

The influence of small-scale disturbances such as gopher mounds on plant population dynamics will depend not just on effects of an initial disturbance, but also on how effects change as a disturbance ages. If effects of soil disturbance persist for multiple years, this has different implications for plant population dynamics and communities than if effects of disturbance only last a single season. Studies that have examined the frequency of gopher disturbance (e.g., Hobbs et al. 2007, Williams et al. 2010) find that re-disturbance can be frequent, with a return time as short as every two to three years (Hobbs et al. 2007). However, few studies have followed the effects of gopher mounds or experimental disturbances through time; Collins (1989) and Williams et al. (2010) each followed plants for 2 yr after an initial disturbance, Jones et al. (2008) examined three ages of mounds, and Forbis et al. (2004) considered mounds of ages up to 20 yr. These studies all found that biotic and abiotic conditions on mounds change through time, and Williams et al. (2010) found that the rate of change (filling in of surrounding vegetation) differed between locations.

*Mimulus angustatus* is a small annual herb that is locally abundant in rocky meadows in the northern California coast range, where this study was conducted (recent revisions suggest a renaming to *Diplacus angustatus*, Barker et al. 2012). Our initial observations suggested that gopher disturbance, along with occasional disturbances from flooding along streams, strongly facilitated the persistence of *M. angustatus* in these meadows. These very small plants have no

obvious seed dispersal mechanisms; thus, local recruitment is likely to be much more important for the population dynamics of *M. angustatus* in meadows than for disturbance-dependent plants with widely dispersed seeds. To begin to understand how gophers influence *M. angustatus* populations, we tested for effects of soil disturbance mediated by different pathways, including effects on floral display and pollination. We also considered how the effects of gopher mounds change in years following the initial disturbance. We used soil disturbance manipulations, observational data, and an experiment to ask the following specific questions:

1. What are the effects of initial soil disturbance on *M. angustatus* density, and are these effects consistent with competition with the most common co-occurring plants?
2. How do plant density and flower size change in years following a single disturbance?
3. Is pollen receipt influenced by disturbance and flower size?
4. Is *M. angustatus* obligately outcrossing and pollen limited? This information is key to interpreting the importance of disturbance through effects on flower size and pollination.

## METHODS

*Mimulus angustatus* (A. Gray) (Phrymaceae; Beardsley et al. 2004, Baldwin et al. 2012) is an annual herb endemic to California. This study was carried out at the Las Posadas State Demonstration Forest in Napa County, California, USA (38.5633° N 122.4181° W). At this site, *M. angustatus* grows in shallow soil in open rocky meadows near a seasonal stream at an elevation of approximately 525 m, and typically blooms from May through June. *Mimulus angustatus* consists of a tiny rosette (stem < 1 cm and leaves < 36 mm; Baldwin et al. 2012) with from zero to seven (N. Underwood and B. D. Inouye, *personal observation*) relatively large magenta tubular flowers with yellow and purple markings. We rarely observed insects visiting *M. angustatus* during our study (some solitary bees, one bumble bee), but we did not devote time specifically to looking for pollinators. By far, the most common other plants in the meadow were *Lasthenia*

*californica* and the grasses *Aira caryophyllea* and *Festuca myuros*, all of which are taller than *M. angustatus* at approximately 15 cm high. Using annual surveys, we found that the frequency of disturbances by pocket gophers (likely *Thomomys bottae*) differed among years, from 1% to 5% of area along our transects. Although a small proportion of the transects was disturbed each year, average return times for re-disturbance may not be very long; large rocks just below the surface make much of the study area unsuitable for gopher activity.

### *Effects of natural and experimental disturbance on M. angustatus density and flower size*

We used permanently marked 20 × 20 cm quadrats along four transects to determine how soil disturbance affects *M. angustatus*. Two of these transects were observational (OT1 and OT2); OT1 included quadrats at each meter for 10 m (a random sample of natural conditions) plus 10 additional pairs of quadrats where one member of the pair was placed on a new naturally created disturbance (pocket gopher mound) and the other on an immediately adjacent undisturbed site. Observational transect OT2 included eight pairs of quadrats placed on gopher mounds and adjacent undisturbed sites. The other two transects were experimental (ET1 and ET2). For both experimental transects, pairs of quadrats were located along each transect in previously undisturbed locations; quadrats in which we created experimental disturbances were paired with immediately adjacent undisturbed controls. We created experimental disturbances in late December or early January by loosening and turning the soil to a depth of approximately 10 cm using a large nail.

We established OT1 in May 1999 and OT2 in May 2001. On the experimental transects, we added new quadrats over the course of the study to examine the effects of disturbances of different ages independent of the year in which the disturbances were made. Six pairs of experimental and control quadrats were established on ET1 in December 1999 and 10 more pairs were added in January 2002. Experimental transect 2 was established with 10 pairs of disturbed and control quadrats in January 2003. When the first six experimental quadrats were established on OT1, both experimental and control quadrats received

one pinch of lightly crushed *M. angustatus* fruits collected from that same meadow the previous summer. This ensured that all quadrats contained at least some *M. angustatus* seeds, although the number of seeds received by each quadrat likely varied widely. We did not add seeds in any other year.

Each spring between 1999 and 2005 (from 25 April to 16 May depending on year, see Appendix S1: Table S2), we surveyed all quadrats that could be re-located. The permanent tags for quadrats on OT2 were lost after two years (to tampering), but all other quadrats were followed for the duration of the study. For each quadrat, we recorded the number of *M. angustatus* plants and flowers/buds, and numbers of individuals of the potential competitors *L. californica* and “grass” (the two grass species were pooled together). We also recorded the percent of disturbed ground (defined as bare soil either raised, as for a pocket gopher mound, or depressed, as for shallow digging by unknown mammals; the overwhelming majority of disturbance was by gophers) and the percent of bare soil (defined as soil not covered by plants, oak leaf litter, or small rocks) for each quadrat. Starting in 2000, we recorded corolla length and width (at the widest point) for all *M. angustatus* flowers open on the day of the survey in all quadrats. For all analyses, we used floral tube length as a measure of flower size; results were very similar for flower width or length times width (not shown).

#### ***Mimulus angustatus* pollination**

*Mimulus angustatus* has yellow pollen and purple stigmas, allowing us to assess pollination in the field by counting pollen grains on stigmas with a hand lens. To examine the effects of flower size and local density of open *M. angustatus* flowers on pollination, we counted pollen grains for 41 randomly chosen flowers (one flower per plant) for plants that were not in our pollination experiment (below) or the quadrats on our transects. We measured flower size and the number of *M. angustatus* flowers open and percent bare ground within a 20 × 20 cm quadrat centered on each flower.

Because the mating system of *M. angustatus* had not previously been examined experimentally (but see Grossenbacher and Whittall 2011 and Grossenbacher et al. 2016 for a

phylogenetically based inference of outcrossing in this taxon), we explored whether *M. angustatus* plants with outcrossed pollen set more seeds than plants with no pollination, and whether *M. angustatus* is pollen limited in the field. In 2003, we created four treatments by factorially crossing pollen supplementation and pollinator exclusion, using small mesh pollinator exclusion cages put over plants while they were still in bud (15–19 plants per treatment). We supplemented pollen by rubbing the anthers from a randomly chosen flower from a different part of the meadow directly on the stigma of the flower to be pollinated; we visually confirmed that plants received pollen grains on the stigma. We returned on five dates between 13 May and 27 May (Appendix S1: Table S2) to pollinate all flowers on the experimental plants as they opened. Most plants had only one flower but a few had multiple flowers, up to a maximum of seven for one particularly productive plant. There was no difference in mean flower size among treatments (results not shown). We collected all fruits after all the plants had senesced, between 7 June and 24 June. Because *M. angustatus* seeds are minute, it was not practical to weigh individual seeds for all plants. Instead, fruits from each plant were weighed together and then seeds were removed and counted.

#### **Analyses**

*Effects of disturbance and competitors on numbers of M. angustatus.*—We addressed this question by fitting structural equation models (SEMs) to data on counts of *M. angustatus*, *L. californica*, and grasses. For this analysis, we used counts from quadrats on all transects in all years except 2006, when new disturbance was not recorded. We carried out an initial fit of the model to all the data, and because conditions in particular quadrats are likely not independent across years, we also fit the model to each year individually. Patterns were largely the same using the full and the yearly data sets; we present the pattern from the full data set here and a summary of the yearly analyses as supplemental information (Appendix S1: Table S1). The initial model considered the number of *M. angustatus* per quadrat as a function of the number of *L. californica*, the number of grass stems, and the percent bare

ground in a quadrat (as a measure of disturbance), with percent bare ground also affecting both grass and *L. californica*. We also fit a reduced model that excluded competitive effects of *L. californica* and grass.

*How plant density and flower size change over time after a single disturbance.*—To address this question, we used only data from quadrats (on all transects) with a known year of initial disturbance, either experimental or a pocket gopher mound. Quadrats that were completely re-disturbed (>90%) were counted as a new disturbance. We examined changes in the number of plants per quadrat as a function of time since disturbance, using a general linear mixed model of log plants per quadrat with quadrat included as a random factor. This resulted in 174 observations in 36 quadrats. The same analysis was used to ask how flower size changed with time since disturbance; we recorded data on flower sizes from a subset of the quadrats used in the analysis of numbers of plants, for a total of 44 observations in 21 quadrats. For analysis of flower size, we only considered data less than three years post-disturbance, because only three flowers were found in quadrats that remained undisturbed longer than two years.

*Effects of disturbance and flower size on pollen receipt.*—We also used an SEM of quadrat-scale data to examine how bare ground, the number of open *M. angustatus* flowers, and flower size influenced the number of pollen grains on *M. angustatus* stigmas. In the initial model, pollen was considered a function of bare ground, *M. angustatus* flower density, and flower size, while flower size and *M. angustatus* flower density were also functions of bare ground.

*Pollination experiment.*—We used a linear model to examine the effects of pollinator exclusion cages and hand pollination on the number of seeds set per flower for each plant. Mean flower size of the focal plant and percent of bare soil within a 20 × 20 cm quadrat centered on each plant were included as covariates. Number of seeds per flower was log-transformed, resulting in approximately normally distributed residuals. There were no significant interactions, so all interactions were dropped from the model with the exception of the interaction of most a priori interest: hand pollination × caging. Linear models using subsets of the data were used

to address the specific a priori hypotheses that *M. angustatus* benefits from receiving pollen from conspecifics (in which case hand pollination should increase seed set for plants in cages, where natural pollination from non-self plants is absent) and that *M. angustatus* was pollen limited in the field (in which case hand pollination should increase seed set when natural pollinators had access to plants).

Flower size could be related to increased seed set in two ways. First, it could increase pollen receipt, which would increase seed set if plants are pollen limited. Second, flower size could be correlated with overall plant vigor or ovule number. Because we did not measure plant size or count ovules, we do not have appropriate data to separate these mechanisms. We were, however, able to test for effects of flower size in the absence of attraction of pollinators by comparing seed set per flower to flower size for only the caged plants. Similarly, we tested for effects of flower size for uncaged plants that did not receive hand pollination, where an effect of flower size on attraction could still operate. A larger effect of flower size for uncaged plants would suggest a role for pollinator attraction.

Analyses used R 3.1.2 (R Development Core Team 2014). Structural equation models were run using the lavaan package, and mixed-effects models were run with the nlme package. Results for linear models used type II SS and contrasts from the car and multcomp packages, respectively.

## RESULTS

Experimental and natural disturbances did not differ in their effects on the number of *Mimulus angustatus* plants present in a quadrat; there was neither an effect of type of disturbance nor an interaction of treatment with type of disturbance (results not shown, but note that these results are not definitive because type of disturbance was partly confounded with transect location).

### *Effects of disturbance and competitors on M. angustatus density*

For all years pooled, disturbance strongly affected local *M. angustatus* density as well as the densities of *L. californica* and grass, but

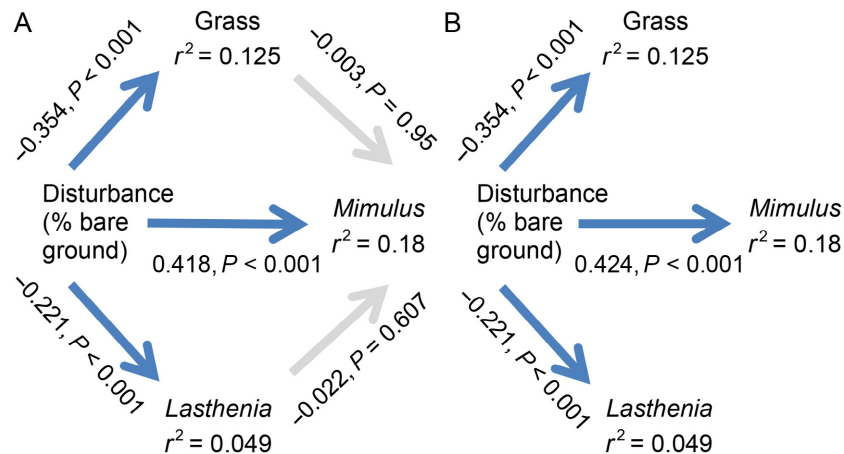


Fig. 1. Structural equation models for effects of disturbance on *M. angustatus* density. Results are shown for 1999–2005 pooled (N = 488); results were very similar in each individual year as well (see Appendix S1: Table S1). Including data from 2006 by substituting bare ground for disturbance did not change results (see text, results not shown). Black arrows indicate significant relationships; gray arrows indicate nonsignificant relationships. Standardized path coefficients and P values are indicated near the arrows. (A) Model including effects of other plants on *M. angustatus* ( $\chi^2 = 1.01$ ,  $df = 1$ ,  $P = 0.315$ , root mean square error of approximation [RMSEA] = 0.004). (B) Model with only effects of disturbance ( $\chi^2 = 1.27$ ,  $df = 3$ ,  $P = 0.735$ , RMSEA = 0). Endogenous (dependent) variables have  $R^2$  values below.

*L. californica* and grass densities were not related to *M. angustatus* density (Fig. 1). The SEM including effects of *L. californica* and grass on *M. angustatus* did not have a significant lack of fit, but was a poorer fit than a model including only effects of disturbance on all three types of plants ( $\Delta AIC = 3.73$ ). Soil disturbance had a positive effect on *M. angustatus* density, but negative effects on both *L. californica* and grass. Results for individual years were qualitatively similar to the results for pooled data; disturbance significantly affected *M. angustatus* positively in all seven years and grass negatively in six out of seven years, but the effect of disturbance on *L. californica* was only significant in two years (Appendix S1: Table S1).

**Effects of disturbance on *M. angustatus* density, flower size, and pollen receipt**

Based on analyses of the transect data, as initial disturbances aged *M. angustatus* density decreased ( $t = -9.04$ ,  $df = 137$ ,  $P = 0$ ), declining to near zero by three years post-disturbance (Fig. 2A). Flower length also decreased with years since disturbance ( $t = -5.91$ ,  $df = 21$ ,  $P = 0$ ), declining by more than 25% (from 26 mm in the

year of disturbance to <20 mm by two years after disturbance) (Fig. 2B). In analyses of individual quadrats centered on flowering focal plants, we found that *M. angustatus* received more pollen when flowers were large (Fig. 3) and when there were more *M. angustatus* flowers in the immediate neighborhood. Flower size was larger in areas with more bare ground (more disturbed areas). While in the data collected to quantify pollen receipt bare ground did not influence the number of open *Mimulus* flowers, these locations were chosen for the presence of flowers, and in other analyses, there was a strong positive effect of bare ground on *Mimulus* density (c.f. Fig. 1).

**Outcrossing and pollen limitation**

Several lines of evidence from our pollination experiment suggest that being visited by pollinators is important for *M. angustatus* to set seeds. Caged unpollinated plants did produce a few seeds, suggesting that perhaps they are able to self. However, the addition of non-self pollen clearly increased seeds per flower overall (main effect of pollination in ANOVA,  $F_{1,58} = 12.31$ ,  $P = 0.0009$ ), with a stronger effect for caged plants without access to pollinators than for uncaged

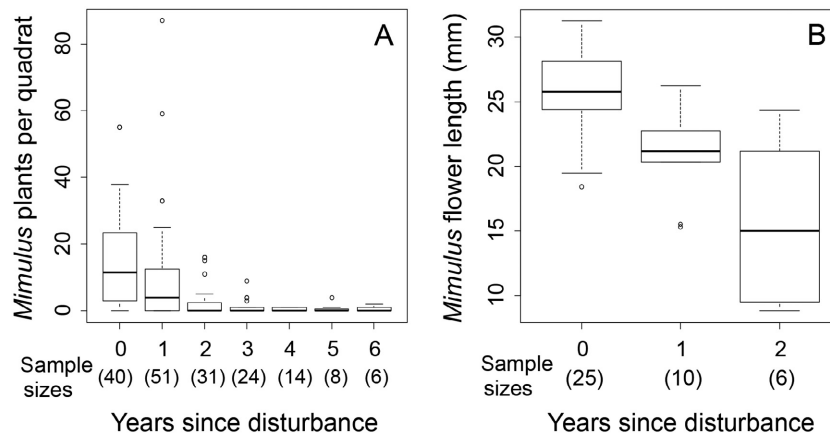


Fig. 2. Changes in (A) *M. angustatus* density and (B) flower size in quadrats vs. years since soil disturbance. Boxes contain 50% of the data, and the whiskers indicate 1.5 times the interquartile range. The number of observations (quadrats) varied with time since disturbance.

plants (marginally significant interaction of pollination with caging,  $F_{1,58} = 2.59$ ,  $P = 0.056$ ). For plants in cages (without access to pollinators), hand pollination significantly increased seed set per plant (main effect of pollination in linear model with only caged plants,  $F_{1,58} = 11.44$ ,  $P = 0.02$ ; compare second and fourth bars in Fig. 4). However, there was only weak evidence for strong pollen limitation; for plants without cages (with access to pollinators), hand pollination increased seed set, but the effect was not significant ( $P = 0.14$ ; compare first and third bars in

Fig. 4). Caging itself did not strongly affect seed set other than through elimination of pollinators, as there was no significant main effect of cage (Fig. 4) and for plants that were hand-pollinated there was no difference in seed set between caged and uncaged plants (with only hand-pollinated plants,  $F_{1,32} = 0.04$ ,  $P = 0.83$ ; compare third and fourth bars in Fig. 4).

Consistent with our observational data suggesting greater pollen receipt for larger plants in more disturbed areas, both flower length and the percent bare ground around a plant in our pollination experiment had strong effects on seed set (flower length:  $F_{1,58} = 12.15$ ,  $P = 0.0009$ ; bare ground:  $F_{1,58} = 18.3$ ,  $P < 0.0001$ ). Consistent with the hypothesis that attraction of pollinators by larger flowers positively influences seed set, in the absence of pollinators (caged plants only) there was no effect of flower length on seeds per flower ( $F_{1,27} = 1.17$ ,  $P = 0.29$ ), while plants receiving pollen only from pollinators (uncaged, no hand pollination) showed a significant positive effect of flower length ( $F_{1,13} = 7.04$ ,  $P = 0.003$ ).

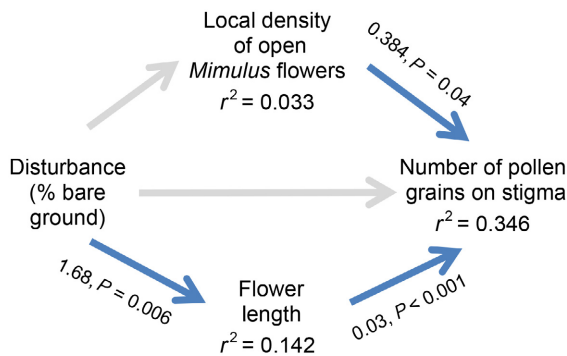


Fig. 3. Structural equation model for effects of disturbance on pollen receipt by *M. angustatus*. Black arrows indicate significant relationships; gray arrows indicate nonsignificant relationships. Standardized path coefficients and  $P$  values are indicated near the arrows ( $N = 46$ ,  $\chi^2 = 2.55$ ,  $df = 1$ ,  $P = 0.110$ ,  $RMSEA = 0.184$ ). Endogenous variables have  $R^2$  values below.

DISCUSSION

Although many previous studies have highlighted the influence of soil disturbance from gophers and other small mammals on plant communities, there are few studies that consider the variety of mechanisms through which these small-scale disturbances may affect plants. Using

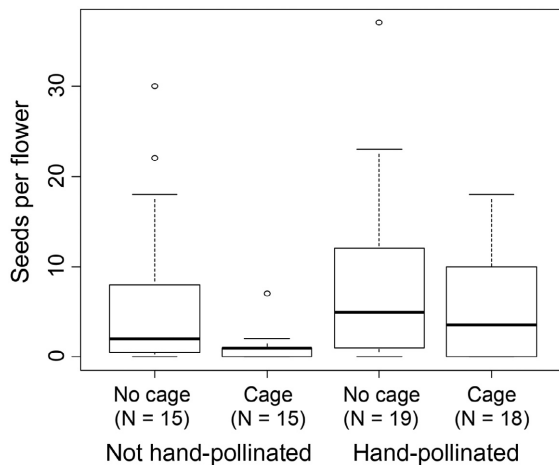


Fig. 4. Seed set for *M. angustatus* with different pollination treatments (caged to prevent insect pollination or not, and hand-pollinated or not). Hand pollination increased seed set, and there tended to be a stronger effect of pollination with caging than without ( $P$  value for the interaction between pollination and caging = 0.056).

experimental and observational data, we found that disturbance strongly affects the density of *Mimulus angustatus*, as well as its flower size and pollen receipt, suggesting that disturbance could influence long-term overall population size in part through effects on pollination.

We found very clear evidence that *M. angustatus* benefits from gopher disturbances, as both our experimental and natural gopher mounds had higher densities of *M. angustatus*. The most common competitors, *Lasthenia californica* and small annual grasses, were negatively associated with disturbance, consistent with what has been seen previously for *L. californica* and *Festuca microstachys* in similar habitats (Hobbs and Mooney 1991, Hobbs et al. 2007). Although these results are consistent with alleviation of competition as a mechanism for the effect of gopher disturbance on *M. angustatus*, the best SEM has no significant path between disturbance and *M. angustatus* density through competitor densities (Fig. 1). This suggests that other mechanisms are at work, the most likely of which are abiotic effects of disturbance, such as changes in soil moisture, texture, nutrients, or temperature. It could also be that the diminutive *M. angustatus* plants are so sensitive to competition that the

presence of *any* competitors prevents germination or growth, which could cause the SEM to indicate the importance of bare ground from disturbance and leave little role for variation in the number of competitors in undisturbed portions of the quadrats. While we did not collect data on abiotic factors such as nutrient availability, previous studies clearly show that gopher mounds are different abiotic environments from surrounding soil (e.g., Inouye et al. 1987, Reichman and Seabloom 2002) and that these differences can influence plants (e.g., Rice 1985, Suding and Goldberg 2001); thus, both competition and abiotic influences deserve further study.

We also found evidence of a novel pathway for effects of small disturbances on *M. angustatus* populations. Several lines of evidence from our study suggest that gopher mounds promote reproduction of *M. angustatus*. Plants in disturbed areas had larger flowers (Fig. 3), consistent with another report of faster growth on gopher mounds (Davis et al. 1991). In this study, we did not measure plant size, which is dominated by flowers. However, we found that larger flowers and flowers in areas of higher flower density receive more pollen grains (Fig. 3). This makes sense in light of the many studies in other systems that have shown that pollinators prefer large flowers or floral displays. If plants on gopher mounds receive more pollen, this could increase seed production or seed quality from plants on mounds, providing a pathway for soil disturbance to contribute to plant density at the population level. The capacity for seeds to contribute to future population densities will of course depend on how long seeds persist in the seed bank relative to rates of disturbance; the age of the seed bank for *M. angustatus* is unknown, although we found that seeds remained viable in the laboratory for at least two years after collection (N. Underwood and B. D. Inouye, *personal observation*).

For pollen receipt to be an important mechanism for effects of disturbance on *M. angustatus*, *M. angustatus* would need to be at least somewhat outcrossing and pollen limited. The genus *Mimulus* contains both selfing and outcrossing species, and while it has been inferred that *M. angustatus* is likely outcrossing (Grossenbacher and Whittall 2011, Grossenbacher et al. 2016), we know of no direct tests of this species'



mating system. Our pollination experiment showed that adding pollen increased seeds per flower, both inside and outside pollinator exclusion cages. Inside cages, the positive effect of hand pollination suggests that *M. angustatus* is indeed outcrossing; plants did not set all the seeds they could with only access to self-pollen. Because we did find some seed set in cages with no pollination, it is possible that *M. angustatus* is not a completely obligate outcrosser, although this could also be a result of imperfect pollen exclusion. We suspect our pollination treatment was variably effective, as some hand-pollinated flowers also did not set any seeds.

Outside of the cages, we found a trend toward a positive effect of hand pollination, suggestive of some pollen limitation, although this was not statistically significant. Another line of evidence suggestive of pollen limitation for the smaller-flowered plants found in undisturbed areas is that for uncaged plants with no hand pollination (i.e., natural conditions), seed set increased significantly with flower size. This is consistent with larger flowers also receiving more pollen in our surveys of unmanipulated flowers (Fig. 3). An alternative explanation for larger flowers setting more seeds is that they have more ovules or are on plants with more resources to mature seeds. However, in this case, the effect of flower size would have nothing to do with pollination and should also hold for caged plants. We did not find support for this interpretation; for caged plants, there was no significant effect of flower size on seed set, although the direction of this relationship was positive. Altogether, we conclude that it is still unclear to what degree smaller *M. angustatus* flowers are pollen limited. Our pollination experiment had relatively low power; thus, it would not detect weak relationships between hand pollination and seed set outside cages, or between flower size and seed set within cages. Our evidence that larger flowers and flowers in disturbed areas set more seeds is quite clear—either way disturbance is influencing reproduction. Although our results suggest that this is at least partly mediated by pollinator attraction, further work is required to confirm this interpretation.

The positive effects of a disturbance on plant density, flower size, pollen receipt, and reproductive success appear to last only a few years in this

location (Fig. 2). We found that *M. angustatus* density declined to very low values by three years after disturbance, and flower size decreased markedly by two years after disturbance. It was also our impression that flowers were paler as disturbances age, although we did not quantify color (N. Underwood, *personal observation*); this could be consistent with reduced nutrient availability given that floral pigments rely on commonly limiting nutrients such as nitrogen and phosphorus (Tanaka et al. 2008) although there is little work on effects of soil nutrient availability on flower color in the wild. Because we took data on disturbances of different ages in many different years and *M. angustatus* is an annual, the results are clearly an effect of disturbance age rather than a temporal trend in the general environment or with plant age. The few previous studies that have followed gopher mounds of different ages have focused on changes in plant community diversity or total plant cover rather than individual plant species traits (but see Collins 1989). We hope that our study encourages more researchers to explore the effects of small local disturbances on the dynamics of plant traits, in order to improve understanding of the mechanisms that affect local population dynamics.

In conclusion, it appears that in the absence of frequent small-scale soil disturbance, *M. angustatus* would likely not persist at our field site, as a large majority of plant recruitment and seed set occur on recent disturbances. There appear to be multiple pathways for these large effects of disturbance on *M. angustatus*, but our study did not test the relative importance of different mechanisms for effects of disturbance and some results about the importance of pollen limitation were inconsistent. Nevertheless, our results suggest that competition is not the most important mediator of disturbance effects and that pollination is an additional pathway that should receive further consideration. Huntly and Inouye (1988) pointed out that, despite a lack of experimental evidence, plants are often assumed to benefit from disturbances like gopher mounds due to release from competition (Huntly and Inouye 1988). It continues to be the case that mechanisms mediating effects of these disturbances are not well understood. There are good reasons to think that a variety of mechanisms, including indirect effects

through mutualists and consumers, are important (Ostrow et al. 2002, Eviner and Chapin 2003). Studies that consider multiple pathways simultaneously are needed to help predict the consequences of changes to these systems, such as reductions in gophers (e.g., with grazing [Stromberg and Griffin 1997] or gopher control), altered precipitation patterns that affect flood frequency, or changes in pollinator abundance.

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