

Effects of Local Conspecific Density on Reproductive Success in *Penstemon digitalis* and *Hesperis matronalis*¹

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ABSTRACT. We investigated the effects of plant density on reproductive success for two insect-pollinated plant species—the native North American wildflower *Penstemon digitalis* (Foxglove-leaved Penstemon), and the showy introduced weed *Hesperis matronalis* (Dame's Rocket). We found no indication that local density (within 3.0 m) affected reproductive success (seeds per fruit, proportion fruit set, total seeds per plant) for either species. *Penstemon digitalis* suffered heavy fruit predation from micro-lepidopterans, and such damage tended non-significantly to increase with density. We discuss the reasons for our results, and suggest that an understanding of those causes is important for conservation.

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INTRODUCTION

Plants in natural populations experience a large range of densities, ranging from isolated plants to densely packed thickets. Such variation in density can have strong effects on plant reproductive success. On the one hand, plants growing in dense patches often compete with one another for resources such as nutrients and water, and this can cause reduced plant size and vigor, reducing seed and fruit set compared to plants with fewer neighbors (Harper 1977). On the other hand are the counter-intuitive results of studies focusing on animal-pollinated plants, which often report increased reproduction for plants in dense populations (Kunin 1992, 1997; Lamont and others 1993; Byers 1995; Agren 1996; Roll and others 1997; Groom 1998; Courchamp and others 1999). This surprising "facilitation" (Rathcke 1983) of reproduction may reflect increased pollinator visitation and reduced selfing rates in high density areas.

An understanding of the effects of plant density, and especially of facilitation of reproduction, is important in regard to at least two major conservation issues; habitat fragmentation and invasive species. Habitat fragmentation is likely to reduce plant density for natives by shrinking habitat patches and degrading habitat quality. If the sorts of facilitative interactions described above are important, this should decrease plant reproductive success, exacerbating the threat of fragmentation to native plants. Invasive species are another topic of concern for which an understanding of the effects of density is important. During the early colonization period, invasives will primarily be in small and isolated populations. The most successful invasive species may be those that are best able to reproduce at low density (Lewis and Kareiva 1993; Crooks and Soule 1999).

To determine whether natives and invasives respond differently to density, and to better understand the effects of plant density on reproductive success, we studied two species: one Ohio native, and one invasive found in Ohio. We chose to study the Ohio native Foxglove Beardtongue (*Penstemon digitalis*), and the introduced

weed Dame's Rocket (*Hesperis matronalis*). Little has been published on the biology of either species, so we also present results of our studies on their natural history and reproductive biology.

SPECIES

Penstemon digitalis

Penstemon digitalis Nutt. (Scrophulariaceae) is a short-lived perennial herb found in moist, open woods and prairies throughout Northeastern North America (Gleason and Cronquist 1991). Adult plants range in height from 20 to over 100 cm, and typically display 1 to 12 flowers at one time on an inflorescence (mean \pm sd = 3.96 ± 2.76 ; N = 116) from late June through July in Northeastern Ohio. Little has been published about the reproductive and pollination biology of the species. The white flowers are self-compatible but require animal visitation to produce seed (see Clinebell and Bernhardt 1998; also RJ Mitchell unpublished data), and are ~15 mm long \times 7 mm wide, with pale purple nectar guides on the floor of the sympetalous corolla. Density varies widely in natural populations, ranging from 0-15 stems/m² (results from the observations described below). Fruits contain a few to over 100 seeds, and unidentified Lepidopteran larvae often damage fruits and stems.

Hesperis matronalis

Hesperis matronalis L. (Brassicaceae) is a short-lived perennial herb of Eurasia that has escaped cultivation to become a common weed of disturbed places in Northeastern North America (Gleason and Cronquist 1991). Adult plants range in height from 50 to over 100 cm, and typically display 1 to >20 showy and presumably insect-pollinated flowers at one time on each inflorescence. Flowering is from mid-May to mid-June in Northeastern Ohio. Little has been published about its reproductive and pollination biology (see Conner and Sterling 1995, 1996). Our pilot work indicates that *H. matronalis* is largely self-incompatible, with hand-cross-pollinated flowers producing 12 seeds/fruit, while selfed or unpollinated flowers produce only 2 seeds/fruit (RJ Mitchell, unpublished data). Flower color is polymorphic in most populations, ranging from purple to

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pink to white, including many intermediates. A wide range of insects visit and presumably pollinate this species, including bees (*Bombus* of several species, *Apis mellifera*, other bee species) and several species of Lepidoptera (including Papilionidae). Densities in natural populations range from 0-48 stems/m² (results from observations described below). Fruits contain a few to over 35 seeds, and other than some occasional evidence of deer browse, we detected no fruit or seed predation on this species.

METHODS

Penstemon digitalis

We observed and collected visitors to *P. digitalis* flowers during summer 1996 and again in 2000. To quantify visitation rates, during 1996 we observed single plants (each with a single stem) for 15 minutes each (N = 14 plants), and recorded the number of flowers probed and the number of flowers on that plant that were available to be visited.

Our study site is on the MetroParks serving Summit County Bike and Hike Trail near Interstate 80 (41°15'35" N, 81°31'16" W). Throughout this area there are extensive but patchy groupings of *P. digitalis* that vary widely in density, and to a lesser extent in microhabitat.

To examine the effects of conspecific density on *P. digitalis* reproduction, in June 1996 we chose twenty pairs of plants in wet meadows and open woods north of Akron, OH. We attempted to match members of each pair for vigor, location, and growing conditions, and members of each pair were within 4-10 m of one another. We chose pairs such that one member of each pair had many conspecific neighbors (mean \pm SE = 57.9 \pm 7.2 stems within 3 m), while the other had an order of magnitude fewer neighbors (5.8 \pm 1.6 stems within 3 m). Other than this categorical variation in local **relative density** (dense versus sparse), the members of a pair were similar in all indicators of plant vigor and attractiveness to pollinators (above-ground dry mass, height, number of open flowers, total season flower production; ANOVA $F_{1,19} < 2.4$, $P > 0.14$ in all cases). We restricted our study to genets with a single stem. For the members of each pair we assessed two measures of the **absolute density** of conspecifics: the number of neighboring flowering stems within three meters of the focal plant, and the mean distance to the three nearest conspecifics in flower.

After fruits had matured in late-summer, but before they had released seeds, we collected the forty inflorescences. In the lab, we noted the number of undeveloped (seedless) and developed (>0 seeds) fruits, and removed and stored developed fruits for later counting. For each fruit we recorded the incidence of microlepidopteran exit holes and frass, scoring such fruits as "damaged." The 1070 flowers on these 40 plants produced 531 fruits that we scored for seed set and insect damage.

To assess the germinability of the seeds resulting from the plants described above, in 1997 we placed 20 seeds from each of 1-6 fruits from each plant (a total of 141 fruits) on moistened filter paper in petri dishes, and

cold stratified the dishes at 4° C for >4 months. Five plants did not produce any viable seeds, and are excluded from this analysis. We scored any seeds with an emerging radicle as germinated.

Hesperis matronalis

In 1996 we studied reproduction for *H. matronalis* plants in three sites in the Cuyahoga Valley, north of Akron: Big Bend Park (41°07'40" N, 81°32'17" W), O'Neill Woods Park (41°09'49" N, 81°35'18" W) and Smith Road (41°08'27" N, 81°33'51" W). Each site was separated by >3.0 km from the others, and had several to many hundreds of *H. matronalis* plants, sometimes in large and continuous patches, other times in scattered clumps. All populations were in successional or ruderal situations, such as open fields, young forest, and roadsides.

We used methods similar to those described above for *P. digitalis* to assess the effects of local density on reproduction. In each site we identified 6 to 21 pairs of plants (35 pairs total) that were matched for size (height, number of stems), flower color, phenological state, and our subjective impressions of plant vigor, but that differed in relative density (dense versus sparse). For each plant, we assessed absolute density using distance to the three nearest neighbors, and the number of flowering stems within 3.0 m of the focal plant, as described for *P. digitalis*. Individual plants were readily distinguishable in most cases, although many had multiple stems.

We collected plants and seeds in mid July 1996, and counted all fruits and flowers produced by each plant, scoring seed production for each fruit. In many cases fruits had dehisced before collection, but we used the number of constrictions in the fruit wall as a surrogate measure of seeds/fruit. This worked well: for a separate sample of undehisced fruits the number of constrictions in the fruit wall was strongly correlated with actual seed counts ($r = 0.98$, $N = 139$, $P < 0.0001$). The 2085 flowers on the 61 plants that survived until harvest and could be relocated produced 1087 fruits that we scored for seed set.

Because so little is known of the pollination biology of this plant species, we took this opportunity to test for differences in seed set and floral morphology among color morphs (purple versus pale, where pale includes white, light pink, and other intermediate colors). To accomplish this, in mid June 1996 we used digital calipers to measure floral traits on the plants described above, following the methods of Conner and Sterling (1995). These traits included petal length, petal width, tallest stamen height, and gynoecium length. We measured two flowers per plant when possible, but could not measure flowers for all plants because some had finished flowering or could not be re-located.

We analyzed data from both studies using JMP 3.26 (SAS Institute Inc. 1995). To assess the effects of relative density on reproductive success, we used two-way mixed model ANOVA. To analyze absolute density and nearest neighbor distance data we used linear regression. Preliminary analysis of the *H. matronalis* data revealed no significant population effects, and we therefore ignored population effects in later analyses. To meet the

assumptions of ANOVA, mass, total seeds, mean nearest neighbor distance, and absolute density were transformed to natural logarithms. To assess effects of flower color for *H. matronalis*, we used a nested analysis, with flower nested within plant, and plant nested within flower color.

RESULTS

Penstemon digitalis

We observed a wide range of bee species visiting *Penstemon digitalis*, including *Bombus fervidus*, *B. vagans*, *B. bimaculatus*, *Apis mellifera*, *Anthidium manicatum*, *Osmia* sp., *Megachile* sp., and *Halictus* sp. Several Lepidopterans (Hesperiidae, Papilionidae) also visited *P. digitalis* flowers, but based on morphology were probably not effective pollinators. During our timed observations, we recorded many visits by small solitary bees (primarily Halictidae; mean \pm se = 2.57 ± 0.77 visits/flower/hour, N = 14), while larger bees (mostly *Bombus* sp., *Megachile* sp., *Osmia* sp.) were much less common (0.51 ± 0.4 ; N = 14). Although both groups of bees foraged for both nectar and pollen, larger bees appeared to be more likely to contact stigmas and effect pollination.

Reproductive success for *Penstemon digitalis* was unaffected by absolute density (Table 1). The variance in reproductive performance explained by absolute density never exceeded 2% for either species, and never approached statistical significance. This pronounced lack of a significant effect of density was evident whether we estimated density using stems within 3.0 m, or mean nearest neighbor distance. In all cases, plants with many near neighbors tended very slightly (and non-significantly) to have better reproductive success. Results are unchanged if plant mass is used as a covariate (analysis not shown).

Relative density also had no significant effect on reproductive success (Table 2). Although non-significant, reproductive success for sparse plants was in all cases worse than that for dense plants, usually by 7 to 13%.

Statistical power analysis (SAS Institute 1995) indicates that samples of 42-146 plants (rather than the 40 plants available to us) would have been necessary to find statistical support for the observed differences between sparse and dense plants, indicating that any effects of relative density are weak. Explained variation for these ANOVA models was reasonably large (Table 2), in part because of "pair" effects that were occasionally significant (see results for fruit set). This indicates that plants of similar size and in similar microhabitats were more similar to one another than were other plants.

Unidentified micro-lepidopteran (Tortricidae) larvae partially or wholly destroyed a substantial fraction of fruits and seeds (Table 2). Furthermore, many inflorescences were damaged by stem and bud feeding caterpillars (for example 80% of stems were damaged in late June 1996 on a random transect; N = 45 stems).

Germinability of the seeds resulting from these plants was unaffected by absolute density (for both stems within 3.0 m, and mean nearest neighbor distance, $F_{1,33} < 0.01$, $P > 0.9$). Likewise, germinability did not differ among relative density categories ($F_{1,15} = 0.01$, $P > 0.9$; mean proportion germinating = 0.25 ± 0.03 for dense, 0.25 ± 0.04 for sparse).

Hesperis matronalis

Results for *Hesperis matronalis* parallel those for *P. digitalis*. Reproductive success was not significantly affected by either measure of absolute density (Table 3), and the amount of variance explained by the regressions was tiny (<3.1%), although the slopes for all the regressions indicated a slight tendency for plants in dense areas to have better reproductive success. Likewise, reproductive success was unaffected by relative density (Table 4), but in this case, responses were inconsistent among the different measures of reproductive success, with sparse plants sometimes doing better, and sometimes worse than dense plants. Although the sample size for this species was larger than for *P. digitalis*, because of the small differences among

TABLE 1

Effects of absolute density and mean nearest neighbor distance on female reproductive success in *Penstemon digitalis*. Statistics are for a linear regression of the response variable on the density measures. Denominator df = 35-38; β represents the standardized regression slope. Predictor variables are both ln-transformed. Seeds/fruit refers to plant mean seeds per fruit, ignoring flowers that did not produce seeds. Fruit Set is the Arcsin ($\sqrt{\text{proportion of flowers that became fruit, including those that were later consumed by caterpillars}}$). Total seeds per plant refers to Log (1 + total number of seeds per inflorescence). Damage = Arcsin ($\sqrt{\text{proportion of flowers that were damaged by caterpillars}}$).

Response Variable	# stems within 3 m			Mean nearest neighbor distance		
	β	R ²	P	β	R ²	P
Seeds/Fruit	-0.028	0.0007	0.87	0.036	0.0013	0.83
Fruit Set	0.065	0.0090	0.56	0.135	0.0184	0.41
Total seeds per plant	-0.010	0.0001	0.95	0.064	0.0041	0.71
Damage	-0.100	0.0100	0.54	0.114	0.0129	0.49

TABLE 2

ANOVA of effects of relative density on female reproductive success in *P. digitalis* (Error $df = 18$.)
Conventions follow Table 1. Boldface indicates statistical significance.

Source	DF	Seeds/Fruit $R^2 = 0.46$		Fruit Set $R^2 = 0.34$		Total Seeds $R^2 = 0.53$		Damage $R^2 = 0.68$		
		F	P	F	P	F	P	F	P	
Density Class	1	1.01	0.33	1.18	0.29	1.7	0.21	4.14	0.056	
Pair	19	0.66	0.81	2.21	0.05	0.84	0.65	1.88	0.09	
<i>Mean \pm se</i>										
Dense		75 \pm 5		0.80 \pm 0.04		6.4 \pm 0.3		0.28 \pm 0.03		
Sparse		67 \pm 6		0.74 \pm 0.04		5.8 \pm 0.3		0.38 \pm 0.03		

means (4-10%) and large variances, sample size would nonetheless have to be doubled or tripled to detect significant effects. Total explained variance for these analyses was reasonably large (50%), but this was mostly because of strong "pair" effects, again indicating that our subjective judgment of plant and site similarity was reflected in reproductive success.

Flower color of *H. matronalis* was unrelated to seed set ($F_{1,55} = 0.06$, $P > 0.8$; mean \pm se seeds/fruit = 10.8 ± 0.82 for pink flowers versus 10.4 ± 1.16 for the others), and also was unrelated to proportion fruit set ($F_{1,55} = 0.35$, $P > 0.55$; $\arcsin \sqrt{(\text{proportion fruit set})} = 0.78 \pm 0.06$ for pink flowers versus 0.72 ± 0.08 for other colors).

However, some aspects of floral morphology differed significantly among *H. matronalis* color classes. For two measures, pink flowers were slightly larger than pale flowers (petal length: 11.09 ± 0.26 mm versus 10.44 ± 0.30 mm, $F_{1,53} = 4.5$, $P = 0.03$; petal width: 8.98 ± 0.17 versus 8.36 ± 0.21 mm, $F_{1,53} = 8.42$, $P = 0.006$). However, other aspects of floral morphology (tallest stamen height, and gynoecium length) did not vary significantly among color classes (in both cases $F_{1,53} < 0.1$, $P < 0.5$).

DISCUSSION

We found no evidence for either competition or facilitation of reproductive success for either species with respect to density, regardless of whether the species are natives or invasives. This result is surprising, given that density effects are often strong (Harper 1977; Kunin 1997). Although power analysis reveals that a much larger design would be needed to detect significance for the minor declines with density that we encountered, studies of other species generally reveal much stronger effects (for example Roll and others 1997), for which our design would have been powerful enough to detect significance. Therefore, we are confident that there was no strong effect of density on reproduction for these species in this year.

One possible reason that we did not detect any competition or facilitation is the spatial scale of our density measures (~ 3 m) may have been inappropriate, and that events on smaller (< 3.0 m) or larger scales (10s or 100s of meters) are important. Inappropriate scale seems unlikely to explain the lack of competitive effects, since examination of our results as a function of number of stems within 1.0 m also shows no hint of any effect of density on reproduction for either species

TABLE 3

Effects of absolute density and mean nearest neighbor distance on female reproductive success in *Hesperis matronalis*.

Statistics are for a linear regression of the response variable on the density measures; β = standardized regression coefficient.

Denominator $df = 53-55$. Predictor variables are \ln -transformed. Seeds/fruit refers to plant mean seeds per fruit ignoring flowers that did not produce seeds. Fruit Set refers to $\arcsin \sqrt{(\text{proportion of flowers that became fruit})}$. Total seeds refers to $\ln(1 + \text{total seeds per plant})$.

Trait	# stems within 3 m			Mean nearest neighbor distance		
	β	R^2	P	β	R^2	P
Seeds/fruit	-0.105	0.011	0.43	0.005	0.0001	0.97
Fruit Set	-0.054	0.003	0.69	0.031	0.0010	0.82
Total seeds/plant	-0.008	0.00001	0.95	0.175	0.0307	0.19

TABLE 4

ANOVA of effects of relative density on female reproductive success in *H. matronalis*. (Error df = 25 for proportion fruit set, 23 for other response variables.) Conventions follow Table 3. Boldface indicates statistical significance.

Source	Seed/fruit R ² = 0.74			Fruit Set R ² = 0.70			Total seeds R ² = 0.55		
	df	F	P	df	F	P	df	F	P
Relative Density	1	0.44	0.51	1	0.89	0.35	1	0.71	0.41
Pair	32	2.10	0.03	28	2.1	0.03	32	0.87	0.64
<i>Means + SE</i>									
Dense		10.66 ± 0.82			0.82 ± 0.06			5.41 ± 0.18	
Sparse		11.50 ± 0.86			0.74 ± 0.06			5.65 ± 0.19	

(unpublished analyses). Inappropriate spatial scale also seems unlikely to explain the lack of facilitative effects, since other studies report strong effects at a scale of 1.0-3.0 m (for example Roll and others 1997).

Another possible explanation for why our results do not show facilitation for seed and fruit production involves seed quality, rather than number. Because *P. digitalis* is self-compatible, it is conceivable that an increase in the selfing rate for sparse plants might mask any effects of density on seed number, but cause a corresponding decrease in seed quality because of inbreeding depression. This does not appear to be the case in our study, since germination rates were not significantly affected by density. However, inbreeding depression may not be exhibited until later stages in the life cycle (Husband and Schemske 1996), so this possibility cannot be ruled out. Note that this possibility does not apply to *H. matronalis*, which is self-incompatible.

For *Penstemon digitalis*, we found that fruit and seed predation have important effects on reproductive success, and some indications that this effect is inversely density-dependent, with more damage on sparse plants ($P = 0.056$). This result deserves further study and verification, and may indicate that plant-herbivore, rather than plant-pollinator interactions, are especially important in sparse populations of this species.

For *Hesperis matronalis*, we found that although flower morphology varies significantly among color morphs (by ~7%), there is no difference among morphs in seed or fruit set. Both color morphs are present in most populations (personal observation), and equality of reproductive success for the color morphs is consistent with the hypothesis that the color morphs are selectively neutral, allowing the polymorphism to persist.

Reproductive responses for both species were insensitive to density, and thus do not support the idea that native and invasive plant species might respond differently. Because our study was observational and short term, it would be inappropriate to extrapolate our conclusions to other life stages or species, but under these conditions we found that the native is not especially

susceptible to effects of fragmentation, and the invasive is not especially good at reproduction at low densities.

Overall, our results provide no support to the proposition that reproduction by these two very different species of wildflower is influenced by the density of conspecifics, although such effects are common in other species. It is currently unclear why competition and facilitation do not occur for these species, but an understanding of these unknown factors will be an important component of future work to understand the dynamics of extinction and colonization, and will therefore be important for conservation efforts.

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