

# From horticultural plantings into wild populations: movement of pollen and genes in *Lobelia cardinalis*

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**Abstract** Understanding the potential movement of genes from horticultural plantings into conspecific wild populations requires fundamental knowledge of pollen flow distances and of the siring abilities of genetically differentiated pollen types on local plants. We addressed these issues using *Lobelia cardinalis*, a native, hummingbird-pollinated species, which is available horticulturally both as wild types and cultivated varieties. Potential pollen and gene flow between relatively isolated populations were measured in an artificial array experiment. Potted plants were placed at discrete distances (50, 100, 500, and 1000 m) from either a local or one of three non-local potted pollen sources and scored for pollen and gene flow. Pollen movement was assessed with a dye analogue. The pollen source population did not significantly influence the results, but dye movement, fruit set, and to a lesser degree fruit volume declined with distance from the pollen source. Even at 1 km away from the pollen source, 20–50% of flowers set fruit, indicating substantial gene movement. Siring

ability of four non-local pollen types on local plants was assessed by comparing paternity success when each type comprised 75, 50, and 25% of the pollen in controlled mixed load pollinations. Pollen type affected the percentage of non-local offspring. A cultivated variety of *L. cardinalis* showed poor siring success on the Virginia maternal plants at all mix ratios. Mating was random in mixes with Virginia pollen and pollen from geographically distant wild-type varieties. Finally, pollen from a neighboring county was significantly favored over local sires. These results demonstrate that pollen movement from horticultural plantings into native populations and the production of hybrid seed on native plants is possible in *L. cardinalis*.

**Keywords** Crop-to-wild gene flow · Gene flow · Pollen competition · Pollen flow · Siring ability

## Introduction

The study of gene dispersal from cultivated and engineered crop plants into related wild populations has been vigorously pursued in recent years due to concerns about the escape of advantageous and weedy traits into the wild (Ellstrand et al. 1999). Hybrids resulting from escaped cultivated genes are documented for maize (Doebley 1990), squash (Kirkpatrick and Wilson 1988), radish (Klinger et al.

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1992), sunflower (Whitton et al. 1997; Snow et al. 2003), and grain crops (Arriola and Ellstrand 1996; Song et al. 2003). Particular interest has been paid to the movement of transgenes into wild populations from genetically engineered crops and its consequences for wild populations (Ellstrand 1988; Raybould and Gray 1993; Snow and Palma 1997; Adam 2003; Muth 2003). Studies with large crop populations have shown that the direction of gene flow is most likely to be into wild populations as a result of asymmetric gene flow from dense cultivated plantings to smaller, patchier wild populations (Wilson and Manhart 1993; Papa and Gepts 2003), similar to mainland-island models of population genetics (see Hedrick 1985).

Little attention has been given to the role of gene flow from horticultural plantings into wild conspecific populations (but see Whelan et al. 2006). The dynamics of horticultural plantings differ because of the smaller sizes of the pollen source populations and the types of artificially selected traits found in horticultural varieties. The magnitude of pollen and gene flow among patchy wild populations is not abundantly documented, but may be linked to floral density (Kunin 1993), interspecific competition for pollinators (Campbell 1985), or structural aspects of the flowers themselves (Campbell and Waser 1989). Gene movement over distances from 100 to 1000 m in natural populations has been estimated at less than 20% of fertilizations but is expected to vary with population size and pollinator type (Levin 1981; Ellstrand and Marshall 1985). The smaller size and patchy nature of many native wildflower populations may make them particularly susceptible to the inflow of genes from gardens containing horticultural selections of conspecifics. These movement dynamics likely resemble those of substructured populations or metapopulations in which the garden planting is considered as one of the subpopulations. Metapopulation studies highlight the complexity of gene flow dynamics based on source and target population sizes and the distance between patches (Richards et al. 1999).

Gene movement into wild systems partially depends on the relative fertilization success of cultivated and wild pollen. Pollinators able to simultaneously carry pollen from multiple donors will deliver mixed pollen loads with a single floral visit. Whenever pollen loads exceed the number of ovules

that can be fertilized, sires compete for access to ovules and the resultant paternity of offspring (Mulcahy 1975; Diaz and Macnair 1999; Skogsmyr and Lankinen 1999). The ratio of local to non-local pollen may determine the rate of success of sires. For example, fertilization by interspecific pollen may require attaining a certain threshold presence in the pollen load (Carney et al. 1994; Hauser et al. 1997) or, conversely, small amounts of non-local pollen may disproportionately reduce seed set (Nagy and Rice 1997).

With increased pressure from conservationists to use more native plants in garden and restoration settings, we need a deeper understanding of both the potential for gene escape and its implications for the genetic diversity of the wild populations. The introduction of non-local genes through their use in horticultural plantings results in secondary contact between previously isolated populations. This gene flow may homogenize the genetic composition of populations through the introduction of new alleles or gene complexes reducing any local genetic differentiation (Hufford and Mazer 2003; McKay et al. 2005). In addition, horticultural selection on flowering plants often emphasizes their main ornamental feature—floral display. Traits such as increased floral size or density or length of bloom period may influence the behavior of the animal pollinators, increasing visitation frequency (e.g. Brown et al. 2002), and therefore potential gene movement (although not always, Li et al. 2004).

Addressing pollen movement concurrently with siring ability of different pollen types in mixed loads can inform the probability of introgression from garden plantings into related native populations. Using artificial arrays of a native, hummingbird-pollinated, ornamental species, *Lobelia cardinalis*, as a model for gene flow dynamics we asked: (1) Are distances of 1 km or less sufficient to impede gene movement between small populations? (2) Is the likelihood of movement across those distances affected by whether the pollen originates from local plants, wild-type horticultural offerings, or a highly selected cultivated variety? We then addressed the potential siring ability among populations in a common greenhouse environment asking: (3) Do local and non-local pollen types have equal access to ovules on local plants, leading to a bias for or against local sires? (4) Do all non-local sires perform

similarly or does the origin of the non-local sire matter? (5) Does the ratio of local to non-local pollen applied to the maternal plant influence the pattern of paternity in the offspring?

## Methods

### Study system

Cardinal flower (*Lobelia cardinalis* L., Lobeliaceae) is a hummingbird-pollinated, perennial herb native to North America. It occurs along streams, ponds and in wet meadows from New Brunswick to Florida, west to Ontario, Kansas, and Texas (Britton and Brown 1970; Strausbaugh and Core 1977). Horticultural use of cardinal flower includes wild-type lineages, which have undergone little artificial selection, and highly selected cultivated varieties. For about three to six weeks in late summer, flowers open sequentially from the base and the stigmas remain receptive for several days ( $2.8 \pm 1.3$  SD days, Devlin and Stephenson 1985) until fully pollinated. Pollen is released into a tube formed by the fused anthers, which simplifies both pollen collection and floral emasculation. Although it is predominantly outcrossing and prone to high cumulative inbreeding depression, *L. cardinalis* is self-compatible and can self-fertilize through geitonogamy (Devlin 1986; Johnston 1992). Each capsule can mature up to 500–600 seeds. For many populations, especially more northern ones, the rosettes must be vernalized before they can elongate their reproductive stems, although this ‘bolting’ can be experimentally manipulated by foliar application of gibberellic acid.

*L. cardinalis* is an important nectar source for its primary pollinator in the east, the territorial Ruby-throated hummingbird (*Archilochus colubris*; Bertin 1982; Devlin and Stephenson 1984).

### Experimental design

#### *Pollen and gene flow*

During two consecutive summers, the potential for gene and pollen movement between isolated populations of wild-type and horticultural selections of *L. cardinalis* plants was determined using artificial

arrays at the University of Virginia’s Blandy Experimental Farm (39°03′49″N, 78°03′52″W), in Boyce, VA. A survey found no *L. cardinalis* populations within a two-kilometer radius from the center of the array. Consequently, pollen movement from wild populations was expected to be minimal. The site regularly supports a summer resident population of hummingbirds through other summer-blooming native and non-native nectar sources such as trumpet creeper (*Campsis radicans*) and mimosa (*Albizia julibrissin*).

Pollen and gene movement were measured using both local and non-local populations as pollen sources. Pollen source populations included local Virginia (VA), and wild-type lineages from Wisconsin (WI), and Mississippi (MS). A cultivated variety, ‘Queen Victoria’ (CV), was added the second summer. The plants for the local VA population were propagated by division of over thirty wild plants from Fluvanna County, VA. Plants for WI, MS, and CV were grown from seed acquired from commercial sources that specialize in providing local seed for native plants and represent commonly available horticultural sources (Appendix). Therefore, WI and MS seeds are representative of the nursery location, although the genetic diversity of the seed lots is not known. The WI and MS sources originate approximately 1100 and 1300 km to the northwest and southwest of the study site population, respectively. Seeds were bulk sown and germinated in the greenhouse under mist. While in the greenhouse, plants were maintained in long-day conditions and fertilized every other week with a 10-10-10 general-purpose fertilizer. Previous work with populations from these areas showed that all non-local populations shared some temporal overlap in flowering with the local population (Johnson 1996). However, plants from northern areas flowered earlier than more southern plants. Consequently, we sprayed all plants in early April with 100 mg/l aqueous gibberellic acid to synchronize flowering phenology of the populations for the arrays. A pilot study with 100 plants comprised of each of the parental populations found that populations responded similarly to gibberellic acid (results not shown). After plants were relocated to the study site in June, they received supplemental water but no additional fertilizer.

Pollen flow and realized gene flow via pollen, i.e. pollen movement that results in seed production (Campbell 1991), were evaluated over a range of

spatial scales using an array design. All plants used in the array trials were potted and in flower. Four recipient sites were chosen at each of four distances from the pollen source—50, 100, 500 m, and 1 km. Each site was near a wooded edge or tree to provide opportunities for perching; all sites had an open visual line so plants could be detected at a distance. Trials were conducted singly and sequentially, and all trials took place when native *L. cardinalis* were flowering. In a single trial, approximately eight pollen source plants from a single parental population were centrally placed in a field. Clusters of three to five emasculated local VA plants were placed at one of the sites at each distance from the pollen source to serve as pollen recipients. VA plants serving as pollen donors were genetically different from those used as pollen recipients. Each recipient cluster had approximately 10–20 receptive (female) flowers while the source display was about twice as large with both male- and female-phase flowers present. In wild populations, clusters of plants typically comprise 5–20 individuals (although lone plants are common, personal observation) with individual plants having a mean of 4.6 flowers open per day ( $\pm 2.3$  SD, Devlin and Stephenson 1985). Therefore, both the plants and the flower cluster sizes of recipients in this study were representative of smaller patches within natural populations of this species.

Each source population was tested in at least three replicated trials each summer. The choice of recipient sites at each distance varied among trials to avoid training the pollinators to a particular foraging pattern. Hummingbirds are known to forage without directionality, preferentially visiting clumps of inflorescences that are more numerous, larger, or closer to their present location or territory (Pyke 1981; Johnston 1991a; Wolf and Hainsworth 1991). Consequently, the experimental configuration was designed to encourage visits to the larger pollen source population serving as a “garden” analogue followed by visits to the recipient VA populations that represented small “wild” populations. During each trial, hummingbird feeders were placed at the three recipient sites without plants at each distance to maintain pollinator visitation to all sites throughout the experiment. The goal of the study was to test the efficiency with which birds delivered pollen given the patchwork of alternate nectar sources on the site, not the efficiency with which birds found the array plants.

Female phase flowers were used to assess realized gene flow via pollen. In order to simplify detection of gene flow events, flowers on recipient plants were emasculated as they opened and trials were initiated with all female-phase recipients. This simplification results in some loss of interpretive power for natural populations because in this design local pollen cannot interfere with deposition of the pollen from the central source population. However, the presence of alternate nectar and pollen sources at the site suggests that the pollen movement found here likely approximates natural conditions.

Because conspecific pollen was only available from the array’s source location, any fruit set observed in the recipient clusters was necessarily the result of gene dispersal from the source. Fruit set and fruit volume were recorded for each pollen recipient. The volume of mature fertilized capsules was calculated from the length, width, and depth of each fruit; capsule volume is correlated with seed number for Virginia plants ( $r = 0.750$ ,  $p < 0.001$ ,  $n = 33$ ; unpublished data).

In order to check for pollen arrival in the event that fruit set did not occur, fluorescent dye was used as a pollen analogue. Fluorescent dye provides an indirect measure of pollinator visitation from source to recipient populations (Kearns and Inouye 1993). Each trial lasted for three days. On the morning of the third day of each experimental trial, we applied dye to the exterior of the anther tube of one or two male-phase flowers on each of the source plants. Foraging hummingbirds received a mix of dye particles and pollen on their heads during their visits to the marked flowers, and both dye and pollen adhered to subsequently visited stigmas of *L. cardinalis*. That evening, the trial was terminated and all plants from the recipient populations were brought indoors and examined for the presence of fluorescent dye particles on the stigmas with a hand-held UV lamp. We waited until the last day of each trial to apply the dye to minimize the dye’s interference with pollen deposition.

#### *Siring success*

A second set of plants from each of the populations used for the gene flow study was propagated and maintained in the greenhouse as described above. In addition, a second Virginia population (VA2) was

grown from seed collected from a wild population in Albemarle County, VA (~32 km from the local VA site). As above, synchronous flowering was induced by misting rosettes with 100 mg/l aqueous gibberellic acid.

To assess the consequences of any competitive or exclusionary effects of one pollen type on another, mixed pollen loads were applied to the stigmas of VA plants with known allelotypes. Ten enzyme systems (AAT, EST, FE, G6PDH, LAP, MDH, PGI, PGM, SKDH, TPI) were screened in order to find systems that were polymorphic among populations. Only one system,  $\alpha$ -esterase (EST), showed sufficient variation for use as a paternity marker; all other systems were null, fixed among all populations, or fixed among all wild populations. Esterase shows Mendelian inheritance in crosses between homozygotes and has been used in other paternity studies of *L. cardinalis* (Johnston 1993). Esterase had four alleles: one allele (C) is fixed in the cultivated population, and three others (F, M, S) occurred with different frequency in the other four populations. Non-FF homozygotes were rare in all populations. Consequently, two VA plants homozygous for different esterase alleles (M and S) were vegetatively propagated into 12 plants (3 MM and 9 SS) to serve as maternal parents. As a consequence, maternal plants were not all genetically independent. Plants from MS, WI and VA2 serving as pollen donors were all FF. All cultivar plants had the CC genotype. VA clones of the alternate allelotype were used as “local” sires. Therefore all crosses onto VA maternal plants produced heterozygous offspring. Esterase allelotypes were determined with ground leaf tissue in a lithium-borate discontinuous running buffer system (Werth 1985) and 1.0 M sodium acetate stain buffer.

The effects of two factors, paternal population and local pollen percentage, were investigated through a series of pollinations. Twelve combinations were utilized (3 ratios  $\times$  4 populations). After the stigmas were exerted, previously emasculated flowers on each maternal plant were pollinated with either: 75% VA plus 25% of one of each non-VA type, 50% VA plus 50% of one of each non-VA type, or 25% VA plus 75% of one of each non-VA type. Each combination of pollen ratio and paternal population was replicated once on each maternal plant for a total of 12 replicate pollinations. Pollen was collected and pooled from several individuals within the sires’ population with

the same homozygous allelotype. Pollen ratios were determined by pollen mass, using an analytical balance. All stigmas were saturated with pollen.

After capsules ripened, fruits were collected. Approximately 50 seeds per fruit were bulk sown in soilless mix (Promix BX) and germinated under mist in long-day greenhouse conditions. After germination, at least 30 progeny representing the range of seedling sizes were transplanted into plug trays filled with soilless mix (128 plugs per tray), reared in constant greenhouse conditions, and fertilized bi-weekly. At least 20 seedlings from each cross type for each maternal parent were chosen for paternity analysis via starch gel electrophoresis (12 maternal plants  $\times$  12 pollinations  $\times$  20 offspring = 2880 individuals).

#### Statistical analysis

##### *Pollen and gene flow*

The effects of distance and pollen source population on the percentage of flowers receiving dye and setting fruit and on fruit volume in each array were investigated through analysis of covariance (PROC MIXED, SAS Institute 2003). Percentage pollen receipt and fruit set were calculated for each recipient population and arcsine square root transformed prior to analysis. Year and source population were treated as fixed factors, trial as a random factor, and distance (ln-transformed) was included as a continuous factor. Fruit volume was averaged on each recipient plant for each trial and maternal plant identity was added as a random factor to the analysis.

##### *Siring success*

We used a replicated goodness-of-fit test (Sokal and Rohlf 1981) within each pollen ratio level to investigate the effects of population on the percentage of seeds sired by local and non-local pollen. Each type of pollination was conducted once on each maternal clone, and the number of local to non-local seedlings scored for each treatment for each mother was the unit of replication included in the replicated goodness-of-fit test. This method tests for differences among populations in siring ability ( $G_H$ ), and the

difference between the number of seeds sired and the number expected with a null model based on the percentage of pollen types applied ( $G_P$ ). Additionally, the total  $G$ -value ( $G_T$ ), which is the sum of the  $G_H$  and  $G_P$  values, can be partitioned into the effects contributed by the different paternal populations ( $G_{PART}$ ). This partitioning can elucidate differential siring performance among the non-local populations. Sequential Bonferroni-adjusted  $t$ -tests (not shown) supported the results of the  $G$ -test with the exception of the lowest significant  $G_{PART}$  value.

Because there were a limited number of maternal genotypes and maternal plants may influence seed and offspring characteristics, the contribution of maternal plants to the observed percentage of VA-sired offspring was investigated with an ANOVA. Maternal plant and clone nested within maternal plant were treated as random factors, and paternal population and pollination treatment were fixed factors (PROC GLM, SAS Institute 2003). The percentage of VA-sired offspring was arcsine-square root transformed to meet the assumptions of the model. Clone nested within maternal plant was not significant in the initial analysis ( $p = 0.214$ ) and consequently was removed from the model. The identity of the maternal plant did not contribute to siring success ( $p = 0.311$ ), but paternal population, pollen ratio, and their interaction were statistically significant (all  $p < 0.001$ ). Due to the significant interaction term, we tested for population differences within each

treatment, and results (not presented) did not differ qualitatively from those of the replicated  $G$ -tests.

## Results

### Pollen and gene flow

Analyses of variance of dye flow and gene flow underscore the importance of distance in gene flow dynamics. The movement of dye and the percentage of fertilized fruits decreased significantly with distance (Table 1, Fig. 1). However, neither dye nor gene dispersal was significantly affected by the pollen source.

Volume of fruit depended on the year, pollen source, and distance of the recipients from the source population (year\*distance\*source,  $F_{2,56} = 4.93$ ,  $p < 0.05$ ). Therefore, separate analyses were conducted for each year. In the first year, the effect of distance on fruit volume varied among the pollen sources (Table 2). This is partially due to larger fruits developing closer to the source on the recipient plants during the trials in which VA was the source (Fig. 2). In the second year, fruit volume decreased with distance from the pollen source and varied among pollen donors (Table 2, Fig. 2). However even in this year, fruit volume, a correlate of seed number, was not as strongly associated with distance from the source population as dye receipt or fruit set.

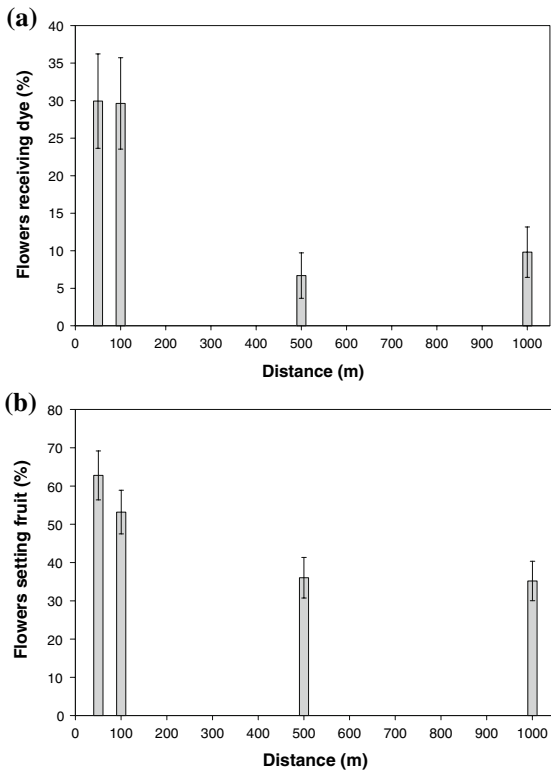
**Table 1** Analysis of variance on percentages of *L. cardinalis* flowers in artificial arrays that received fluorescent dye and that set fruit with pollen from four different sources across four distances over two years

Source of variation	Dye receipt			Fruit set		
	Num df	Den df	<i>F/Z</i>	Num df	Den df	<i>F/Z</i>
Year	1	35	0.01	1	59	5.13*
Distance	1	35	13.47***	1	59	17.60***
Source	3	7	3.27	3	15	3.09
Trial (source)	–	–	0.97	–	–	1.52
Distance × year	1	35	0.29	1	59	2.24
Year × source	2	35	0.29	2	59	2.11
Source × distance	3	35	3.48	3	59	2.38
Distance × year × source	2	35	0.32	2	59	1.22

*Z*-values are given for random factors; *F*-values are given for fixed factors. Numerator and denominator degrees of freedom are given for each

\* $p < 0.05$

\*\*\* $p < 0.001$



**Fig. 1** Mean (SE) percentage of *L. cardinalis* flowers in artificial arrays that received fluorescent dye (a) and set fruit (b) at four distances from the pollen source. Data averaged over two years

Siring success

Populations differed significantly in their siring success, as shown by the replicated goodness-of-fit test ( $G_H$ , Table 3). Pooling across all populations, the offspring paternity ratio did not conform to the null

model expectations in any of the pollination treatments ( $G_P$ , Table 3). However, the direction of paternity bias changed depending on the paternal pollen source (Fig. 3). The most dramatic difference from expectation occurred in all crosses including CV pollen; CV pollen was almost completely unsuccessful in siring ovules even in the treatment where 75% of the pollen applied was CV pollen. In contrast, in all ratios the VA2 pollen was favored over the local pollen to sire seeds. At each ratio level, the majority of the lack of fit is attributable to the performance of VA2 and CV pollen, although in the 50% treatment, WI sired fewer seeds than expected, and the MS population sired a disproportionate number of seed when its pollen was in the minority ( $G_{PART}$ , Table 3). In control crosses with 100% non-local pollen, F1 progeny were formed for all populations.

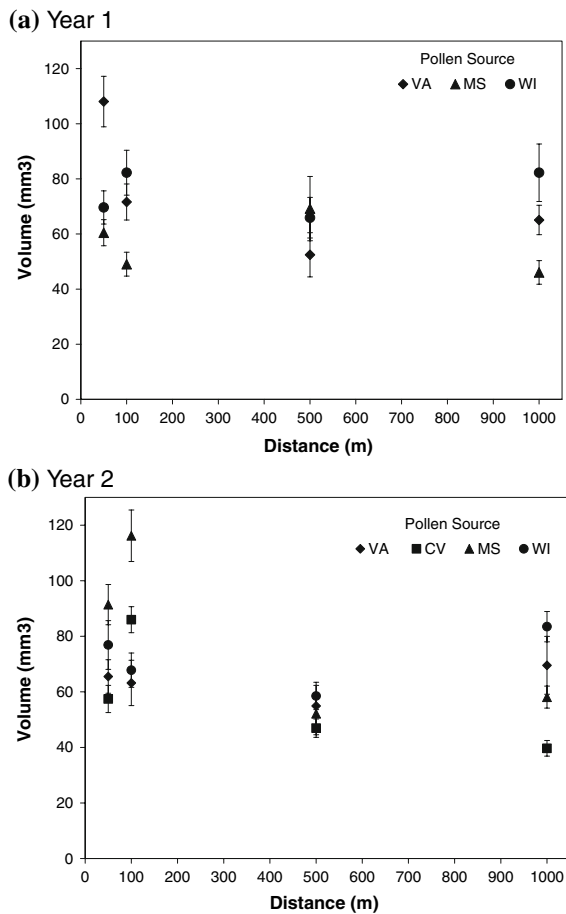
The near failure of the CV pollen in fertilizing ovules in pollen mixes prompted an investigation of the viability of the pollen. We cultured pollen in Brewbaker–Kwack solution (Brewbaker and Kwack 1963) on glass slides for 4 h and found that fewer CV pollen grains germinated than those from other populations, and those that did not germinate were frequently deformed (personal observation). Pollen from a few plants from each population was analyzed with an Elzone particle counter. All grains were approximately the same size (21–24 microns), but the CV flowers showed a bimodal distribution (with the second peak between 14 and 18 microns) that was not seen in the other populations. Consequently, CV pollen contributions may have contained more grains per sample, but potentially fewer of those grains were viable. The CV plants also had fewer pollen grains

**Table 2** Analysis of variance on *L. cardinalis* fruit volume in artificial arrays. Fruit was set with pollen from four different genetic sources and compared across four distances from the source, analyzed separately by year

Source of variation	Year 1			Year 2		
	Num df	Den df	F/Z	Num df	Den df	F/Z
Distance	1	35	0.01	1	10	5.44*
Source	2	7	3.46	3	8	4.08*
Trial (source)	–	–	0.64	–	–	0.66
Plant	–	–	1.02	–	–	0.67
Source × distance	2	35	3.57*	3	10	3.54

Z-values are given for random factors; F-values are given for fixed factors. Numerator and denominator degrees of freedom are given for each

\* $p < 0.05$



**Fig. 2** Mean (SE) volumes of *L. cardinalis* capsules on VA target plants in artificial arrays at four distances from four different pollen sources over two consecutive years

per flower (139,000) compared to VA (246,000), WI (220,000), and MS (302,000).

## Discussion

### Pollen and gene flow

This study explores the potential for pollen from garden plantings to travel a kilometer and fertilize plants in other populations. Pollen from *L. cardinalis* easily traveled that distance between array populations, and the lack of discrimination among three of four pollen sources shown in this study suggests that genes from many horticultural plantings will be able to fertilize plants in nearby wild populations.

The array study, used to model the movement of pollen and genes between relatively isolated populations, revealed that pollinators did not behave differently when offered non-local pollen sources, but that the distance between source and recipient drove the deposition of pollen. The largest percentage of dye was deposited on flowers nearest the pollen source, yet on average the plants 1000 m away still received dye on about 10% of the flowers during the one-day dye trials. Realized gene flow as evidenced by fruit set was also influenced by distance from the pollen source, although even 1000 m away from the pollen source, 20–50% of flowers set fruit. Given this long distance pollen movement, it is possible that fruit set at 1000 m may be from pollen originating outside the array. However, the similar pattern of decline of fruit set and dye deposition at greater distances suggests that fruit set is largely due to pollen from the source population in the array. These pollen and gene movements occurred despite plentiful hummingbird nectar and pollen sources other than *Lobelia* throughout the study site.

The decline in pollen (as assessed by dye movement) and realized gene flow with distance is consistent with many studies of animal-pollinated plants (Smyth and Hamrick 1987; Klinger et al. 1991; Ellstrand 1992) including studies using dye as a pollen analogue (Waser and Price 1982; Waser 1988; Rademaker and DeJong 1998; but see Thomson et al. 1986). Small populations nearest a pollen source often receive the highest amount of pollen deposition and gene immigration (Ellstrand and Marshall 1985; Klinger et al. 1992; Richards et al. 1999). Hummingbird-pollinated plants typically show long distance pollen movement, with regular dispersal beyond 100 m (Linhart 1973; Webb and Bawa 1983; Schulke and Waser 2001). Schulke and Waser (2001) found that pollen movement declined rapidly within the first 50 m from the source, but then declined slowly out to 400 m, a pattern similar to our observation that movement declined by half between 100 and 500 m.

Other plant studies have measured hybridization rates from cultivated plants to wild relatives. Tracking a marker allele from cultivated to wild sunflowers found hybrid offspring in greater than 25% of nearest populations, 10–15% hybrids at 200 m from the source and about 1% at 1000 m (Arias and Rieseberg 1994). Hybridization rates in radish ranged from 14

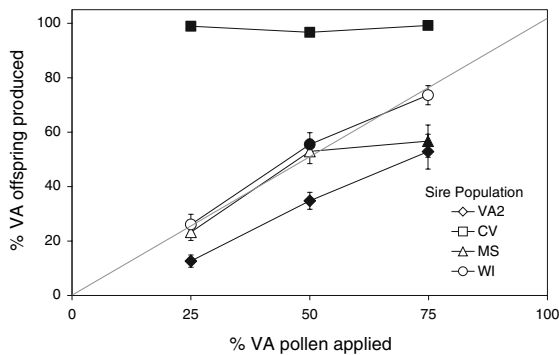
**Table 3** VA pollen was mixed in three different ratios with pollen from four other populations and applied to stigmas of VA plants. Actual paternity of offspring compared with

expected paternity based on ratio of pollen applied was tested with replicated goodness-of-fit tests

% VA	$G_H$	$G_P$	$G_T$	$G_{PART}$	Pop
25	585.68*	92.70*	678.38*	31.05*	VA2
				645.81*	CV
				0.55	MS
				0.97	WI
50	351.87*	40.30*	392.17*	30.55*	VA2
				356.18*	CV
				1.08	MS
				4.36*	WI
75	214.77*	20.74*	235.51*	72.43*	VA2
				116.28*	CV
				46.59*	MS
				0.21	WI

$G_H$  = test for heterogeneity among populations;  $G_P$  = test for difference from expected ratio pooling across populations;  $G_T$  = combination across populations and expected value;  $G_{PART}$  =  $G_T$  partitioned among populations showing contribution of each population to observed differences

\* $p < 0.05$



**Fig. 3** Mean percentage (SE) of VA *L. cardinalis* offspring produced from mixed pollen loads with different percentages of four non-local populations. Diagonal line indicates expected values given no difference in performance of local and non-local pollen on local stigmas. Filled symbols are significantly different from expectations based on partitioned  $G$ -test (see Table 3)

to 100% within 1 m of the pollen source, but hybrids were still detectable 1000 m away (Klinger et al. 1991). While these studies focused on gene flow from large populations, Campbell (1991) found 16% of offspring within the study site of a non-agricultural plant represented gene flow from multiple sires outside the site.

### Siring success

Non-local pollen must be competitive on stigmas containing local pollen grains in order to fertilize plants in natural populations. In this study, non-local populations differed dramatically in siring ability when in competition with local pollen in controlled crosses. In *L. cardinalis*, VA2 pollen was favored at each mix ratio, while CV pollen was strongly rejected as sires on VA plants. However, CV pollen was able to fertilize local ovules when it is the only pollen source, suggesting its poor performance when in competition with local pollen was due to its low viability (possibly associated with introgression during development of the variety, Bowden 1948). In all cases except the CV pollen, there was a positive relationship between the percentage of non-local pollen applied to the stigmas and the percentage of offspring sired (even if the percentage differs from the expected value under the hypothesis of equal fertilization success), suggesting increased pollen flow will result in hybrid seed production. The mechanism for the differential siring success observed in this study is not known; it could be due to either competitive processes among pollen grains (before fertilization) or to relative fitness of the

embryos sired by different pollen sources (after fertilization).

The results, in combination with the geographic distance among populations, suggest that degree of relatedness among populations may have influenced the outcome of the crosses. A previous study with *L. cardinalis* showed no difference in seed production between selfed and outcrossed fruits, but did show inbreeding depression for survival, probability of flowering, and flower number (Johnston 1992). Here VA2, the nearest (~32 km) to the home site of the VA plants, was consistently favored, suggesting early acting inbreeding depression in crosses between individuals from the same population and heterosis in between-population crosses. The siring success of WI and MS crosses did not differ from expectations of random fertilization suggesting a balance between heterosis and outbreeding depression. These results are similar to those found in other studies of interpopulation crosses (Lynch 1991; Galloway and Etterson 2005; Willi and Van Buskirk 2005) in which a bias toward non-local paternity diminishes with increasing genetic and geographic distance.

#### Experimental constraints

The need for a tractable experimental design necessitated several departures from natural conditions: male sterile plants in recipient patches, flowering synchrony among populations in the arrays, and pollen-saturated stigmas in the pollen ratio experiment. In the array study, these experimental constructs enhanced the probability of detecting pollen and gene movement from larger, garden-like patches into the smaller patches designed to mimic wild populations. However, the difference is primarily one of *probability* rather than *possibility* of gene movement; our experimental results indicate that gene movement under these conditions is indeed possible.

Gene flow in the wild is strongly influenced by the proximity and functional gender of neighbors (Kunin 1993). In the arrays, male function of the recipient plants was eliminated in order to permit detection of movement from the source plants. Natural populations would have neighboring males competing with

distant males for access to ovules. Under these conditions, as the number of intervening flowers increases between the source population and the recipient population, the proportion of source population pollen on the pollinator is expected to diminish (Waser and Price 1982). As a consequence of not including pollen-bearing flowers in the recipient populations, the probability of detecting long-distance pollen movement is enhanced in the experimental arrays relative to a natural population. However, populations of *L. cardinalis* are often small and sparsely distributed. Under these conditions of reduced input of conspecific pollen, dispersal may be similar to that predicted under the experimental conditions.

The potential for pollen movement from the focal to the recipient populations in the experimental arrays was likely greater than that from comparable horticultural settings because the phenology of all populations was experimentally synchronized. *L. cardinalis* plants from different regions have slightly different bloom periods when grown in a common environment. In a previous study, northern plants (i.e. WI) grown in a Virginia garden setting had full phenological overlap with field grown local Virginia plants while plants from a southern origin (comparable to MS) had 64% overlap (Johnson 1996). However, in order to randomize the use of populations in experimental arrays we artificially synchronized the flowering phenology of all populations. Without this synchronization, phenological differences between populations would have limited the potential for pollen movement among populations.

In the pollen ratio study, we saturated recipient stigmas with the pollen loads and saturation is likely to lead to competition among pollen grains for available ovules. In a study in two natural populations of *L. cardinalis*, Johnston (1991b) found that plants undergoing natural pollination produced 86% and 48% as many fruits as those receiving supplemental pollination, suggesting that these tested populations were pollen-limited. Under pollen-limited conditions, the likelihood of fertilization will be more similar for all pollen grains. These conditions could enhance fertilization success for pollen like that of CV, which did not fare well under the pollen-saturated conditions in the study.

## Implications

The potential for genes from horticultural plantings to arrive at and introgress into wild populations is a function of numerous ecological factors. This study investigated two of them, the influence of distance in gene movement and siring abilities under competition, through pollination arrays and mixed pollen load crosses in *L. cardinalis*. Despite the constraints imposed by the experimental design, the results show that the distance pollen is carried does not differ between local and non-local pollen sources, and that fruit set is substantial even 1 km away from a pollen source. Pollen source did not influence quantity and had little effect on quality of the fruits formed in the arrays. The low fertilization success of the CV pollen in mixes suggests that ornamental plantings differ in their ability to affect natural populations but that wild-type horticultural varieties, such as those used in restoration plantings, show competent siring ability. These results highlight the importance of considering plant origin and seed provenance in conservation planning. Since pollen flow in natural populations is anticipated at distances greater than 1 km and since plants are readily fertilized by most non-local pollen, even when in competition with local pollen, our results suggest that pollen from garden plants can contribute to hybrid seed formation on wild plants and warrant the investigation of hybrid performance in natural populations.

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## Appendix 1: seed sources and locations

### Seeds for Mississippi population

Barbara Bridges

Southern Perennials & Herbs

Tylertown, MS 39667

(latitude 31°06'55"N, longitude 90°08'10"W)

### Seeds for Wisconsin population

Little Valley Farm

5673 Snead Creek Road

Spring Green, WI 53588

(latitude 43°10'36"N, longitude 90°04'11"W)

### Seeds for CV 'Queen Victoria'

Thompson and Morgan

PO Box 1308

Jackson, NJ 08527-0308

(cultivated variety)

### Seeds for VA2 population

Bentivar

Albemarle County, VA

(latitude 38°06'00"N, longitude 78°25'34"W)

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