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ENVIRONMENTAL EFFECTS ON POLLEN–PISTIL COMPATIBILITY BETWEEN *PHLOX CUSPIDATA* AND *P. DRUMMONDII* (POLEMONIACEAE): IMPLICATIONS FOR HYBRIDIZATION DYNAMICS[1]

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Postpollination mechanisms of reproductive isolation can critically influence the amount of gene flow between hybridizing species. While much evidence exists for genetically based pollen–pistil incompatibility, we show that environmental variation also influences the postpollination performance of heterospecific pollen in the annual *Phlox* hybrid system. Thus, the environmental segregation of species can influence hybridization dynamics. We found that *P. cuspidata* was restricted to soils of low Ca concentrations in the field and performed better under experimentally low Ca; *P. drummondii* was able to inhabit high-Ca soils and sometimes performed better in this environment. To determine whether soil Ca influenced pollen–pistil compatibility in a manner that alters pollen siring success, single-donor pollinations were performed in a completely factorial crossing design between species, maternal Ca environments, and paternal Ca environments. Maternal and paternal environments interacted in their effects on pollen–pistil compatibility for both inter- and intraspecific crosses, such that pollen performance was highest when mothers and fathers were grown in different soil Ca environments. These results suggest that when *Phlox* species predictably inhabit different environments, environmental heterogeneity can impede the processes of speciation and local adaptation by enhancing the performance of pollen dispersed across species and environments.

Key words: local adaptation; maternal growth environment; Polemoniaceae; pollen environmental origin; pollen–pistil compatibility; reproductive isolation; speciation.

Closely related species that produce hybrids less frequently than expected under random mating are wonderful systems for understanding mechanisms that reduce gene flow between species and ultimately for understanding how species integrity is maintained (Lewis, 1953; Harper et al., 1961; Grant, 1966, 1981; McPeek and Wellborn, 1998). Postpollination, but prefertilization, mechanisms of reproductive isolation are especially important in plants because initial mate choice is mediated by potentially unreliable third parties such as pollinators or wind and because postzygotic reproductive isolation is costly. Recent research has identified diverse mechanisms of reproductive isolation between species (Weiblen and Brehm, 1996; Rieseberg and Carney, 1998; Bradshaw and Schemske, 2003; Ramsey et al., 2003; Coyne and Orr, 2004). One question arising from this work is how the action of these mechanisms depends on environmental variation present in natural populations. For example, does the compatibility between individuals of different species depend on the environments in which each developed?

Both the maternal and the paternal environments are capable of influencing hybridization dynamics via their effects on pollen–pistil compatibility. Changes in the maternal growth environment that affect stylar chemical composition can alter pollen performance (Searcy and Macnair, 1990; Marshall and Diggle, 2001) because pollen grains partly rely on stylar environment for tube growth (Labarca and Loewus, 1972, 1973; Sanders and Lord, 1992; Taylor and Hepler, 1997). Pollen performance can also be influenced by the environment in which pollen grains develop (Young and Stanton, 1990; Quesada et al., 1995; Delph et al., 1997; Travers, 1999). When pollen grains produced by plants growing in different environments are deposited together on stigmas, grains derived from the environment that increases the rate of pollen tube growth sire disproportionately more seed. For example, *Cucurbita* pollen had longer tubes and sired disproportionately more seed when produced in high nutrients (Lau and Stephenson, 1993; Jóhannsson et al., 1994) and in cool temperatures (Jóhannsson and Stephenson, 1998). Similarly, pollen grains with faster growing tubes, which were produced by nondefoliated plants, sired more seed in *Alstroemeria aurea* (Aizen and Raffaele, 1998) and *Silene vulgaris* (Delph et al., 1997). Therefore, the environment can affect pollen performance within species. How these environmental effects influence postpollination mechanisms of reproductive isolation between species has not yet been examined.

When different species inhabit different environments, environmental effects are likely to be especially important influences on hybridization dynamics (e.g., Ridgway and McPhail, 1984; Rice and Salt, 1988; Diehl and Bush, 1989; Dodd, 1989; Nagel and Schluter, 1998; Rundle et al., 2000).

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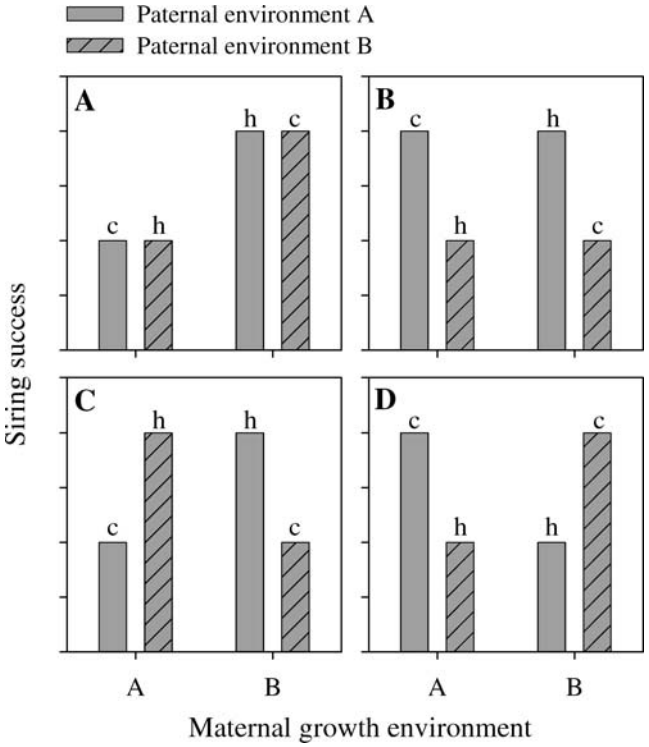

Fig. 1. Four different possible effects of maternal growth environment and pollen environmental origin on relative pollen siring success. The two middle bars in each panel represent heterospecific matings (h), and the two outer bars in each panel represent conspecific matings (c) when hybridizing species reliably inhabit different environments. Consider the following four scenarios: (A) Hybrids are more frequent in maternal plants growing in environment B. (B) Hybrids are more frequent in paternal parents from environment A. (C) Hybrids are more frequent when maternal and paternal parents are from different environments. (D) Hybrids are less frequent when maternal and paternal parents are from different environments. See the introduction for detailed explanations.

First, pollen performance may depend primarily on the maternal growth environment (Fig. 1A), which influences the fertilization success of both conspecific and heterospecific pollen, such that pollen will sire more seeds when dispersed to maternal plants inhabiting a particular environment (i.e., environment B in Fig. 1A). When the two species inhabit different environments, more hybrid seeds will be mothered by the species growing in environment B. However, mothers in both environments A and B will produce the same proportion of hybrid progeny.

Second, pollen performance may depend on the environment in which the pollen developed (Fig. 1B). If pollen grains sire more seed when derived from plants grown in environment A, then more hybrids will be sired by the species that inhabits environment A. When species predictably inhabit different environments, this environmental effect on pollen performance will lead to asymmetrical patterns of hybridization because hybrid progeny will be fathered by the species in environment A and mothered by the species in environment B. The environmental effects described in these two examples (Fig. 1A, B) therefore determine which species is more likely to be the maternal and paternal parent of hybrid offspring. This distinction is important when the fitness of hybrids—and thereby their future contribution to gene flow between species—depends on which species is the mother, as is the case in many hybrid systems (Nagy, 1997; Campbell and Waser, 2001; Coyer et al., 2002; Klips and Culley, 2004; Van der Velde and Bijlsma, 2004; Chapman et al., 2005).

Maternal growth environment and pollen environmental origin can also interact to affect postpollination pollen performance. Such interactions may either decrease or increase the degree of reproductive isolation between species that predictably inhabit different environments. For example, if pollen performance is higher when mothers and fathers are derived from different environments (Fig. 1C), and if different species inhabit different environments, then environmental effects would enhance the performance of heterospecific pollen, thereby decreasing the degree of reproductive isolation between species. Alternatively, the environment would increase the degree of reproductive isolation between species if pollen performance were higher when mothers and fathers are derived from the same environment (Fig. 1D). The outcome of the environmental effects on hybridization dynamics outlined in Fig. 1 will depend strongly on the degree to which different species inhabit different environments and indeed would be important only when the two species are environmentally segregated.

Soil Ca concentration is particularly likely to influence postpollination mechanisms of reproductive isolation. Not only is Ca essential for pollen germination and subsequent pollen tube growth (Brewbaker and Kwack, 1963), it can also influence the direction of tube growth. Pollen tubes of some species grow chemotropically toward higher exogenous Ca concentrations in vitro (Mascarenhas and Machlis, 1962, 1964; Rosen, 1968). This chemotropic response to Ca is applicable to in vivo pollen performance because the concentration of Ca has been shown to increase from the top to the bottom of the pistil (Mascarenhas and Machlis, 1962, 1964; Chichiriccò et al., 2002). Thus, if the concentration of Ca in the soil affects either the Ca gradient within pistils or the Ca concentration in pollen grains, then it may affect pollen–pistil compatibility.

Here we examine the effects of variation in soil Ca concentrations on pollen–pistil compatibility between *Phlox cuspidata* and *P. drummondii* (Polemoniaceae), two hybridizing species that occur within the range of pollen dispersal of each other but which also are spatially segregated to some degree. First, we quantified variation in soil Ca concentrations in natural plant populations, and we determined whether the two species inhabited soils with different Ca concentrations. Next, we tested whether each species was adapted to its native Ca concentration. We then performed single-donor pollinations to determine whether postpollination mechanisms of reproductive isolation are influenced by soil Ca level. Combined, our results reveal whether Ca environmental effects on pollen–pistil compatibility operate so as to increase or decrease the predicted hybridization frequency.

MATERIALS AND METHODS

Phlox hybrid system—*Phlox cuspidata* and *P. drummondii* (Polemoniaceae) are two closely related annual plant species whose ranges overlap in southeastern Texas (Erbe and Turner, 1962). We conducted the study in three sites in Bastrop County, Texas (Lot: 30°05′08″ N, 97°21′30″ W; Pat: 30°04′35″ N, 97°21′26″ W; Vic: 30°05′45″ N, 97°21′13″ W). In each of these sites, *P. cuspidata* and *P. drummondii* occurred within the distance of pollen

dispersal of each other, as indicated by observed interspecific pollinator visitation (L. Ruane, personal observation) and the presence of hybrids. In this region of overlap, these species have distinct vegetative and floral morphologies (Levin, 1970). *Phlox cuspidata* is relatively diminutive in stature with smaller pink flowers and relatively short styles (mean ± SE: 0.66 ± 0.10 mm; L. Ruane, unpublished data). It is self-compatible and has been documented to have high selfing rates in the field (Levin, 1978, 1989a). In contrast, *P. drummondii* is taller and displays larger red flowers with much longer styles (mean ± SE: 1.56 ± 0.06 mm; L. Ruane, unpublished data) and is self-incompatible.

Despite differences in these species' flower colors (Levin and Schaal, 1970; Levin, 1985) and limited pollen dispersal distance (Levin and Kerster, 1974; Levin 1981, 1989b), pollen is dispersed to heterospecific stigmas, as evidenced by interspecific pollinator visitation and the existence of hybrids in natural populations (Erbe and Turner, 1962; Levin, 1967, 1975; Ferguson et al., 1999; L. Ruane, personal observation). The formation of hybrids is encouraged by *P. cuspidata*'s and *P. drummondii*'s coinciding flowering intervals (from early March to late May) and their mutual use of lepidopteran pollinators (Erbe and Turner, 1962; Grant and Grant, 1965; Levin, 1967, 1970, 1975). Hybrids can be identified in the field by their magenta corolla (Levin, 1970).

Within sites, *P. cuspidata* and *P. drummondii* are both well within the distance of pollen dispersal of each other, yet they grow in spatially segregated patches (D. Levin, University of Texas at Austin, personal communication; L. Ruane, personal observation). Hybrids, which were found in all our study sites, typically occur sporadically without an obvious preference for *P. cuspidata* or *P. drummondii* patches (L. Ruane, personal observation). The degree to which *P. cuspidata*'s patch and *P. drummondii*'s patch were separated from each other varied across sites. Of the three sites we examined, the two species were separated by only 10 to 25 m in two of the sites (Lot and Vic) and by approximately 50 m in the third site (Pat). Seeds are ballistically dispersed and typically travel less than 3 m from the maternal plant. Thus, both seed dispersal and environmental factors may contribute to the spatial segregation in this hybrid system.

Edaphic variation in natural populations—To identify and quantify edaphic characteristics that differed between the locations of these two species, we collected soil inhabited by *P. cuspidata* and by *P. drummondii* within each of the three sites in 2003 and 2004. In 2003, soil samples were collected along a 16-m linear transect through the middle of each species' distribution within three sites where these species co-occur (Lot, Pat, and Vic). Along each transect, 0.5 L of soil was collected every 4 m. These five samples were then mixed to yield a composite sample from that site. Four composite samples were collected to serve as replicates.

To obtain a better estimate of the scale of variation within each species' distribution, a different soil sampling technique was used the following year. In 2004, soil samples were collected every meter for a total of 10 m through the middle of each species' distribution within three sites (Bob: 30°07′00″ N, 97°18′34″ W, Pat, and Vic). Urban development prevented sampling of the Lot site in 2004. Each soil sample was analyzed separately for NO_3, P, K, Ca, Mg, Na, S, pH, and conductivity by the Soil, Water and Forage Testing Laboratory at Texas A&M University in College Station, Texas, USA.

Local adaptation to native Ca concentrations—Because *P. cuspidata* and *P. drummondii* were sometimes nonrandomly distributed with respect to soil Ca level (an environmental factor that is likely to affect postpollination pollen performance), we recorded a component of fitness of both species raised under low and high soil Ca levels to determine whether they were locally adapted to their native Ca environments. Seeds of each species were collected from three sites (Lot, Pat, and Vic) in 2003 and germinated at 12°C in a dark growth chamber (Harris Environmental Systems, Andover, Massachusetts, USA). All 432 plants (2 species × 3 sites × 2 environments × 36 replicates) were grown in nutrient-lacking perlite to ensure that plants did not receive supplemental Ca from their soil medium. As soon as radicles had emerged, the photoperiod was gradually increased to 12 h a day, and the temperature was gradually increased to 22°C. Calcium treatments were initiated approximately 6 wk after germination, when the second set of true leaves was fully expanded, and were continued throughout the experiment.

Low (200 ppm) and high (1000 ppm) soil Ca levels were applied by saturating pots with 15–20 mL of diluted calcium carbonate (Liquid Lime, Aggrene, Superior, Wisconsin, USA) twice a week. These Ca levels were chosen to best approximate low and high levels of Ca in the sites from which the species were derived in 2003 (see Results). While these levels span the range of soil Ca concentrations in the field, neither concentration is toxic (Nixon, 1964). On nontreatment days, all plants were either watered with deionized water or fertilized with 25% Hoagland's solution modified such that sodium nitrate replaced calcium nitrate (Hoagland and Arnon, 1938; Nixon, 1964). This modification, which eliminates Ca while maintaining an adequate nitrogen concentration, increases the concentration of sodium to approximately 100 ppm for plants in both treatments. This increase in sodium is unlikely to be toxic because these populations were derived from soils that had close to 200 ppm sodium (Appendix S1, see Supplemental Data accompanying online version of this article). After 4.5 mo of growth, plants were harvested. The sum of reproductive structures (number of fruits + flowers + developed buds) was used as an estimate of performance rather than the number of seeds because hand pollinations were necessary to set fruits in the self-incompatible *P. drummondii*.

Environmental effects on in vivo pollen performance—When plants in the different Ca treatments began to flower (approximately 10 wk after germination), experimental hand pollinations were begun to determine the effects of maternal growth environment and pollen environmental origin on postpollination pollen performance. Pollinations were conducted between plants derived from the same site; pollinations between individuals derived from different sites were not performed. Crosses were fully factorial with respect to species, paternal environment, and maternal environment, such that conspecific and heterospecific pollinations were conducted for each combination of maternal and paternal Ca environments. Pollinations were performed by emasculating freshly opened flowers and dusting dehisced pollen grains from a single donor over the entire surface of a receptive stigma. The number of pollen grains transferred to each stigma was estimated to range from 200 to 400. This excess amount of pollen (200–400 grains for every three ovules) ensured that incomplete seed set was not due to an insufficient number of pollen grains. A subset of flowers from each species, which were emasculated but not pollinated, failed to set seed, indicating that emasculation effectively prevented self-fertilization.

To estimate fertilization success at different times after pollination, each pollination was performed twice daily, and styles were harvested 8 and 10 h after pollen was placed on the stigma. Eight and 10 h were chosen on the basis of preliminary trials, which indicated that these times were long enough for pollen germination and even ovule fertilization. To minimize the effects of factors other than time on pollen performance, the same maternal and paternal genotypes were used for pollinations performed on the same day that differed only in the amount of time that pollen was in contact with the pistil. Thus, each individual participated in a minimum of two pollinations. Overall, 84% of both pollen recipients and pollen donors were used in six or fewer pollinations, including both harvest times.

One complete replicate of pollinations (2 maternal species × 2 paternal species × 2 maternal growth environments × 2 pollen environmental origins × 2 harvest times = 32 pollinations) was performed for 12 different plants from each of the three sites, for a total of 36 replicates and 1152 pollinations. One complete replicate was performed each morning between 0700 and 1030 h over a period of 36 d.

To determine the effects of maternal growth environment and pollen environmental origin on in vivo pollen performance, styles were harvested 8 or 10 h after a pollination and fixed in 70% ethanol, which prevents further pollen germination and tube growth (Kearns and Inouye, 1993). Each style was then stained for viewing pollen tubes with fluorescence microscopy (Pittman and Levin, 1989; Bixby and Levin, 1996; Plitmann and Levin, 1996). Styles were placed in 8 N NaOH for 13–16 h, washed with deionized water, placed in Tris-glycine buffer (pH 8.3) for 1.5 h, then in 0.005% toluidine blue (w/v in deionized water) for 15 min, and finally in decolorized aniline blue (0.1% aniline blue in 0.1 M K_3PO_4) for 23 h. Stained styles were placed on slides, and in vivo pollen performance was assessed by quantifying pollen germination and pollen tube growth. The total number of pollen grains on each stigma was recorded. Because of the abundance of pollen grains on the stigma, it was impossible to count the total number of pollen grains that had germinated on each stigma. Instead, pollen germination success was assessed by calculating the proportion of stigmas on which one or more pollen grain(s) had germinated. Pollen tube growth was estimated by calculating the proportion of pollen grains deposited on the stigma whose tubes had reached the base of the style (number of pollen tubes at stylar base/number of pollen grains on stigma). We were also able to assess pollen fertilization success because ovules developed normally when styles were harvested just above the ovary (Levin, 1985). Three-week-old fruits, which developed after styles were harvested 10 h after pollen deposition,

TABLE 1. Analysis of variance (A) of the level of Ca (ppm) in soils inhabited by *Phlox cuspidata* and *P. drummondii* in 2003 and 2004 in three sites in Bastrop County, TX, USA and (B) of the fitness of *P. cuspidata* and *P. drummondii* when grown in low (200 ppm) and high (1000 ppm) soil Ca in the growth chamber.

(A) Soil Ca level	2003			2004		
Source	df	MS	*F*	df	MS	*F*
Site	2, 18	3 273 556	60.7343**	2, 54	16 743 481	95.3748**
Species	1, 18	970 589	18.0074**	1, 54	7 742 987	44.1059**
Site × species	2, 18	425 803	7.8999**	2, 54	9 274 781	52.8313**

(B) Fitness			
Source	df	MS	*F*
Site	2, 292	1179	2.4608*
Species	1, 292	763	1.5924
Ca	1, 292	788	1.6457
Site × species	2, 292	2465	5.1462**
Site × Ca	2, 292	499	1.0410
Species × Ca	1, 292	9913	20.6952**
Site × species × Ca	2, 292	1100	2.2972

Note: Fitness represents the sum of reproductive structures (number of fruits + flowers + developed buds). MS = mean square. *$P < 0.1$, **$P < 0.05$.

were collected to determine the proportion of fruit set, the number of seeds sired, and the number of seeds aborted.

Analyses—All analyses were performed using the computer program JMP, version 4.0 (SAS Institute Inc., Cary, NC, USA). Two-way ANOVAs were performed to determine whether *P. cuspidata* and *P. drummondii* inhabited soils containing different Ca concentrations in 2003 or 2004. Site (Lot, Pat, and Vic in 2003; Bob, Pat, and Vic in 2004) and species (*P. cuspidata* and *P. drummondii*) were the main effects. We were not able to make comparisons between years because the Soil, Water and Forage Testing Laboratory slightly modified their extraction protocol during that time.

A three-way ANOVA was performed to determine whether *P. cuspidata* and *P. drummondii* individuals were locally adapted to their native Ca environment. Site (Lot, Pat, and Vic), species (*P. cuspidata* and *P. drummondii*), and Ca environment (low and high) were the main effects, and our estimate of fitness—total number of reproductive structures—was the dependent variable. A significant species × Ca interaction would indicate that the effect of soil Ca environment on fitness depended on the plant species. Local adaptation would be apparent if a species had higher reproduction when grown in the Ca environment that it inhabited in the field. Differences between treatment means were analyzed using a priori contrasts to determine the environment in which each species had higher fitness.

Multi-way ANOVAs were performed to determine the effects of maternal growth environment and pollen environmental origin on seven postpollination pollen performance traits. These traits represent multiple stages of reproduction after pollen grains reach the stigma. They include the proportion of stigmas with pollen germination 8 and 10 h after pollination, the proportion of pollen tubes at the base of the style 8 and 10 h after pollination, the proportion of fruit set, the number of seeds sired, and the number of fertilized ovules aborted. The proportion of pollen tubes that had reached the base of the style 8 and 10 h after pollination was arc-sine square-root transformed. In the ANOVA, site (Lot, Pat, and Vic), cross type (*P. cuspidata* × *P. cuspidata*, *P. cuspidata* × *P. drummondii*, *P. drummondii* × *P. drummondii*, and *P. drummondii* × *P. cuspidata*), maternal growth environment (low and high Ca), and pollen environmental origin (low and high Ca) were the main effects. We tested for interactions between maternal growth environment and pollen environmental origin to determine whether the effect of maternal growth environment on pollen performance depended on the environment in which pollen developed. We also tested for interactions between cross type and environment (maternal growth environment and pollen environmental origin) to determine whether environmental effects on pollen performance were independent of maternal and paternal species.

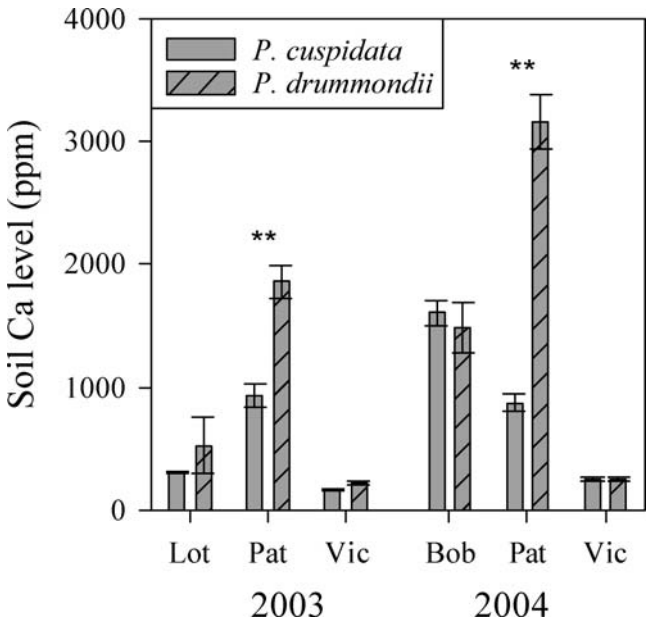

Fig. 2. Mean concentration of Ca in soils inhabited by *Phlox cuspidata* and *P. drummondii* in Lot, Pat, and Vic sites in April of 2003 and 2004. Standard errors are given. Asterisks indicate significant differences between soils inhabited by each species within sites (**$P < 0.01$). Overall, soils inhabited by *P. cuspidata* had significantly lower concentrations of Ca compared with the soils inhabited by *P. drummondii*; however, this difference was only significant in one site (Pat) each year.

RESULTS

Edaphic variation in natural populations—*Phlox cuspidata* and *P. drummondii* were nonrandomly distributed with respect to several soil characters other than Ca. Soils inhabited by *P. cuspidata* had lower potassium concentrations and lower pH in both 2003 and 2004, lower phosphorous concentrations in 2004, and lower magnesium concentrations in 2003 compared with soils inhabited by *P. drummondii* (Appendix S1, see Supplemental Data with online version of this article). Although several characters differed between soils inhabited by *P. cuspidata* and *P. drummondii*, our main focus is on soil Ca because of its likely effect on pollen–pistil compatibility.

On average, *P. drummondii* inhabited soils of significantly higher Ca concentrations compared with the concentration in soils inhabited by *P. cuspidata* (Table 1A; 86% higher in 2003 and 79% higher in 2004 when data were pooled across sites), but the magnitude of the difference in soil Ca concentration inhabited by the two species varied across sites (Fig. 2). In both 2003 and 2004, the concentration of Ca in soils inhabited by *P. drummondii* was only significantly different in Pat (Fig. 2), where the highest Ca concentration occurred for this species.

Local adaptation to native Ca concentrations—We found evidence of local adaptation to soil Ca level. Specifically, when pooled over all sites, low soil Ca was associated with 27% higher fitness of *P. cuspidata* (which inhabits only soils with lower Ca), but with 17% lower fitness of *P. drummondii,* compared with their fitness in the high-Ca treatment (Fig. 3). The difference in response of the two species to soil Ca level was significant, as indicated by a significant interaction

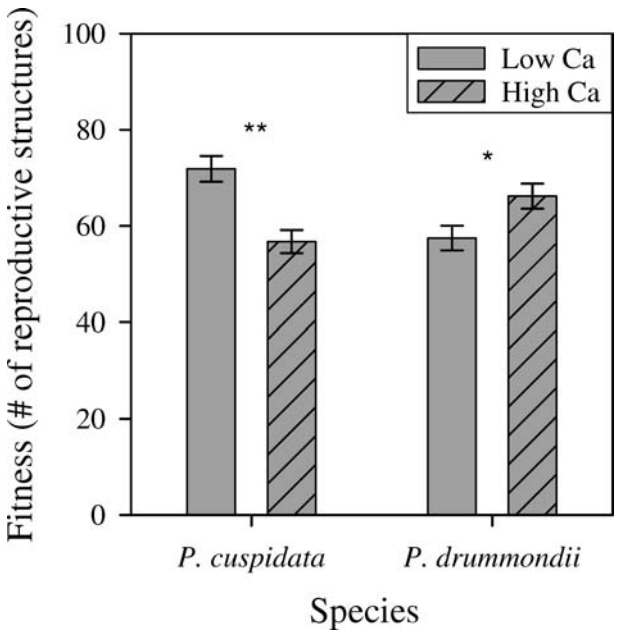

Fig. 3. Means and standard errors of fitness (sum of reproductive structures: number of fruits + flowers + developed buds) of *Phlox cuspidata* and *P. drummondii* individuals grown in low (200 ppm) and high (1000 ppm) soil Ca. Asterisks indicate significant differences between treatment means: $*P < 0.05$, $**P < 0.01$.

between species and Ca (Table 1B). *Phlox cuspidata* from all three populations had significantly higher fitness when grown in low Ca. However, only *P. drummondii* from one population (Vic) produced more reproductive structures on high Ca. In a different experiment, however, we found that individuals in other *P. drummondii* populations may also be locally adapted to high Ca. Specifically, *P. drummondii* individuals derived from Pat—the population that inhabited the highest Ca levels—produced significantly more seeds per fruit when grown in high compared with low soil Ca (mean ± SE: 2.73 ± 0.11 vs. 2.28 ± 0.15, respectively; L. Ruane, Harvard University, unpublished data).

Environmental effects on in vivo pollen performance—Cross type significantly influenced the majority of postpollination pollen performance traits that we measured (Table 2). *Phlox drummondii* pollen outperformed *P. cuspidata* pollen in pistils of both maternal species. Compared with *P. cuspidata* pollen, *P. drummondii* pollen sired more seeds per fruit in *P. cuspidata* pistils (mean ± SE: 1.72 ± 0.11 vs. 1.20 ± 0.11, respectively) and in *P. drummondii* pistils (1.34 ± 0.11 vs. 0.20 ± 0.05). The effects of maternal and paternal environment on pollen–pistil compatibility were similar for all four crosses, as indicated by the nonsignificant interaction between the environmental factors and cross type for all pollen performance traits (Table 2). Thus, we examined the effects of soil Ca environment on pollen–pistil compatibility across all cross types.

Pollen–pistil compatibility was significantly affected by both the environment in which pollen was produced and the growth environment of the maternal plant (Table 2, Fig. 4). Pollen environmental origin affected early stages of pollen performance—including pollen germination and tube growth. Pollen produced in low soil Ca had higher germination (at 8 and 10 h) and a greater proportion of tubes at the base of the style (8 h) (Table 2, Fig. 4). The growth environment of the maternal plant, however, had more pronounced effects at later stages in the reproductive process and ultimately determined pollen siring success. Specifically, pollen had higher fertilization success—set more fruit, sired more seeds, and had fewer aborted seeds—when mothers were grown in high Ca (Table 2, Fig. 4).

Importantly, maternal growth environment and pollen environmental origin interacted to affect four postpollination pollen performance traits. The effect of the maternal growth environment depended on the environment in which pollen developed for pollen germination (at 10 h), proportion of tubes at stylar base (10 h), proportion of fruit set, and number of seeds sired (Table 2). For these traits, pollen grains had higher performance when mothers and fathers were from different Ca

TABLE 2. Analysis of variance of seven postpollination pollen performance traits, which were measured following conspecific (*Phlox cuspidata* × *P. cuspidata* and *P. drummondii* × *P. drummondii*) and heterospecific (*P. cuspidata* × *P. drummondii* and *P. drummondii* × *P. cuspidata*) pollinations between individuals grown in low (200 ppm) and high (1000 ppm) soil Ca. *F* values are presented.

Trait	df	Site	Cross	ME	PE	ME × PE	Cross × ME	Cross × PE	Cross × ME × PE
Pollen germ. (8 h)[a]	548	1.3271	6.0131**	0.0587	2.7292*	0.2702	0.7518	0.8063	0.8556
Pollen germ. (10 h)[a]	539	0.8620	4.4459**	1.0468	3.6304*	7.8781**	0.5089	0.0747	2.5985*
Prop. tubes at base (8 h)[a]	539	1.4179	16.3730**	1.8784	4.2952**	0.5673	1.8837	0.3181	1.3453
Prop. tubes at base (10 h)[a]	533	2.9996*	13.0909**	2.6622	0.0342	4.3661**	0.6646	1.1073	0.6113
Prop. fruit set	553	0.1979	49.0166**	15.0129**	1.1770	4.3827**	0.3909	0.8289	0.2230
No. seeds sired per fruit	551	0.1609	44.9963**	15.8839**	0.4935	2.7717*	1.5112	0.9565	0.0581
No. seeds aborted per fruit	257	0.2764	1.3022	6.2186**	0.3059	0.2325	0.5430	1.4173	1.3121

Note: Site, cross type (cross), maternal growth environment (ME), and pollen environmental origin (PE) were the main effects. Maternal growth environment × pollen environmental origin interaction (ME × PE) was included to determine whether the effect of maternal growth environment on in vivo pollen performance depended on the environment in which pollen developed. The cross type × maternal growth environment and cross type × pollen environmental origin interactions (cross × ME and cross × PE) were included to determine whether environmental effects on pollen performance were consistent across all cross types. $*P < 0.1$, $**P < 0.05$.

[a] Pollen germination (pollen germ.) and the proportion of tubes at the base of the style (prop. tubes at base) were measured 8 and 10 h after hand pollination. Pollen germination indicates the proportion of stigmas on which one or more pollen grain(s) had germinated. The proportion of tubes at the base of the style was calculated by dividing the number of pollen tubes at the stylar base by the number of pollen grains on the stigma.

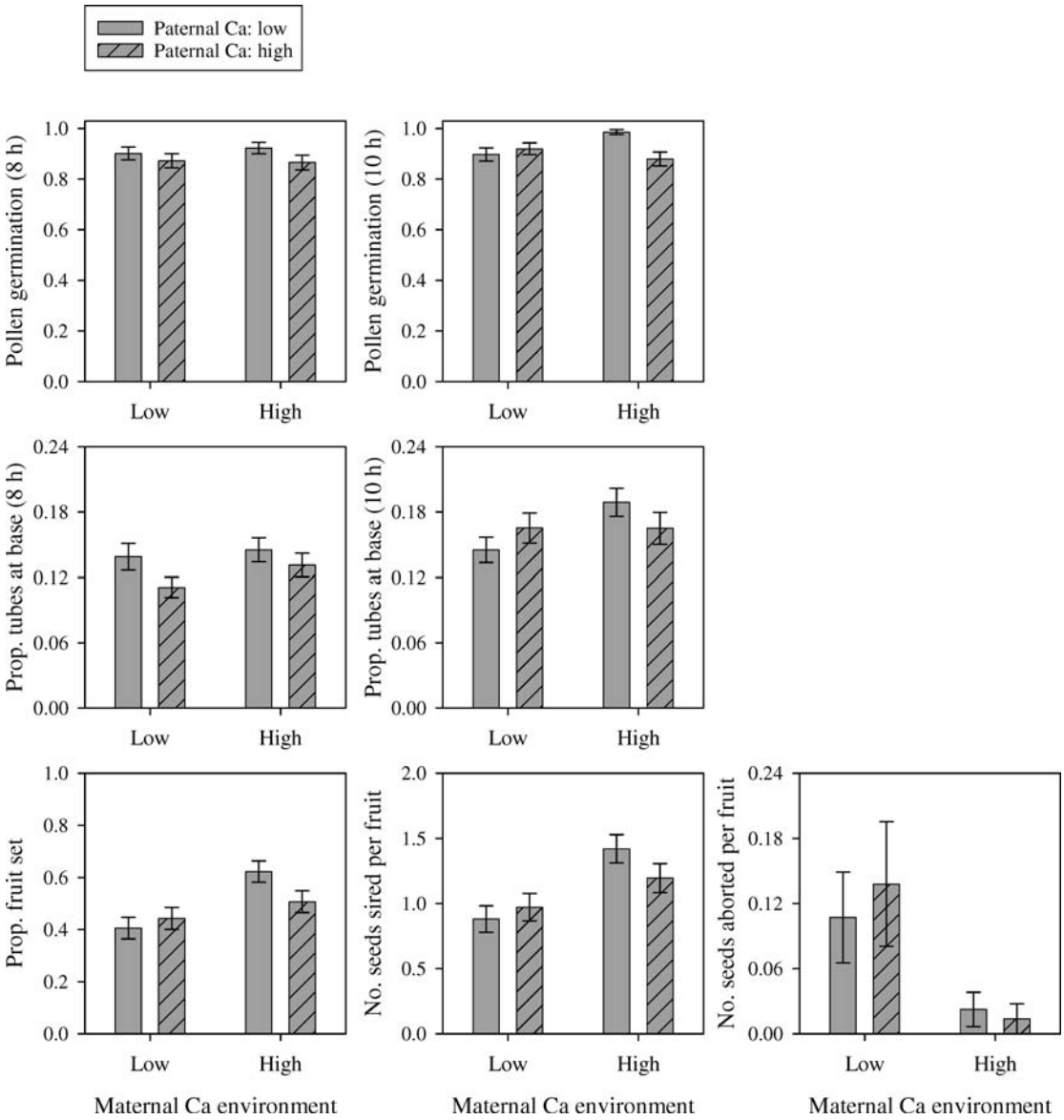

Fig. 4. Means of seven postpollination pollen performance traits, which were measured after conspecific (*Phlox cuspidata* × *P. cuspidata* and *P. drummondii* × *P. drummondii*) and heterospecific (*P. cuspidata* × *P. drummondii* and *P. drummondii* × *P. cuspidata*) pollinations between individuals grown in low (200 ppm) and high (1000 ppm) soil Ca. Pollen germination and the proportion of tubes at the base of the style were measured 8 and 10 h after hand pollination. Pollen germination indicates the proportion of stigmas on which one or more pollen grain(s) had germinated. The proportion of tubes at the base of the style was calculated by dividing the number of pollen tubes at the stylar base by the number of pollen grains on the stigma. Means were pooled over the cross type, and bars indicate SEs. Significant differences are indicated in Table 2.

environments and specifically when pollen came from low Ca but maternal plants were grown in high Ca (Fig. 4).

DISCUSSION

We found that naturally occurring variation in soil Ca level is capable of influencing hybridization dynamics in the annual *Phlox* hybrid system via its effects on pollen–pistil compatibility. First, the two species were nonrandomly distributed across soils with different Ca concentrations in one of our study sites, with *P. drummondii* capable of inhabiting soils with very high Ca concentrations and *P. cuspidata* being more restricted to areas of low Ca. Adaptation of *P. cuspidata* to low Ca and the lower relative performance of *P. drummondii* in low Ca would preserve, or even augment, this nonrandom association over time. Second, environmental effects on pollen–pistil compatibility operated such that pollen that developed in low-Ca environments had higher fertilization success on stigmas that developed in high-Ca environments. Thus, given the environmental associations of the two species, the observed environmental effects would enhance the performance of *P. cuspidata* pollen on stigmas of *P. drummondii* and thereby facilitate hybrid production.

The mechanism by which soil Ca level affected postpollination pollen performance may be explained by the incorpo-

ration of Ca from the soil into pistils and/or pollen grains (e.g., Searcy and Macnair, 1990). The concentration of Ca in pistils and pollen is likely to influence in vivo pollen performance because of the prominent role of Ca in the mechanics of pollen germination and tube growth (Mascarenhas and Machlis, 1962, 1964; Brewbaker and Kwack, 1963; Rosen, 1968; Chichiriccò et al., 2002). Thus, the increased proportion of fruit set and number of seeds sired by pollen in pistils of maternal plants grown in high Ca may be attributed to the incorporation of more Ca into the pistils of maternal plants growing in soils with higher Ca levels.

The consequences of interactions between maternal growth environment and pollen environmental origin on hybridization dynamics will depend strongly on the degree to which species are nonrandomly distributed with respect to the relevant environmental factor. We do not know the extent to which the species are environmentally segregated outside of our study sites, but in Pat—where *P. cuspidata* inhabited soils of significantly lower Ca concentrations, and *P. drummondii* inhabited soils with very high Ca concentrations—one would predict that *P. cuspidata* pollen from low Ca would perform well in *P. drummondii* pistils in high Ca. In this manner, the environmental effects would operate to facilitate hybridization in this site. However, in locations where the species were not strongly associated with different Ca environments, environmental effects are not expected to alter hybridization dynamics because pollen from low-Ca areas and pistils in high-Ca areas are equally likely to be either species.

Data from our experimental pollinations predict that environmental variation would enhance hybridization in Pat (the site with the most environmental segregation) but not in Lot and Vic (the sites with relatively little environmental segregation). However, there was a similar frequency of hybrids across all sites (approximately 0.5% in all sites; L. Ruane, Harvard University, personal observation). The two species were separated by a greater distance in Pat (50 m) compared with Lot and Vic (10–25 m), which would make hybridization less likely in Pat. While many other factors doubtlessly contribute to variation in the frequency of hybrids among sites, our experimental pollinations indicate that Ca heterogeneity could also be important in sites where species are associated with different Ca environments. The strong association of each species with a different Ca environment in Pat may cause the hybridization frequency in that site to be higher than expected based solely on the distance between each species' patch.

Effects of interactions between maternal and paternal environments on fitness components (Galloway, 2001a, b) can have interesting consequences for processes of local adaptation within a species as well. Because the environmental effects we observed on pollen–pistil compatibility were consistent across all cross types (i.e., conspecific and heterospecific), they are expected to increase the success of matings between conspecific individuals growing in different environments. Environmental effects such as those seen in this study have the potential to impede the processes of local adaptation of each of these species to their own local Ca environment. Thus, environmental effects on pollen–pistil compatibility can influence processes of local adaptation.

The influence of the environmental effects that we observed on hybridization frequency would also depend on heterospecific pollen competition. In the *Phlox* hybrid system, we observed that one species—*P. drummondii*—performed better within the pistils of both species. This is likely to be because *P. drummondii* has longer styles than *P. cuspidata*, and the pollen is correspondingly more efficient at growing long distances quickly (Kiang and Hamrick, 1978; Brandvain and Haig, 2005). Therefore, in this case, while the association of *P. cuspidata* with low Ca (that is, the performance-enhancing environment for pollen) would increase its chance of siring seeds on *P. drummondii* seed parents, it may not ensure it. Moreover, pollen from one species can introduce allelochemicals that hinder the germination, tube growth, and fertilization success of the other species' pollen (Kanchan and Chandra, 1980; Murphy and Aarssen, 1989, 1995). If the performance of heterospecific pollen is affected by the presence of conspecific pollen, then environmental effects on hybridization dynamics will change when heterospecific and conspecific pollen grains are forced to compete for ovule fertilization.

The timing of pollen deposition will also be important for determining the performance of heterospecific pollen. While the association of *P. cuspidata* seed parents with low Ca would enhance the performance of *P. drummondii* pollen from high-Ca locations, whether *P. drummondii* actually sires *P. cuspidata* seeds will depend on the timing of pollen deposition by *P. cuspidata* compared with that of *P. drummondii*. Heterospecific pollen—regardless of its environmental origin—may only be capable of siring seed when it reaches the stigma before conspecific pollen (Spira et al., 1996; Snow et al., 2000). With self-compatible species such as *P. cuspidata*, this is likely to be an important issue. Experiments are currently underway to investigate how environmental effects on pollen–pistil compatibility are affected by interspecific pollen competition and by the relative timing of heterospecific pollen deposition.

In conclusion, environmental effects on pollen–pistil compatibility can influence hybridization dynamics between species that inhabit different environments. In the annual *Phlox* hybrid system, pollen grains and pistils were generally more compatible when mothers and fathers were from different Ca environments. These observed effects of the soil Ca environment on pollen–pistil compatibility suggest that this environmental factor could increase the number of hybrids produced in sites where *P. cuspidata* and *P. drummondii* inhabit soils of different Ca concentrations. In addition to facilitating hybridization between species, the increased compatibility of pollen grains and pistils from different Ca environments can also impede the process of local adaptation within species by enhancing the performance of pollen dispersed across environments. Thus, environmental effects on pollen–pistil compatibility can influence fundamental ecological processes of hybridization and local adaptation.

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