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Pollen competition and environmental effects on hybridization dynamics between *Phlox drummondii* and *Phlox cuspidata*

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Abstract Pollen competition between species strongly influences hybridization dynamics in plants. By performing single- and mixed-donor pollinations, we show that soil Ca alters the outcome of interspecific pollen competition in the annual *Phlox* hybrid system of *Phlox cuspidata* and *P. drummondii*. In the absence of interspecific pollen competition, heterospecific pollen siring success of both species was influenced most strongly by the maternal growth environment, such that hybridization was facilitated when heterospecific pollen was deposited on stigmas of maternal plants growing in high Ca soils. When heterospecific pollen was forced to compete against conspecific pollen, however, the maternal growth environment did not influence hybridization, but the environmental origin of heterospecific pollen did, and this effect depended on the maternal species. Pollen of *P. drummondii* was more effective at outcompeting *P. cuspidata* pollen and preventing hybridization in *P. drummondii* dams when *P. cuspidata* pollen was derived from low Ca. Pollen competition within pistils of *P. cuspidata* was unaffected by pollen Ca environment. In situations in which *P. cuspidata* grows in lower soil Ca than *P. drummondii*, as has been documented in one population, these results suggest that the competitive ability of heterospecific pollen will be diminished by environmental effects of soil Ca. Thus, the environment in which pollen develops can influence interspecific pollen competition and hybridization frequency.

Keywords Hybridization · Interspecific pollen competition · Population differentiation · Post-pollination pollen performance · Reproductive isolation · Soil calcium environment

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Introduction

Mechanisms of reproductive isolation reduce the occurrence or success of heterospecific gametes, resulting in the production of fewer hybrid offspring than expected under random mating (Rieseberg and Carney 1998; Bradshaw and Schemske 2003; Ramsey et al. 2003; Coyne and Orr 2004). By identifying a diversity of isolating mechanisms responsible for decreased gene flow between species, past research has helped us understand how closely related species maintain separate identities in the absence of geographical isolation. The efficiency with which these mechanisms maintain species integrity, however, depends on biotic and abiotic variation that pervades natural systems (Ruane and Donohue 2007). Here, we examine the effects of edaphic variation on pollen siring success within the context of interspecific pollen competition.

While many studies have documented environmental effects on post-pollination pollen performance within species (Young and Stanton 1990; Quesada et al. 1995; Travers 1999; Marshall and Diggle 2001), the effects of environmental variation on pollen–pistil compatibility between two different species have only recently been explored. In a recent study of the annual *Phlox* hybrid system, we found that, in the absence of interspecific pollen competition, edaphic variation in soil Ca influenced in vivo performance of both conspecific and heterospecific pollen (Ruane and Donohue 2007). While traits expressed early after pollen deposition (i.e. pollen germination and tube growth rate) were affected by the paternal Ca environment, the maternal Ca environment largely determined pollen siring success (i.e. proportion fruit set, number of seeds sired and number of seeds aborted; Ruane and Donohue 2007). Moreover, after pooling conspecific and heterospecific pollinations, paternal and maternal Ca environments frequently interacted to affect pollen–pistil compatibility, such that pollen performance was highest when mothers and fathers were grown in different soil Ca environments (Ruane and Donohue 2007). These results suggest that gene flow across environments or species—when species predictably inhabit different Ca environments—may be facilitated by environmental effects on pollen–pistil interactions.

Soil Ca level is likely to be an especially important edaphic factor to influence pollen performance. In a range of species, both pollen germination percentage and pollen tube growth rate increased as the concentration of exogenous Ca concentration increased (Brewbaker and Kwack 1963). Moreover, the natural Ca gradient in pistils (Mascarenhas and Machlis 1962, 1964) has been shown to encourage pollen germination, increase pollen tube growth rate and direct pollen tubes towards the ovules (Chichiriccò et al. 2002, Holdaway-Clarke and Hepler 2003). Because this Ca gradient determines pollen germination and growth, then the difference in Ca concentration between pollen and the pistil may predict pollen–pistil compatibility.

Soil Ca is likely to influence not only the pollen performance of individual species but also pollen interactions between these two *Phlox* species. These two species are non-randomly distributed with respect to soil Ca level in some populations, such that *P. cuspidata* appears to be restricted to soils with low Ca concentrations, while *P. drummondii* can inhabit soil with high Ca concentrations (Ruane and Donohue 2007). Each species is also adapted to the soil Ca environment in which it tends to grow, with *P. cuspidata* having higher reproduction when grown in soil with low Ca, and *P. drummondii* having higher reproduction when grown in higher Ca, based on experimental studies under controlled conditions (Ruane and Donohue 2007). Therefore, the two species may have different pollen qualities in part due to the different Ca environment in which each species tends to grow. A given species may compete more effectively against heterospecific pollen and prevent hybridization if the

heterospecific pollen is derived from an environment that decreases its competitive ability. In this way, edaphic variation in soil Ca level has the potential to alter the outcome of competitive interactions between *P. cuspidata* and *P. drummondii* pollen grains that are competing for ovule fertilization.

In this paper, we investigate how edaphic variation in soil Ca level alters outcomes of interspecific pollen competition between *P. cuspidata* and *P. drummondii*. Single-donor heterospecific pollinations (Ruane and Donohue 2007) reveal the effects of maternal and paternal soil Ca environments on the number of seeds sired by heterospecific pollen in the absence of interspecific pollen competition. Mixed-donor interspecific pollinations measured the proportion of progeny sired by heterospecific pollen that was derived from either low or high Ca, when that pollen competed against conspecific pollen in pistils of maternal plants grown in either low or high soil Ca. We compared single- and mixed-donor data to determine if environmental effects on heterospecific pollen siring success depend on the presence of interspecific pollen competition. We also compared competitive outcomes of mixed-donor experiments to determine how soil Ca of the maternal parent and the heterospecific pollen donor influenced hybridization frequency in the presence of interspecific pollen competition.

Materials and methods

Phlox *hybrid system*

Phlox cuspidata and *Phlox drummondii* (Polemoniaceae) are two closely related annual plant species whose ranges overlap in southeastern Texas (Erbe and Turner 1962). In this region of overlap, these species have distinct vegetative and floral morphologies (Levin 1970). *P. cuspidata* is relatively diminutive in stature with smaller pink flowers and shorter styles (mean ± SE, 0.66 ± 0.10 mm, $N = 4$). 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, $N = 4$) 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; Ruane and Donohue 2007). 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).

The fitness of hybrids in this system is low, but it depends on which species acts as the maternal parent. Hybrids sired by *P. cuspidata* are seed-sterile (0 seeds set, $N = 134$ pollinations) and have low paternal fitness (0.22 ± 0.11 seeds sired per pollination by hybrid pollen within *P. cuspidata* pistils, $N = 32$ pollinations and 0 seeds sired by hybrid pollen within *P. drummondii* pistils, $N = 45$ pollinations). On the other hand, hybrids sired by *P. drummondii* do produce seeds (*P. cuspidata* and *P. drummondii* each sired an average of 0.41 ± 0.07 hybrid seeds per pollination, $N = 45$ pollinations). Moreover, hybrids sired by *P. drummondii* are capable of siring seeds when deposited on stigmas of

P. cuspidata (0.72 ± 0.18 seeds sired per pollination, $N = 32$) and *P. drummondii* (0.18 ± 0.08 seeds sired per pollination, $N = 45$). Thus, introgression of alleles from one species to the other can occur through backcrossing, via pollen of both types of hybrids and via fertilization of hybrid seeds that matured in *P. drummondii* dams.

Populations of seed collection

Seeds were collected in April 2003 from three populations where *P. cuspidata* and *P. drummondii* co-occur (Lot, Pat, and Vic) in Bastrop County, TX (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 these and other populations where the ranges of these species overlap, *P. cuspidata* and *P. drummondii* occur within pollen dispersal distance of each other, but they tend to grow in spatially segregated patches (D. Levin, personal communication, L. Ruane, personal observation). Hybrids, which were present at equal frequencies (approximately 0.5%) in each of our study populations, typically occur sporadically without an obvious preference for *P. cuspidata* or *P. drummondii* patches, suggesting that pollen from each species can be dispersed to styles of the other species under field conditions. The degree to which *P. cuspidata*'s patch and *P. drummondii*'s patch were separated from each other varied across populations. Of the three populations we examined, the two species were separated by only 10 to 25 m in two of the populations (Lot and Vic) and by approximately 50 m in the third population (Pat). Seeds are ballistically dispersed, and typically travel less than 3 m from the maternal plant (Levin and Kerster 1968). Thus, both seed dispersal and environmental factors may contribute to the spatial segregation in this hybrid system.

Analyses of soil collected from our three study populations—Lot, Pat and Vic—revealed that *P. cuspidata* and *P. drummondii* were non-randomly distributed with respect to soil Ca level in one population. In both 2003 and 2004, the concentration of Ca in soils inhabited by *P. drummondii* was significantly higher than the concentration of Ca in soils inhabited by *P. cuspidata* in Pat, with *P. drummondii* capable of inhabiting locations with very high soil Ca (the highest Ca concentration observed in our study sites), while *P. cuspidata* was not. In Lot and Vic, these species did not inhabit soils with significantly different Ca concentrations (Ruane and Donohue 2007).

Plant growth environments

Field-collected seeds were grown in a common environment (growth chamber) for one generation before initiating the pollination experiment. Thus, differences between populations can be interpreted as genetically based differences rather than field maternal effects.

To determine the effects of maternal and paternal soil Ca environments on heterospecific pollen competition, we performed experimental pollinations using *P. cuspidata* and *P. drummondii* individuals grown in low and high soil Ca. Seeds were germinated at 12°C in a dark growth chamber (Harris Environmental Systems, Inc., Andover, Massachusetts, USA). All plants (2 species × 3 populations × 2 environments × 34 genotypes) were grown in nutrient-lacking perlite to insure that they did not receive supplemental Ca from their soil medium. Once radicles had emerged, the photoperiod was gradually increased to 12 h a day and the temperature was gradually increased to 22°C. Ca treatments were initiated approximately six weeks after germination, when the second set of true leaves was fully expanded, and were continued throughout the experiment. Sibling pairs were grown in both low (200 ppm) and high (1,000 ppm) soil Ca. Full-sibling pairs for

self-compatible *P. cuspidata* and half-sibling pairs for self-incompatible *P. drummondii* were replicated across environments in order to minimize genetic differences between individuals grown at each soil Ca level. Treatments were applied by saturating pots with 15–20 ml of diluted calcium carbonate (Liquid Lime, Aggrene, Superior, Wisconsin, USA) twice a week.

We chose 200 ppm and 1,000 ppm Ca because these levels best approximate low and high Ca levels in the populations from which the species were derived in 2003 (Ruane and Donohue 2007). While these levels span the range of soil Ca concentrations in the field, neither concentration is toxic (Nixon 1964). On non-treatment 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 (Ruane and Donohue 2007).

Pollination treatments

Once plants in the different Ca treatments began to flower (9–10 weeks after germination), experimental hand pollinations were begun to determine the effects of maternal growth environment and pollen environmental origin on heterospecific pollen siring success. Pollinations were conducted between plants derived from the same population. 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 3 ovules) insured 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.

Mixed-donor pollinations were performed to assess the performance of heterospecific pollen when in competition with conspecific pollen. Interspecific pollinations were fully factorial with respect to maternal environment and heterospecific pollen environmental origin. The environmental origin of conspecific pollen was always the same as the maternal growth environment, which is an ecologically realistic scenario whereby conspecific matings usually occur between individuals growing in a similar Ca environment, especially when the two species inhabit different Ca environments. This design allowed us to isolate the effects of heterospecific pollen environmental origin on hybridization frequency when mothers are grown in both low and high soil Ca.

Results from these mixed-donor pollinations were compared to a subset of the data from a single-donor experiment (Ruane and Donohue 2007) in order to determine whether interspecific pollen competition alters the environmental effects on heterospecific pollen siring success. This subset of data measures heterospecific pollen siring success in the absence of interspecific pollen competition. Single-donor experimental heterospecific pollinations were performed in a factorial crossing design between maternal environment (low and high soil Ca) and heterospecific pollen environment (low and high soil Ca) for both maternal species (Fig. 1). Heterospecific pollen siring success was assessed by counting the number of seeds sired by heterospecific pollen when styles were harvested 10 h after pollen deposition. By harvesting the styles, we were able to better determine differences in the compatibility between pollen and pistils from different Ca environments. Sample sizes for the single- and mixed-donor experiments were very similar, the genotypes

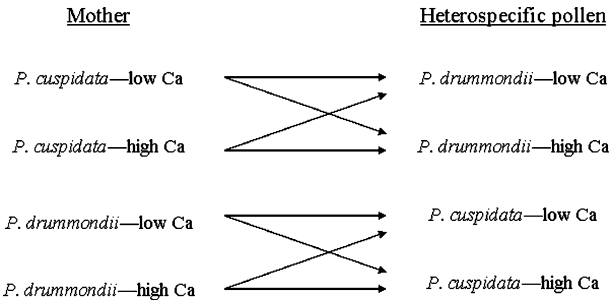

Fig. 1 Experimental design. Single-donor heterospecific pollinations and mixed-donor interspecific pollinations were performed in a factorial crossing design between maternal environment (low and high soil Ca) and heterospecific pollen environment (low and high soil Ca) for both maternal species. For mixed-donor pollinations, conspecific pollen always came from an unrelated plant that was growing in the same soil Ca environment as the maternal plant being pollinated. See text for details

were the same, and the Ca treatments and growth conditions were identical. Detailed methods and results for the single-donor pollination experiment are presented in Ruane and Donohue (2007).

A factorial crossing design between maternal growth environment and heterospecific pollen environmental origin was also used to determine heterospecific pollen siring success in the presence of interspecific pollen competition. Mixed-donor pollinations were performed by emasculating freshly opened flowers and dusting approximately the same number of conspecific and heterospecific dehisced pollen grains over the entire surface of a receptive stigma. Preliminary trials revealed that visual inspection was enough to insure that an equivalent number of pollen grains from each species was deposited on each stigma. Care was taken to insure that conspecific pollen used for both species was not closely related to the maternal plant. Conspecific and heterospecific pollen grains were deposited one after the other, separated by 2–3 s. To eliminate the possible effect of application sequence on relative pollen performance, we randomized the order in which each pollen type was applied.

For mixed-donor pollinations, each of the eight mixed-donor treatments depicted in Fig. 1 (2 heterospecific crosses × 2 maternal growth environments × 1 conspecific pollen environmental origin × 2 heterospecific pollen environmental origins = 8 treatments) was replicated 20–30 times—depending on the population—for a total of 592 pollinations over a period of 17 days. Each maternal genotype received exactly two experimental pollinations that differed only in the environmental origin of heterospecific pollen.

In the presence of interspecific pollen competition, heterospecific pollen performance was determined by measuring the proportion of hybrid progeny sired within each fruit that resulted from each experimental pollination. Fruits, which took approximately 21 days to mature, were collected in November and December of 2004, and every seed (1,405 in total) was planted in April of 2005. Seeds were germinated in a dark growth chamber at 12°C; seeds reluctant to germinate were watered with 600 ppm GA to encourage germination. Once radicles had emerged, the photoperiod was gradually increased to 12 h a day and the temperature was gradually increased to 22°C. After seven weeks, the progeny were moved into a glasshouse (26°C ± 2°C). Within two weeks of being in the glasshouse, individuals began to flower. Progeny flower color was observed to determine its paternity, as hybrids are easily identified by their magenta corollas (Levin 1970, 1985). We were not able to

determine the paternity of seeds that did not germinate. This lack of data, however, is unlikely to change our results because only 6.3% of seeds did not germinate, the seeds that did not germinate may not be viable, and hybrid seeds and parental seeds have the same germinability (Levin 1985).

Analyses

All analyses were performed using JMP version 4.0 (SAS Institute Inc., Cary, North Carolina, USA). A multi-way ANOVA was performed to determine the effects of maternal growth environment and heterospecific pollen environment on the number of seeds sired per fruit following single-donor heterospecific pollinations and on the proportion of seeds sired by heterospecific pollen following mixed-donor interspecific pollinations. In both models, population nested within species (Lot, Pat and Vic), maternal species (*P. cuspidata* and *P. drummondii*), maternal growth environment (low and high Ca) and heterospecific pollen environment (low and high Ca) were the main effects. Tests for interactions between maternal species and maternal growth environment, and between maternal species and heterospecific pollen environment, were performed to determine whether environmental effects on pollen performance were consistent across maternal species. We also tested for an interaction between maternal growth environment and heterospecific pollen environmental origin to determine if the effect of heterospecific pollen environmental origin on pollen performance depended on the growth environment of the maternal plant. To analyze the cause of the interactions, we conducted ad hoc comparisons across treatments.

We also tested for population differentiation in hybridization propensity and environmental effects. Specifically, we tested for interactions between population, maternal environment and heterospecific pollen environment to determine if environmental effects were consistent across populations. We conducted these tests pooled across maternal species, with population nested within species, because there were non-significant three- and four-way interactions between maternal species, population and maternal/paternal environment, indicating that population differentiation for environmental effects did not differ between the maternal species. When we did see population effects, we analyzed each species separately with population, maternal environment and heterospecific pollen environment as the main effects in the fully factorial ANOVA.

The proportion of hybrid progeny produced following mixed-donor interspecific pollinations was arc-sin square-root transformed. The data presented are weighted by the number of seeds within each fruit that flowered; however, statistical significance of effects in the model was the same for non-weighted data.

The single-donor analysis presented in this paper differs from that presented in the previous paper (Ruane and Donohue 2007) because: 1) this analysis includes only heterospecific pollinations, whereas the previous analysis included both conspecific and heterospecific pollinations, and 2) this analysis includes additional interaction terms in order to make the single- and mixed-donor analyses comparable. These changes caused differences in the statistical significance levels of some effects between the former (Ruane and Donohue 2007) and present single-donor analyses. In particular, the marginally significant interaction between the maternal and paternal environments for the number of seeds sired per fruit in the previous paper (Ruane and Donohue 2007) was not detected in this analysis.

Results

Maternal species significantly influenced heterospecific pollen siring success in the absence and presence of interspecific pollen competition (Table 1). In both cases, *P. cuspidata* mothers produced more hybrids than did *P. drummondii* mothers. Following single-donor pollinations, heterospecific pollen sired between 1.5 and 2 seeds per fruit within *P. cuspidata* pistils, but fewer than 0.5 seeds per fruit within *P. drummondii* pistils (Fig. 2a, b). The same pattern held following mixed-donor pollinations. When heterospecific pollen competed against conspecific pollen, *P. drummondii* pollen fertilized an average of 55% of *P. cuspidata*'s ovules, while *P.cuspidata* pollen fertilized an average of 27% of *P. drummondii*'s ovules (Fig. 2c, d).

Maternal growth environment significantly affected heterospecific pollen siring success following single-donor, but not mixed-donor, pollinations (Table 1, Fig. 2a, c). When heterospecific pollen was deposited alone on the stigma, both *P. cuspidata* and *P. drummondii* mothers produced more hybrid seeds when grown in high—compared to low—Ca soils; however, this effect was only significant for *P. cuspidata* mothers derived from Vic ($F = 15.60$, error DF = 261, $P < 0.05$; Fig. 2), as indicated by the significant interaction between population and maternal environment (Table 1). Thus, for single-donor pollinations, maternal soil Ca environment only impacted heterospecific pollen siring success in one maternal species in one population. Maternal growth environment had no effect on

Table 1 Analysis of variance of heterospecific pollen siring success in the absence and presence of interspecific pollen competition

	(A) Single-donor heterospecific pollinations			(B) Mixed-donor interspecific pollinations		
	df	MS	F	df	MS	F
Pop[MS]	4	1.09	1.1355	4	4.21	4.4434*
MS	1	164.66	171.8583*	1	73.01	77.1097*
ME	1	6.95	7.2562*	1	0.95	1.0082
HPE	1	0.27	0.2786	1	0.02	0.0165
MS x ME	1	0.79	0.8259	1	1.00	1.0600
MS x HPE	1	1.24	1.2936	1	4.56	4.8167*
ME x HPE	1	1.24	1.2936	1	1.60	1.6864
Pop[MS] x ME	4	2.38	2.4829*	4	1.12	1.1876
Pop[MS] x HPE	4	0.53	0.5530	4	0.80	0.8406
Pop[MS] x ME x HPE	4	0.28	0.2943	4	0.14	0.1523
MS x ME x HPE	1	0.09	0.0962	1	0.01	0.0126
Residual	261	0.96	–	497	0.95	–

(A) When heterospecific pollen grains were deposited alone on *Phlox cuspidata* and *P. drummondii* stigmas, the number of seeds sired per fruit was recorded. (B) When conspecific and heterospecific pollen grains were deposited together on *P. cuspidata* and *P. drummondii* stigmas, the proportion of hybrids produced per fruit was recorded. Population nested within species (Pop[MS]), maternal species (MS), maternal growth environment (ME) and heterospecific pollen environment (HPE) were the main effects of each ANOVA. We included interaction terms to determine: 1) if environmental effects on pollen performance differed between maternal species (MS × ME, MS × HPE and MS × ME × HPE), 2) if the effect of heterospecific pollen environmental origin on hybridization frequency depended on the growth environment of the maternal plant (ME × HPE), and 3) if environmental effects on hybridization dynamics were consistent across populations (Pop[MS] × ME, Pop[MS] × HPE and Pop[MS] × ME × HPE). MS = mean square. $^{*}P < 0.05$

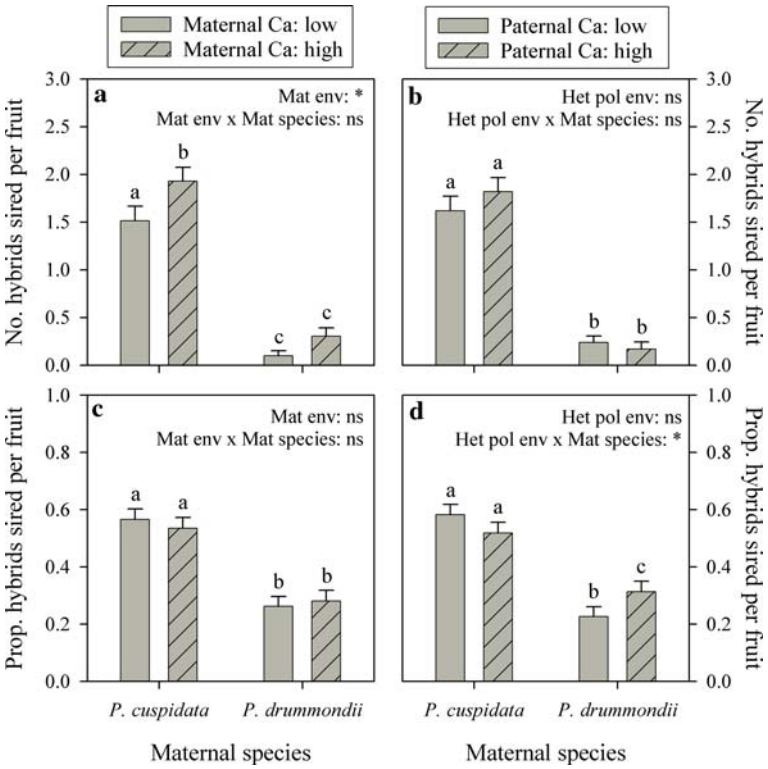

Fig. 2 Means and standard errors of the number of hybrid seeds set after single-donor heterospecific pollinations (upper panels) and of the proportion of hybrid seeds set after mixed-donor interspecific pollinations (lower panels). Effect of maternal (left) and paternal (right) soil Ca environment (200 and 1,000 ppm) on the number of hybrid progeny produced by *Phlox cuspidata* and *P. drummondii*. For single-donor pollinations, data were collected from fruits that matured after styles were harvested 10 h after pollen deposition. Mat env = Maternal environment; Mat species = Maternal species; Het pol env = Heterospecific pollen environment. $*P < 0.05$. Different letters indicate significant differences. For single-donor pollinations, different letters indicate $P < 0.05$. For mixed-donor pollinations, the significant environmental effect within *P. drummondii* mothers was $P < 0.1$ for the complete parametric model, and was verified with a Kruksal–Wallis test ($P = 0.06$)

heterospecific pollen siring success when heterospecific and conspecific pollen grains were deposited together on stigmas (Table 1, Fig. 2c).

Heterospecific pollen environment had no effect on heterospecific pollen siring success following single-donor pollinations (Table 1, Fig. 2b). Following mixed-donor pollinations, however, heterospecific pollen environment and maternal species significantly interacted to affect the proportion of hybrid progeny produced (Table 1). When *P. cuspidata* was the maternal plant, heterospecific pollen siring success was slightly but not significantly higher when derived from low—compared to high—Ca soils ($F = 2.12$, error DF = 497, $P = 0.15$; Fig. 2d). When *P. drummondii* was the maternal plant, heterospecific pollen siring success was marginally significantly higher when derived from high—compared to low—Ca soils ($F = 2.72$, error DF = 497, $P < 0.1$; $P = 0.06$ based on a non-parametric Kruskal–Wallis test; Fig. 2d). Therefore, pollen environment influenced hybridization propensity in *P. drummondii*, but not in *P. cuspidata*.

The proportion of hybrid progeny produced following mixed-donor (but not single-donor) pollinations varied significantly among populations (Table 1). This difference was primarily driven by *P. drummondii* dams, as the proportion of hybrid progeny produced by *P. drummondii* dams derived from Vic was 122% and 99% lower than that produced by *P. drummondii* dams derived from Lot ($F = 12.24$, error DF = 497, $P < 0.01$) and Pat ($F = 6.60$, error DF = 497, $P < 0.05$), respectively. Population had a marginally significant effect on the proportion of hybrid progeny produced by *P. cuspidata* dams, with individual females derived from Pat being more likely to hybridize (less likely to exclude heterospecific pollen) compared to individuals from Lot ($F = 2.75$, error DF = 497, $P < 0.1$) and Vic ($F = 3.86$, error DF = 497, $P < 0.1$).

The lack of significant interactions between population and environment in the presence of interspecific pollen competition (Table 1) indicates that environmental effects on hybridization dynamics were consistent across populations when both conspecific and heterospecific pollen were deposited on stigmas.

Discussion

We found that interspecific pollen competition altered environmental effects on heterospecific pollen siring success in the annual *Phlox* hybrid system, and that the environment in which heterospecific pollen was derived influenced interspecific competitive outcomes. When heterospecific pollen was deposited alone on *P. cuspidata* and *P. drummondii* stigmas, maternal growth environment significantly affected heterospecific pollen siring success in one population (Table 1), as heterospecific pollen sired more seeds when deposited on stigmas of maternal plants grown in high—compared to low—soil Ca (Fig. 2a). When conspecific and heterospecific pollen grains were forced to compete for ovule fertilization, however, maternal environment no longer influenced the proportion of hybrids formed, but instead heterospecific pollen environment and maternal species significantly interacted to impact the proportion of hybrid progeny produced. Specifically, *P. drummondii* pistils were more resistant to heterospecific pollen derived from low Ca soil, but *P. cuspidata* pistils were not significantly affected by the soil Ca form which heterospecific pollen was derived.

Pollen competition altered predictions about whether environmental effects would promote or impede hybridization in the annual *Phlox* hybrid system. In the absence of interspecific pollen competition, the maternal environment was a reliable predictor of hybridization in one population (Vic), with hybridization frequency being highest when maternal plants were grown in high-Ca soil. Hybridization would be facilitated in both *P. cuspidata* and *P. drummondii* in populations where these species inhabit high Ca soils. However, in populations where *P. drummondii* inhabits soils of significantly higher soil Ca levels than *P. cuspidata* (i.e. Pat in 2003 and 2004; Ruane and Donohue 2007), this environmental effect is expected to increase the number of hybrids mothered by *P. drummondii*. In contrast, in the presence of interspecific pollen competition, the paternal environment of the heterospecific pollen—not the maternal environment—had a greater effect on hybridization. The magnitude and direction of this effect depended on the maternal species, with *P. cuspidata* pollen siring fewer hybrid progeny when derived from low-Ca soils. In locations in which *P. cuspidata* inhabits low Ca soils and *P. drummondii* inhabits high Ca soils (i.e. in Pat in 2003 and 2004, Ruane and Donohue 2007), the environmental origin of heterospecific pollen would decrease the proportion of hybrids

produced, thereby increasing the degree of reproductive isolation between these species. Thus, environmental effects can influence the outcome of interspecific competitive interactions among pollen and thereby alter hybridization dynamics. The environmental effects were somewhat weaker in the presence of interspecific pollen competition than in its absence, however (Ruane and Donohue 2007). Thus, environmental effects may be expected to influence gene flow across environments within species somewhat more strongly than gene flow across species that inhabit different environments.

This study demonstrates that the environment from which the heterospecific pollen was derived can influence the probability of hybridization, when conspecific pollen is derived from the same environment as the maternal plant. We were not able, however, to determine the three-way interactions between the environment of the pistil, conspecific pollen, and heterospecific pollen. The ecological context represented by our design was the case when the two species inhabit different environments, or when they both inhabit the same environment, but not when both species inhabit both environments. The design therefore allowed us to test whether the environment of the heterospecific competitor altered its competitive ability, and thereby whether habitat segregation of the two species can influence hybridization. However, it is possible that both species are distributed over a hetereogeneous environment, and that conspecific pollen may also be derived from either environment. In such cases, competitive outcomes may depend on whether the conspecific pollen was derived from the same or a different environment as the maternal plant.

The pronounced difference in hybridization dynamics with and without pollen competition makes it clear that competitive outcomes will also depend on the relative timing of heterospecific versus conspecific pollen deposition. While our data suggest that 55% of seeds produced by *P. cuspidata* mothers and 27% of seeds produced by *P. drummondii* mothers will be hybrid offspring, this is only expected when pollen grains from each species are deposited on the stigma simultaneously by the same pollinator. Although this is possible given that lepidopteran pollinators have been observed traveling between these species (Erbe and Turner 1962; Grant and Grant 1965; Levin 1967, 1970, 1975; L. Ruane, personal observation), it is also likely that conspecific and heterospecific pollen will be deposited at different times during stigma receptivity. Competitive outcomes will also depend on the degree to which the competing conspecific pollen is genetically related to the maternal plant. When inbreeding depression impedes the performance of conspecific pollen, heterospecific pollen siring success can increase as the degree of relatedness between the competing conspecific pollen and the maternal plant increases (Levin 1989b; Montalvo 1992; Cruzan and Barrett 1993). In the case of self-incompatible species, such as *P. drummondii*, self-pollen is not even capable of fertilization. Thus, heterospecific pollen may have an advantage if the competing *P. drummondii* conspecific pollen is closely related to the maternal plant. Experiments are currently underway to determine how heterospecific pollen siring success is impacted by the relative timing of heterospecific versus conspecific pollen deposition and by the degree to which competing conspecific pollen is genetically related to the maternal plant.

Interestingly, we found population differentiation in the frequency at which *P. drummondii* mothers produced hybrid progeny following mixed-donor pollinations. Only 16% of the progeny produced by *P. drummondii* mothers derived from Vic were hybrids, compared to 35% and 31% for *P. drummondii* mothers derived from Lot and Pat, respectively. This could be due to faster absolute growth rates of *P. drummondii* pollen from Vic, or to increased resistance of *P. drummondii* Vic pistils to heterospecific pollen. Analyses of single-donor data revealed that *P. drummondii* pollen from Vic grew faster than the other populations in conspecific pistils, but that it did not grow faster than the

other populations in heterospecific pistils. Therefore, Vic *P. drummondii* pollen does not necessarily have a faster absolute growth rate. However, the pistils of *P. drummondii* mothers allowed for the faster growth of both conspecific and heterospecific pollen tubes when derived from Vic compared to those derived from Lot and Pat. Specifically, conspecific pollen derived from Vic had significantly faster tube growth rates 8 h ($F = 5.94$, error DF = 124, $P < 0.05$) and 10 h ($F = 7.16$, error DF = 125, $P < 0.01$) after pollen deposition compared to conspecific pollen derived from Lot and Pat. Moreover, heterospecific pollen also had significantly faster tube growth rates 8 h after pollen deposition when derived from Vic compared to heterospecific pollen derived from Lot and Pat ($F = 7.44$, error DF = 130, $P < 0.01$). Despite the faster growth rates of both species' pollen tubes in Vic *P. drummondii* pistils, conspecific tubes grew 36% faster than heterospecific tubes for individuals derived from Vic, compared to only 24% and 7% faster for individuals derived from Lot and Pat, respectively. Thus, pistils of *P. drummondii* from Vic appear to facilitate conspecific pollen tube growth relative to heterospecific pollen tube growth more than in the other populations. If these patterns from single-donor pollinations are consistent in mixed-donor pollinations, then an increase in the relative discrimination of pistils against heterospecific pollen may have helped to decrease the proportion of hybrids produced by *P. drummondii* mothers derived from Vic in this experiment.

In conclusion, environmental effects on heterospecific pollen siring success altered the outcome of interspecific pollen competition in the annual *Phlox* hybrid system, and genetically based population differentiation for interspecific pollen competitive ability was detected. In the absence of interspecific pollen competition, the maternal growth environment impacted heterospecific pollen siring success, but in the presence of interspecific pollen competition, the environmental origin of heterospecific pollen affected hybridization frequency in one maternal species. Pollen of *P. drummondii* was more effective at outcompeting *P. cuspidata* pollen and preventing hybridization in *P. drummondii* dams when *P. cuspidata* pollen was derived from low Ca, but hybridization in *P. cuspidata* mothers was unaffected by the environment of heterospecific pollen origin. In situations in which *P. cuspidata* grows in lower soil Ca than *P. drummondii*, as has been documented in one population, these results suggest that the competitive ability of heterospecific pollen in *P. drummondii* dams will be diminished by environmental effects of soil Ca. Thus, the environment in which pollen developed can influence interspecific pollen competition and hybridization frequency.

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