Physiological differences among two *Penstemon* species and their hybrids in field and common garden environments

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Summary

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• Hybrids can exhibit unique combinations of the physiological traits of their parents. These particular combinations may influence hybrid fitness and the evolutionary trajectory of a hybrid zone.
• Here, a hybrid zone between *Penstemon newberryi* and *Penstemon davidsonii* along an elevational gradient was examined, and physiological traits of parents and hybrids were measured in their native environment and a common garden. Gas exchange rates of nine different crosses were also measured.
• Alpine *P. davidsonii* had less negative pre-dawn water potential and lower water use efficiency (WUE) than its montane relative *P. newberryi* in a common garden and in field measurements. The species difference in WUE was attributable to lower conductance in *P. newberryi* in the field, but to a higher photosynthetic rate in this species in the common garden. The alpine species took less time to produce mature fruits and reached maximum photosynthetic rate at a lower temperature. Natural hybrids were intermediate for most characters. F_1 hybrids had lower conductance than progeny of natural hybrids.
• The intermediate WUE of natural hybrids may be one factor that allows them to persist in intermediate environments. Comparisons of different crosses suggest that the genotypic composition of hybrids influences their physiological performance.

Introduction

Hybrid zones are regions where diverse genotypes are produced as a result of the cross-fertilization of closely related species (Stebbins, 1959; Arnold, 1997). Over time, a hybrid zone may be stable, result in the evolution of a new hybrid species, strengthen reproductive isolating mechanisms between the two parent populations, or reduce diversity as a result of the breakdown of reproductive barriers. These outcomes depend on the combination of genetic and environmental factors that influence hybrid fitness. F_1 hybrids often exhibit hybrid vigor as a result of dominance or overdominance (Rieseberg & Carney, 1998), but they can also show poor growth or survival (Abbo & Ladizinsky, 1994). Second-generation hybrids (F_2 hybrids) are expected to show reduced fitness if genetic

incompatibilities derive from epistatic interactions between alleles at different genes (Dobzhansky, 1937; Muller, 1942), and several studies of plants have provided evidence for such hybrid breakdown (e.g. Fishman & Willis, 2001; Fritz *et al.*, 2006; Johansen-Morris & Latta, 2006). The performance of reciprocal F_1 plant hybrids may differ (hereafter referred to as 'reciprocal effects') as a consequence of mitochondrial or chloroplast genes inherited through the mother or the interaction of those cytoplasmic genes with nuclear genes (Galloway & Fenster, 1999; Wu & Campbell, 2006), as well as differences in the maternal environment. Hybrids often have characters that are intermediate between those of the pure parents (Rieseberg & Ellstrand, 1993), although transgressive morphological characters that go beyond the limits of either parent are also frequently observed in plants (Rieseberg *et al.*, 1999).

In addition to genetic background, environmental factors also help to determine hybrid fitness. In several recent studies of plant hybrid zones, the fitness of various hybrids was found to depend heavily on the environment (e.g. Emms & Arnold, 1997; Wang *et al.*, 1997; Campbell & Waser, 2007). As environmental factors can play such a strong role, it is critical to examine traits of plants that may influence the performance of hybrids in particular environments. The physiological performance of various hybrid classes relative to their parents may help to explain their relative survival in different habitats and thereby the structure of natural hybrid zones (Johnston *et al.*, 2001; Ludwig *et al.*, 2004).

Photosynthetic rate, water use efficiency (WUE), temperature of maximum photosynthetic rate, water potential, and specific leaf area (SLA) are examples of ecophysiological traits that can correlate with survival or other fitness components in different environments, so they can be important indicators of environmental selection (Gurevitch, 1992; Sandquist & Ehleringer, 1997; Ackerly *et al.*, 2000; Heschel & Riginos, 2005; Angert, 2006; Agrawal *et al.*, 2008). Although there is a rich literature on these traits for related species (Wright *et al.*, 2004), physiological traits of hybrids between wild (rather than crop) species have rarely been studied. In most cases, we do not even know whether they are intermediate or transgressive (but see McArthur *et al.*, 1998; Schwarzbach *et al.*, 2001; Lexer *et al.*, 2003; Brock & Galen, 2005; Campbell *et al.*, 2005). Data on the physiological traits of different types of hybrid crosses, such as F_1 versus advanced generation hybrids, are especially rare, particularly for gas exchange studies of photosynthesis (Campbell *et al.*, 2005; Wu & Campbell, 2007).

Most studies of natural hybrid systems have examined functional traits only in one environment in the glasshouse (but see Brock & Galen, 2005; Campbell *et al.*, 2005), although studies of commercial crop hybrids often use multiple experimental conditions (Monclus *et al.*, 2005). Gas exchange traits are known to be highly plastic depending on environmental conditions (Cordell *et al.*, 1998; Caruso *et al.*, 2006; Golluscio & Oesterheld, 2007). Thus, it is important to measure plants under multiple conditions to assess their relative physiological performance.

The objective of this study was to determine whether physiological traits of parents and hybrids differed and whether those differences corresponded to differences in the environment in which each plant type occurs. Our study system was a large natural hybrid zone between an alpine and a montane species of *Penstemon* along two elevational gradients in the eastern Sierra Nevada, CA, USA. We used both field studies and measurements of potted plants under common conditions to determine how the habitats of the plant types differed, to identify physiological differences among the parent species and hybrids, and to determine whether these differences are likely to be genetic. Water relations, gas exchange traits related to photosynthesis, and phenological traits such as time between flowering and fruiting are expected to vary with elevation

because the montane environment is dominated by drought- and heat-tolerant plants such as *Artemisia tridentata*, while the alpine zone consists of mat-forming plants and has snow melting on the ground throughout a shorter summer growing season.

We first characterized the environment in terms of temperature and vapor pressure deficit (VPD), and then addressed three questions.
• Do the parental species and hybrids have different water potentials, SLAs, and reproductive phenologies in their native habitats?
• Do parents and hybrids have different rates of gas exchange when grown in a common environment, and in field populations?
• Are hybrids intermediate or transgressive for various physiological traits and do the gas exchange rates of hybrids differ depending on the type of cross?

We analyzed nine types of crosses, including F_1 hybrids with crosses in both directions, crosses between natural hybrids, and backcrosses of natural hybrids to the parental species, for which data on gas exchange are particularly scarce. Inclusion of these types allowed us to test for hybrid vigor in the F_1, reciprocal effects, and differences between first-generation and natural hybrids.

Materials and Methods

Study system

Penstemon davidsonii E. Greene is a perennial wildflower found in the alpine zone of the Sierra Nevada mountain range. It has blue-violet flowers and forms mats at ground level. Its close relative, *Penstemon newberryi* A. Gray, occurs in granite and metamorphic rock outcrops in the montane zone of the Sierra Nevada mountains. It has fuchsia-colored flowers and is *c.* 20 cm tall. The two species form extensive hybrid zones where their ranges overlap (Clausen *et al.*, 1940; Chabot & Billings, 1972; Datwyler & Wolfe, 2004). We have studied this hybridization along two elevational transects on the eastern slope of the Sierra Nevada, above Bishop along the Piute Pass trail (from 37°14′22″N 118°36′24″W to 37°14′44″N 118°40′36″W) and east of Yosemite National Park, in Lee Vining Canyon (from 37°56′08″N 119°10′42″W to 37°58′15″N 119°11′27″W). Along the Piute transect, *P. newberryi* grows mainly between 2400 and 3025 m elevation. *Penstemon davidsonii* grows mainly between 3450 and 3800 elevation, and morphological hybrids are abundant in the intermediate region. The elevational ranges of the species are shifted *c.* 150 m downslope along the Lee Vining transect, where frequent storms move through Tioga Pass. Classification of plants into pure parental and hybrid populations was based on detailed studies of morphology along the Piute transect, where the plant types differ markedly in floral circumference, inflorescence height, stamen exsertion, and leaf

area (Kimball, 2008). The two parent species are visited by many of the same pollinators, which may allow hybridization (Kimball, 2008). Previous work indicates that F_1 and later generation hybrid crosses produce as many seeds as conspecific crosses (Kimball *et al.*, 2008).

Field measurements

Environmental data During summer 2005, HOBO meters (Onset Computer Corporation, Bourne, MA, USA) were deployed to measure temperature and relative humidity every 15 min at four locations along the Piute transect (at 2906, 3292, 3419, and 3537 m elevation) and three locations along the Lee Vining transect (at 2382, 2888, and 3316 m elevation). The lowest elevation HOBO meters along both transects were in the range of *P. newberryi*, the highest meters were in the range of *P. davidsonii*, and the intermediate meters were in the hybrid zones. HOBO meters were housed in small wooden shelters, ventilated and shielded from radiation, and placed 50 cm above ground level. VPD of the air was calculated from temperature and relative humidity (Murray, 1967). The locations were compared with respect to daily maximum and minimum temperatures and VPDs.

Water potential During summer 2005, we compared plants of the two species and natural hybrids in the field with respect to several traits. First, we measured pre-dawn (Ψ_{PD}) and midday (Ψ_{MD}) shoot water potential with a Scholander style pressure chamber (Model 1000; PMS Instrument Company, Corvallis, OR, USA). We measured shoot water potential because leaves were too small to measure individually. Measurements were conducted in one *P. newberryi*, one hybrid, and two *P. davidsonii* populations along the Lee Vining transect and four *P. newberryi*, four hybrid, and two *P. davidsonii* populations along the Piute transect. All populations were measured when the plants were in full bloom, between 16 June and 29 July 2005. Measurements were taken on dry clear days on vegetative stems of individual plants between 02:00 and 06:00 h for pre-dawn values, and between 10:00 and 14:00 h for midday values. In each population we subsampled eight plants at each time and used the average value as a single replicate in analyses. Each mean value for Ψ_{PD} and Ψ_{MD} was analyzed using a general linear model with categorical factors of transect and plant type (*P. newberryi*, *P. davidsonii*, or natural hybrid), along with date of sampling as a covariate. As it did not rain > 0.1 cm on any date during the sampling period (based on the nearby station of Bishop; http://cdo.ncdc.noaa.gov/dly/DLY?stnid = 20002386), any effect of date would likely show up as a decrease in water potential across the season. Inclusion of date in the model allowed us to test for such a seasonal effect and to determine if there was an additional effect of plant type. We did not include a transect × plant type interaction because of the lack of replicate populations of the parental types along the Lee Vining transect.

Specific leaf area Leaves from 122 plants spread across 12 populations along the Piute transect and four populations along the Lee Vining transect were collected to calculate SLA (the ratio of leaf area to leaf mass). Leaves were scanned, and SLA calculated using IMAGE TOOL (Wilcox *et al.*, 2002). Leaves were then dried and weighed. The mean SLA for each population was analyzed as a two-way ANOVA with the fixed effects of plant type (*P. newberryi*, *P. davidsonii*, or natural hybrid) and transect of origin.

Phenology We measured reproductive phenology along with physiological traits because differences between plant types may reflect adaptations to distinct environmental conditions. During the growing season of 2005, we recorded the phenological stage of plants at various elevations every 2 wk, noting whether the majority of plants were in bud, in bloom, with immature fruits, or with mature fruits, and whether the population consisted of *P. newberryi* or *P. davidsonii*, or was mixed with hybrids. The number of days from flowering to fruit maturation was calculated, and populations were compared by ANOVA with plant type and transect as fixed factors.

Gas exchange measurements

Plants from cuttings of the two species and natural hybrids Trait differences between taxa in the field can reflect genetic differences, spatial differences in the environment, or effects of the maternal environment during an earlier growth period. To eliminate current environmental effects, we measured gas exchange traits for potted plants under common conditions. In summer 2003, we collected 300 stem cuttings from plants along the Piute transect. These cuttings were placed in water until they rooted, and then planted in 4-inch pots with a 1 : 1 mixture of pumice and potting soil (Sunshine Soil Mix, Sun Gro Horticulture, Vancouver, Canada). Plants were transplanted into 1-gallon pots after 1 month and placed outside at the University of California, Irvine (UCI). The potted plants were watered regularly and fertilized with Miracle Grow (The Scotts Company, Marysville, OH, USA; 15-30-15 NPK with trace elements) every 3 wk. Gas exchange measurements were taken on potted plants while they were blooming during the spring of 2005 using a portable gas exchange system with full-spectrum lamps (Li-Cor 6400; Li-Cor, Lincoln, NE, USA).

We measured instantaneous photosynthetic rate (A_{max}), transpiration (E), stomatal conductance (g_c), and internal CO_2 concentration (c_i). Intrinsic WUE was calculated as A_{max}/g_c, which was highly correlated with A_{max}/E ($r = 0.845$, $n = 30$) and with c_i (-0.865). Unless otherwise noted, light was set at a photosynthetically active radiation (PAR) intensity of 1800 µmol m^{-2} s^{-1}, leaf temperature at 26°C (close to ambient in the common garden), and CO_2 at 37 Pa (ambient). Measurements were taken on plants in a randomized order between

10:00 and 14:00 h. Leaf to air VPD during measurement was similar for the three plant types ($F_{2,30} = 2.69$, $P = 0.085$) and ranged from 1.37 to 2.38 kPa.

The leaves of these species are small (average of 1.73 cm² for *P. newberryi* and 0.66 cm² for *P. davidsonii*), so, in order to achieve measurable carbon flux, we placed a small branchlet consisting of several overlapping leaves in the leaf chamber at one time, a common method for plants with small leaves (Ding *et al.*, 1991; Royer *et al.*, 2005; Maherali *et al.*, 2006). Leaf area in the cuvette was determined in two ways. First, the branchlets were scanned in the overlapping configuration they formed while in the chamber, and silhouetted leaf area was calculated. However, it is possible that overlapping leaves still photosynthesized, so we also separated each leaf and calculated total leaf area. SLA did not differ significantly between the species, so this method was similar to determining carbon gain per unit of leaf mass. The two methods of calculating leaf area yielded the same conclusions, so we only report results for silhouetted leaf area.

Variables were analyzed by ANOVA, with plant type as a fixed factor, supplemented with two planned comparisons, using the CONTRAST statement in Proc GLM of sas (SAS Institute, Cary, NC, USA). The first comparison was between the two parent species. The second comparison was between the mean of the hybrids and the mid-parent value (the average of the mean values of *P. newberryi* and *P. davidsonii*) to test for hybrid vigor. In the absence of environmental effects, first-generation hybrids would not differ from the mid-parent value if physiological characters combined additively. Including VPD as a covariate in the analyses did not change the results, so we present only the ANOVA.

To determine the light intensity at which maximum photo-synthetic rate was reached, in a separate experiment we measured photosynthetic rate at PAR intensities of 0, 50, 100, 500, 1000, 1500, 1700, 1900, 2100, 2300, and 2500 μmol m⁻² s⁻¹. Light compensation point and light saturation were calculated using Photosyn Assist (Dundee Scientific, Scotland, UK), and plant types were compared by ANOVA. The two species did not differ in light saturation point ($F_{2,20} = 0.164$, $P = 0.850$) or light compensation point ($F_{2,20} = 2.159$, $P = 0.144$). We used a PAR intensity of 1800 μmol m⁻² s⁻¹ in all other measurements, as it is a typical saturating light intensity for alpine plants (Korner, 1999; Campbell *et al.*, 2005).

We also measured the photosynthetic rate of *P. newberryi*, *P. davidsonii*, and natural hybrids every 2°C from 4 to 35°C to determine whether they differed in the temperature at which they reached maximum photosynthetic rate. Potted plants were taken from the common garden outside at UCI and placed in a walk-in growth chamber with grow lamps, and were allowed 1 h to equilibrate to 4°C. Light in the leaf chamber was set at a PAR intensity of 1800 μmol m⁻² s⁻¹. The temperature in the chamber was slowly raised by 2°C increments, and plants were given 15–30 min to equilibrate to each new temperature before a measurement was taken.

For each individual, we noted the temperature at which maximum photosynthetic rate was reached and analyzed those temperatures by ANOVA with plant type as a fixed factor. As temperatures in the field decrease with elevation (see Results), we expected that, if there was a difference, the alpine *P. davidsonii* would reach A_{max} at a lower temperature than *P. newberryi*.

Plants grown from seeds generated by hand-pollination To compare gas exchange among natural hybrids, F_1 plants, and various backcrosses, we grew different types of plants from seed. During spring 2004, we hand-pollinated three types of plants (*P. davidsonii*, *P. newberryi*, and natural hybrids) that themselves were grown from cuttings in pots at UCI. For each study transect, we made nine crosses representing all factorial combinations of the three types as the mother crossed with the three types as the father. These crosses represent pure parents, reciprocal F_1 hybrids, reciprocal backcrosses, and crosses between natural hybrids. For each cross, 10–15 mother plants were crossed with 1–6 different father plants. Seeds were germinated in spring 2005 and plants were grown outside in pots at UCI. Growing plants from seed allowed us to eliminate any effects of early maternal environment that may have influenced the performance of cuttings. During fall 2006, we measured instantaneous gas exchange rates of the nine cross types (when none of the plants was flowering). As in the previous potted plant measurements, environmental levels were controlled, and all plant types were measured over the same range of VPD (from 1.11 to 2.42 kPa), which did not differ significantly depending on the cross type ($F_{8,116} = 0.504$, $P = 0.851$). A_{max}, E, and WUE were analyzed by two-way ANOVA with type of mother and type of father as fixed factors. WUE was again calculated as A_{max}/g_c, which was highly correlated with A_{max}/E ($r = 0.880$, $n = 125$) and c_i (−0.977). We performed four planned comparisons for all gas exchange variables. First, we compared the mean values of the two pure parental crosses. Next, we compared the reciprocal F_1 hybrids to see whether the direction of the cross influenced gas exchange. The third comparison tested for effects of hybrid generation by comparing the average value of both F_1s and the value of later generation hybrid (natural hybrid-by-natural hybrid) crosses. The final comparison, to test for hybrid vigor, was between the average of the F_1s and the mid-parent value.

Field populations To determine whether gas exchange differences among the two species and hybrids seen in the common gardens could also be detected under field conditions, we took gas exchange measurements on three populations along the Lee Vining transect during the summer of 2007. The three populations represented *P. newberryi* at 2382 m, morphological hybrids at 2888 m, and *P. davidsonii* at 3505 m elevation. Each population was measured between 10:00 and 13:00 h on a sunny day when plants were in full bloom (16 May for *P. newberryi*, 12 June for hybrids, and 27 June for

Table 1 Average daily maximum and minimum temperatures and vapor pressure deficits (VPDs) at four elevations along the Piute transect and three elevations along the Lee Vining transect during summer 2005

Transect	Elevation	Plant type	Min temp (°C)	Max temp (°C)	Min VPD (kPa)	Max VPD (kPa)	Days with highest max temp	Days with highest max VPD
Piute	2906	*Penstemon newberryi*	6.42	31.32	0.47	4.53	55	68
Piute	3292	Hybrid	3.48	30.63	0.06	3.75	25	13
Piute	3419	Hybrid	3.67	24.20	0.24	2.72	1	0
Piute	3537	*Penstemon davidsonii*	0.85	17.72	0.20	1.67	0	0
Lee Vining	2382	*Penstemon newberryi*	12.08	29.59	0.70	3.70	73	73
Lee Vining	2888	Hybrid	5.30	23.04	0.21	2.40	0	0
Lee Vining	3316	*Penstemon davidsonii*	4.92	22.55	0.30	2.28	0	0

The plant type growing at that particular site is noted. The last two columns list the number of days that each site had the highest maximum temperature and VPD when compared with all other sites along the same transect.

P. davidsonii). The distances between populations and differences in phenology prevented us from making same-day measurements in all populations. These field results must be viewed with caution, as any observed differences in gas exchange could reflect seasonal changes as well as differences in spatial environment and genetic background.

As before, we measured instantaneous A_{max}, E, g_c, and c_i. Intrinsic WUE was again calculated as A_{max}/g_c, which was highly correlated with A_{max}/E ($r = 0.954$, $n = 42$) and c_i ($r = -0.997$, $n = 42$). Light was set at a PAR intensity of 1800 μmol m^{-2} s^{-1}, leaf temperature at 26°C (close to ambient; range 23–29°C), and CO_2 at 37 Pa. A_{max}, E, g_c, and WUE were analyzed by ANCOVA with plant type (*P. newberryi*, natural hybrid, or *P. davidsonii*) as a fixed categorical factor and leaf to air VPD as a covariate. We included this covariate to eliminate one source of variation across the season, as the three populations differed slightly in VPD values during measurement (*P. newberryi* mean = 2.537 kPa, hybrid mean = 2.384 kPa, *P. davidsonii* mean = 3.052 kPa; $F_{2,39} = 67.80$, $P < 0.0001$).

Results

Field measurements

Environmental data The high-elevation sites were cooler and moister than the low-elevation sites. Average daily maximum and minimum temperatures and VPDs were highest for the *P. newberryi* site, and lowest for the *P. davidsonii* site, with the hybrid site(s) intermediate, for both transects (Table 1). These habitat differences provided the basis for expectations of differences in the physiological traits of parents and hybrids if they are adapted to these different sites.

Water potential On average, *P. newberryi* populations had more negative pre-dawn water potentials than *P. davidsonii* populations, and hybrid populations were intermediate ($F_{2,9} = 6.50$, $P = 0.018$; Fig. 1). Differences among plant types
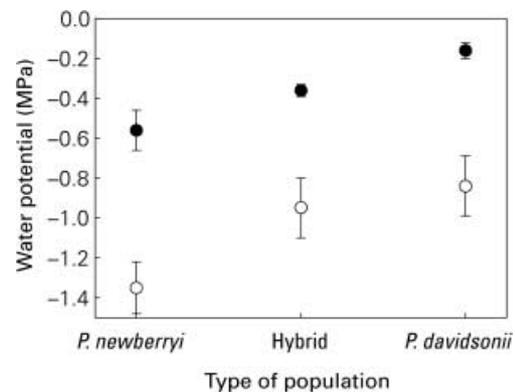
Fig. 1 Average pre-dawn (Ψ_{PD}) water potential (closed circles) and midday (Ψ_{MD}) water potential (open circles) for five populations of *Penstemon newberryi*, five hybrid populations, and four populations of *Penstemon davidsonii*. Values are means ± 1 SE.

were not significant for midday water potential ($F_{2,9} = 1.65$, $P = 0.245$; Fig. 1). Water potentials did not change systematically over the 4-wk measurement period, as judged by the absence of a covariate effect in the overall model ($P > 0.25$ for both pre-dawn and midday measurements). Neither water potential measurement differed between the two study transects (both $P > 0.25$). A two-way factorial ANOVA with plant type and time of measurement (but with date and transect removed because they had negligible effects in the earlier analysis) detected both main effects but not an interaction, reflecting the similarity in pattern for the two times of day.

Specific leaf area *Penstemon davidsonii* had smaller leaves than *P. newberryi*, but leaf weight followed the same trend, so that there was no significant difference in SLA among the two species and hybrids (overall means = 0.059, 0.073, and 0.063 cm^2 mg^{-1} for *P. davidsonii*, hybrids, and *P. newberryi*, respectively; $F_{2,12} = 1.56$, $P = 0.25$). This trait also did not

Table 2 (a) *F*-values from an ANOVA comparing gas exchange measurements of *Penstemon newberryi*, naturally occurring hybrids, and *Penstemon davidsonii* grown from field cuttings in pots at University of California, Irvine. (b) *F*-values from an ANCOVA comparing measurements from populations of *P. newberryi*, naturally occurring hybrids, and *P. davidsonii* at three different elevations (2382, 2888, and 3505 m) along the Lee Vining study transect

Source of variation	df	Photosynthetic rate (*A*)	WUE (*A/g*)	Stomatal conductance (*g*)
(a) Potted plants				
Type	2	21.76**	8.24*	5.64*
P. newberryi vs *P. davidsonii*	1	39.17**	15.4*	2.89
Parents vs hybrids	1	3.15	1.52	7.88*
Error	28			
(b) Field plants				
Type	2	6.32*	6.51*	11.71
VPD	1	9.82*	6.87*	17.67
Error	40			

Type of plant was a categorical factor and vapor pressure deficit (VPD) was a covariate. *, $P < 0.05$; **, $P < 0.0001$. WUE, water use efficiency.

differ significantly between the Piute and Lee Vining transects ($P = 0.64$).

Phenology Populations at low elevation initiated flowering earlier than high-elevation populations. The time from flowering to fruit maturation was longer in low-elevation populations than in high-elevation populations. It took flowers of *P. newberryi* populations *c.* 60 d to dehisce fruit after full bloom, while the process took *P. davidsonii* populations only 38 d (ANOVA $F_{2,7} = 73.69$, $P < 0.0001$; *P. newberryi* vs *P. davidsonii* $F_{1,7} = 85.205$, $P < 0.0001$). Time to fruit maturity was intermediate for hybrid populations (mean = 54 d).

Gas exchange measurements on potted plants

Plants from cuttings of the two species and natural hybrids For plants grown from cuttings, *P. newberryi* had greater WUE than *P. davidsonii* (Table 2, Fig. 2). c_i values corresponded with WUE results (*P. newberryi* mean c_i = 17.58 Pa; hybrid = 23.94 Pa; *P. davidsonii* = 28.26 Pa). For these plants grown in pots, WUE was higher for *P. newberryi*, in part because this species had a threefold higher photosynthetic rate than *P. davidsonii* (Table 2). Natural hybrids had fairly high stomatal conductance which was significantly greater than the mid-parent value, but the effect of high *P. newberryi* photosynthetic rates outweighed this difference such that natural hybrids were intermediate for WUE (Table 2, Fig. 2).

We calculated the temperature at which maximum photosynthetic rate was reached from photosynthesis–temperature curves. When the values were compared by ANOVA, *P. newberryi* had higher photosynthetic rates than *P. davidsonii* at all temperatures (Fig. 3). *Penstemon davidsonii* reached maximum photosynthetic rate at a lower temperature than *P. newberryi* (Fig. 3, $F_{2,19} = 3.7965$, $P = 0.041$; *P. newberryi* vs *P. davidsonii* $F_{1,19} = 5.823$, $P = 0.026$). The temperature optimum of natural hybrids was higher than that of either parent and significantly higher than the mid-parent value (Fig. 3; mid-parent vs

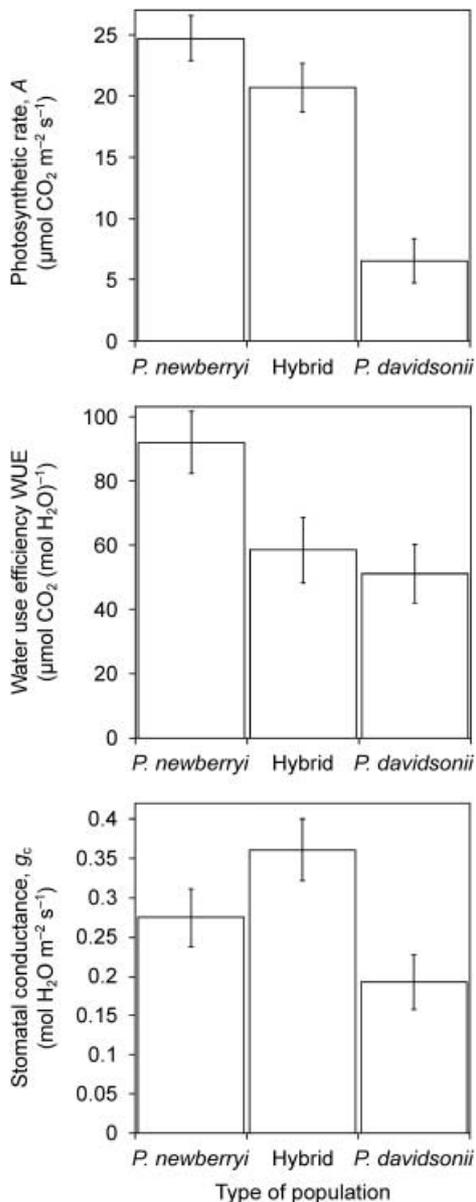**Table 3** Comparisons of gas exchange rates of nine cross types

Source of variation	df	Photosynthetic rate (*A*)	WUE	Conductance
Maternal	2	2.31	3.06	3.34*
Paternal	2	7.88*	1.03	4.63*
Maternal × paternal	4	1.43	0.75	1.68
D × D vs N × N	1	19.94**	0.46	11.83*
D × N vs N × D	1	0.98	0.02	0.87
H × H vs F$_1$	1	1.99	2.21	5.61*
F$_1$ vs parents	1	0.55	2.36	1.92
Error	116			

F-values are reported for two-way ANOVAs with type of mother and type of father as fixed factors, supplemented with four planned comparisons. D, *Penstemon davidsonii*; N, *Penstemon newberryi*; H, natural hybrid. The first letter indicates the identity of the mother and the second letter indicates the identity of the father. *, $P < 0.05$; **, $P < 0.0001$. WUE, water use efficiency.

hybrid $F_{1,19} = 6.359$, $P = 0.017$). Photosynthetic rates were lower for *P. davidsonii* individuals in the growth chamber (Fig. 3) than at comparable temperatures for individuals measured in pots outside (Fig. 2), possibly because of differences in the individuals measured or, more likely, because light intensities were lower in the growth chamber (average PAR intensity = 17 μmol m^{-2} s^{-1}) than outside (average PAR intensity = 1800 μmol m^{-2} s^{-1}). However, light intensities inside the leaf chamber were set to a PAR intensity of 1800 μmol m^{-2} s^{-1} as in all other experiments.
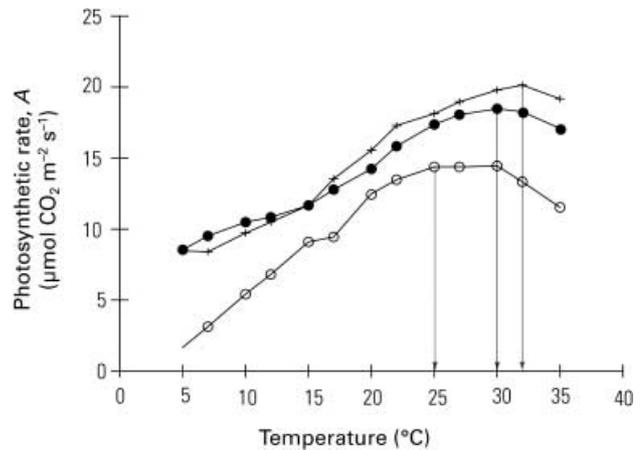
Plants grown from seeds generated by hand-pollination For plants grown in pots from seeds produced by hand-pollinations, gas exchange rates of hybrids varied depending on the cross type (Fig. 4, Table 3). Plants with *P. newberryi* fathers had higher A_{max} than plants with other fathers. *Penstemon davidsonii* had lower A_{max} than *P. newberryi* (Fig. 4, Table 3). The F$_1$ versus pure parents comparison indicated no hybrid vigor, nor

Fig. 3 Photosynthesis–temperature curves as measured with a Li-Cor 6400. Seven to ten individuals of *Penstemon davidsonii* (open circles), *Penstemon newberryi* (closed circles), and naturally occurring hybrids (crosses) grown from cuttings in pots at University of California, Irvine were measured at all temperatures in a growth chamber. Values are means. Arrows indicate the average temperature at which individuals of each type reached maximum photosynthetic rate (*A*).

Fig. 2 Instantaneous photosynthetic rate (carbon assimilation, or A_{max}), intrinsic water use efficiency (WUE = carbon assimilation/ stomatal conductance, or A_{max}/g_c), and stomatal conductance (g_c) of *Penstemon newberryi*, *Penstemon davidsonii*, and naturally occurring hybrids for plants growing in pots at University of California, Irvine. Values are means ± 1 SE.

was there any difference between F_1 and later generation (natural hybrid-by-natural hybrid) crosses. Plants with *P. newberryi* mothers and fathers had the highest stomatal conductance rates (Fig. 4, Table 3). Later generation hybrids had significantly higher rates than the two F_1 hybrid crosses (Fig. 4, Table 3), which could be a result either of natural selection for higher conductance acting on hybrids in the field, or of epistatic interactions between genes with different alleles fixed in the two parents that show up as a consequence of recombination in later generation crosses. The results for intrinsic WUE were

similar to those for plants grown from cuttings in that *P. newberryi* tended to have higher WUE than *P. davidsonii*. However, in this case, the effect of mother was only marginally significant (Table 3). We detected no reciprocal effects; for all of the gas exchange variables, F_1 hybrids with *P. newberryi* as the mother performed similarly to F_1 hybrids with *P. davidsonii* as the mother (Table 3).

Field populations In the field, photosynthetic rate and stomatal conductance differed significantly across the three sets of measurements (one from each population taken during its peak growth time; Table 2b). Whereas both of these variables changed with leaf to air VPD (Table 2b), for any given VPD, photosynthetic rates measured in the *P. davidsonii* population were higher than those measured in the *P. newberryi* population, with the hybrid population intermediate (Fig. 5). Although these results departed from the patterns seen for plants in pots, results for WUE did follow the prediction based on those earlier results. WUE (A_{max}/g_c) was again highest for the measurements taken from *P. newberryi* and lowest for those taken from *P. davidsonii*. Leaf internal CO_2 concentration supported the A_{max}/g_c results (*P. davidsonii* mean $c_i = 21.37$ Pa; hybrid = 18.23 Pa; *P. newberryi* = 16.99 Pa). As we mentioned earlier, field gas exchange measurements may reflect seasonal changes and should be viewed with caution.

Discussion

Mid-elevation *P. newberryi*, mid-elevation natural hybrids, and alpine *P. davidsonii* had differences in gas exchange traits, water potential, and reproductive phenology that are consistent with the environmental differences in their native habitats.
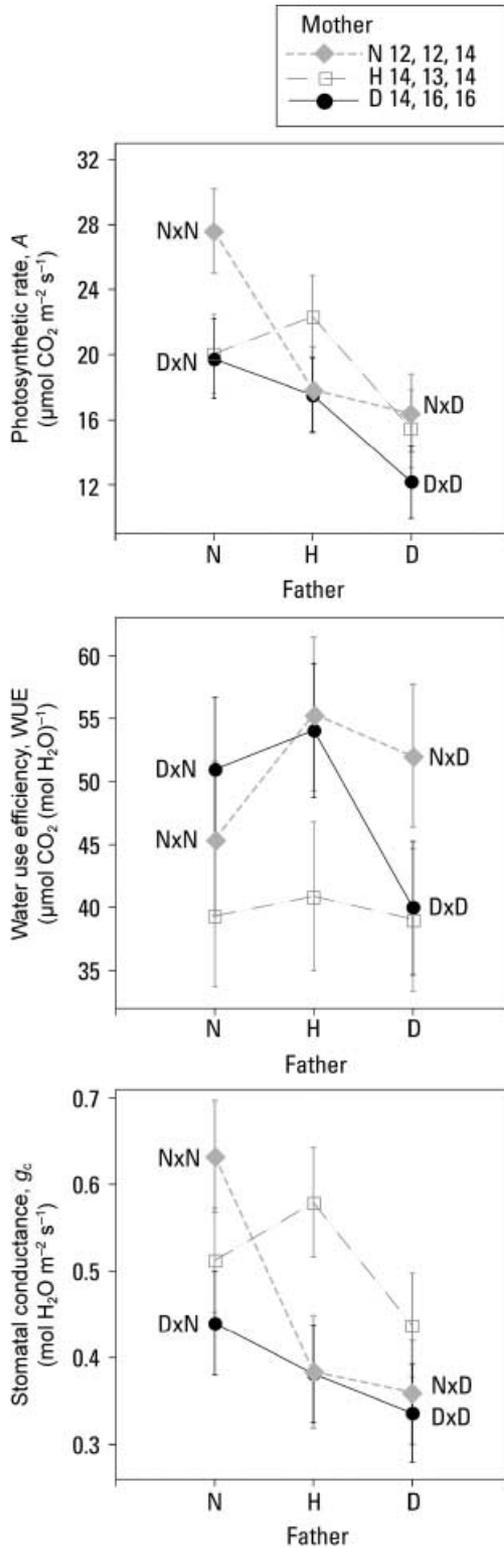
Fig. 4 Instantaneous photosynthetic rate (carbon assimilation, or A_{max}), intrinsic water use efficiency (WUE = carbon assimilation/ stomatal conductance, or A_{max}/g_c), and stomatal conductance (g_c) of nine plant types grown from seed in pots at University of California, Irvine. The identity of the mother is given in the key and the x-axis
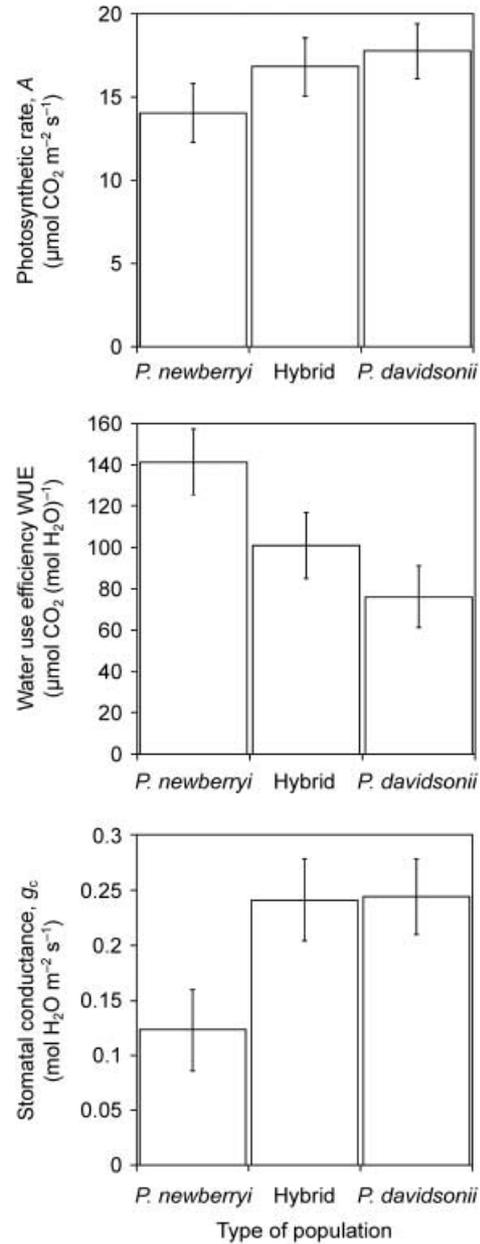
Fig. 5 Instantaneous photosynthetic rate (carbon assimilation, or A_{max}), intrinsic water use efficiency (WUE = carbon assimilation/ stomatal conductance, or A_{max}/g_c), and stomatal conductance (g_c) of *Penstemon newberryi* (2382 m elevation), *Penstemon davidsonii* (3505 m), and naturally occurring hybrid populations (2888 m) along the Lee Vining transect. Values are means ± 1 SE.

indicates the identity of the father, where: N, *Penstemon newberryi*; D, *Penstemon davidsonii*; H, naturally occurring hybrids. Pure parents and reciprocal F_1 hybrids are labeled where the first letter indicates the identity of the mother and the second letter indicates the identity of the father. Sample sizes are listed after each mother in the same order as the data are presented on the x-axis. Values are means ± 1 SE.

This raises the possibility that the two parent species and the natural hybrids may have physiological and phenological adaptations to different elevations. Such adaptations could help to maintain the structure of the hybrid zone by determining elevational range limits.

Species differences

Pre-dawn water potential measurements corresponded with temperature and VPD such that air and soil conditions became cooler and moister with increasing altitude. Although none of the water potential measurements was very low compared with other alpine and montane plant species (Korner, 1999), midday measurements tended to be more negative in low-altitude *P. newberryi* populations than in alpine *P. davidsonii* populations.

Penstemon newberryi populations required more time to set fruit than alpine *P. davidsonii* populations. Time from pollination to fruit dehiscence was also greater for the potted individuals of *P. newberryi* than *P. davidsonii* when grown under common conditions (Kimball *et al.*, 2008). Such differences in timing have also been demonstrated for other species (Woodward, 1990; Asselin *et al.*, 2003). For example, *Mimulus cardinalis* individuals were able to survive when planted at elevations higher than the natural altitudinal range, but were unable to reproduce in the short growing season (Angert & Schemske, 2005).

Based on gas exchange measurements, *P. newberryi* had higher intrinsic WUE than *P. davidsonii*. Although this difference was consistent across all experiments in both the field and the common garden, the manner by which *P. newberryi* achieved higher WUE differed depending on the environment. In the field, *P. newberryi* had higher WUE (A_{max}/g_c) as a result of very low g_c. Penstemon davidsonii had much higher g_c in the field, and its ability to maintain high g_c also allowed for higher A_{max} but still resulted in lower WUE than *P. newberryi*. For measurements of well-watered potted plants, *P. newberryi* had higher WUE than *P. davidsonii* as a result of higher A_{max}. Relative rates of g_c were switched in potted plants such that *P. davidsonii* had lower g_c than *P. newberryi*. The potted plants in the common garden at UCI were well outside the natural elevational ranges of these plants, and the differences in gas exchange rates in the field and in pots suggest genotype-by-environment interactions. Plants with high intrinsic WUE can have increased fitness in dry environments (Geber & Dawson, 1990; Dudley, 1996; Heschel & Riginos, 2005), like the habitat experienced by *P. newberryi*. Comparisons of populations from different sites often find that WUE is inversely related to soil moisture availability (Heschel *et al.*, 2002; McKay *et al.*, 2003), but also that WUE is highly plastic (Caruso *et al.*, 2006; Golluscio & Oesterheld, 2007).

Instantaneous gas exchange measurements were usually made with the Li-Cor gas exchange system set to a leaf temperature of 26°C, which was close to ambient during the time of measurement. Temperature response curves indicate that *P. davidsonii* reached A_{max} at 24.4°C compared with 30.6°C for *P. newberryi*. Alpine plants typically have lower optimal temperatures for photosynthesis (Field *et al.*, 1982; Korner, 1999) and also higher respiration rates than their lowland relatives when grown in warm environments, indicating that alpine species never fully acclimate to high temperatures (Korner, 1999). These dependences of photosynthetic rate on measurement conditions, and on whether plants were raised in the field or the common garden, while not surprising, underscore the importance of measuring hybrid physiological performance under more than one set of conditions, in contrast to most studies of wild hybrids.

Hybrid performance

Natural hybrids between these two species of *Penstemon* were intermediate for Ψ_{PD}, Ψ_{MD}, A_{max}, intrinsic WUE (except when grown in pots from seed), and reproductive phenology. For the photosynthesis–temperature curves, natural hybrids had higher photosynthetic rates than the parents, but all other measures of A_{max} were intermediate. These intermediate traits may help explain why hybrids can persist at intermediate elevations, which are also intermediate in several environmental conditions including temperature and VPD. The intermediacy of natural hybrids can reflect the combination of genes from parental species as well as subsequent environmental selection. Studies of natural hybrids in other genera have produced a range of outcomes for ecophysiological traits. Natural hybrids of subspecies of *Artemisia tridentata* had intermediate respiration and pre-dawn and midday water potential (McArthur *et al.*, 1998). Natural hybrids of two species of pines had higher WUE than either parental species (Silim *et al.*, 2001). The species *Helianthus anomalus*, which is thought to have arisen through diploid hybrid speciation, had some ecophysiological traits that were similar to those of one or the other parent species and was transgressive for other ecophysiological traits (Schwarzbach *et al.*, 2001; Ludwig *et al.*, 2004).

Hybrid performance reflects how the genes combine in their expression as well as the characteristics of the habitats where hybrids are found (Arnold, 1997), so we might expect hybrids of different genetic backgrounds to perform differently. Our comparisons allowed tests for heterosis, reciprocal effects in the F_1, and comparisons between F_1 and advanced generation hybrids. Although the F_1 hybrids had slightly higher mean WUE than the parents, they were not significantly different from the mid-parent value for any gas exchange trait and so showed no heterosis in ecophysiology. Few other such tests exist for wild (as opposed to crop plant) species, but F_1 hybrids between two species of *Ipomopsis* showed strong heterosis in instantaneous WUE measured in a common field garden (Campbell *et al.*, 2005). Studies of *Ipomopsis* have also detected strong reciprocal effects where gas exchange traits depend on which species is used as the cytoplasmic parent

(Wu & Campbell, 2007), whereas no such reciprocal effects were evident for these *Penstemon* F_1 hybrids, even though they were observed for survival (Kimball *et al.*, 2008).

Natural hybrids potentially represent the result of advanced generation crosses and backcrosses as well as selection on those recombinant genotypes in the field, so they might exhibit physiological traits that increase fitness. When grown in pots, the F_1 *Penstemon* hybrids had significantly lower conductance (g_c) and slightly higher WUE (although not significantly so) than natural hybrid-by-natural hybrid crosses. It is not clear whether natural hybrids would have higher g_c than F_1 hybrids in the field, but differences in potted plants do indicate that natural hybrids have different g_c values from F_1 hybrids when grown in a common environment. This study joins a small number of studies that have tested for, and found, differences in physiological traits of hybrids depending on the type of hybrid cross. For example, backcrosses to *Iris fulva* had lower A_{max} than other types of hybrids between that species and *Iris brevicaulis* (Johnston *et al.*, 2001). Such results highlight how critical it is to include diverse types of hybrids with known genetic background in future studies of other systems and not to rely entirely on natural hybrids to assess physiological traits.

This study identified several physiological traits that differed among the parent species and hybrids and that corresponded with differences in environmental conditions across the elevational gradient. Differences in WUE were maintained in common conditions, despite suggestions of genotype-by-environment interactions in the underlying traits of A_{max} and g_c. The results suggest the hypothesis that *P. newberryi* is unable to flower and set seed during the short growing season at high elevations, that the lower WUE of *P. davidsonii* prevents it from flourishing at low elevations, and that hybrids survive particularly well at intermediate elevations. A reciprocal transplant experiment has provided partial support for this hypothesis, in that F_1 hybrids had higher survival than other plant types in a mid-elevation garden and *P. davidsonii* did not survive at low elevation (Kimball *et al.*, 2008). However, the low-elevation species *P. newberryi* actually had lower survival than F_1 hybrids in the low-elevation garden (Kimball *et al.*, 2008), suggesting that traits other than those investigated here also influence patterns in survival. Overall, the difference in physiological performance of F_1 hybrids and natural hybrids lends some support to the 'evolutionary novelty' model for hybrid zones, in which hybrids of certain genetic backgrounds can perform exceptionally well in particular environments (Arnold, 1997).

Acknowledgements

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