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## Environmental stressors differentially affect leaf ecophysiological responses in two *Ipomopsis* species and their hybrids

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**Abstract** The recombination that follows natural hybridization may produce hybrid genotypes with traits that are intermediate or extreme relative to the parental species, and these traits may influence the relative fitness of the hybrids. Here we examined leaf ecophysiological traits that may influence fitness patterns in a natural plant hybrid zone. We compared the biochemical photosynthetic capacity of *Ipomopsis aggregata*, *I. tenuituba*, and early generation hybrids, as well as their photosynthetic responses to varying light and temperature, two abiotic factors found to differ among sites along the hybrid zone. In general, ecophysiological traits expressed in these plants were consistent with their natural habitat, even when grown under common greenhouse conditions. *I. tenuituba* reached higher photosynthetic rates ( $A$ ) at higher light levels than *I. aggregata*, and also had a higher optimal temperature for photosynthesis ( $T_{opt}$ ). This suite of traits may reflect adaptations to the more exposed, rocky sites where *I. tenuituba* is found, compared to the more vegetated, mesic *I. aggregata* site. Hybrids had characters that were largely intermediate or *tenuituba*-like, but particular individual hybrids were extreme for some traits, including light saturation level, light-saturated  $A$ , and  $T_{opt}$ . Many of these traits are consistent with adaptations reported for plants found in warm, dry sites, so they may put certain hybrids at an advantage at the relatively xeric center of the natural hybrid zone.

**Keywords** Environment-dependent selection · Hybridization · *Ipomopsis* · Temperature response · Photosynthesis

### Introduction

Natural hybridization has long been recognized as an important factor in the evolution of flowering plants (Anderson 1949; Grant 1981). The recombination that accompanies hybridization can produce hybrid individuals with a range of genotypes. Although many recombinant genotypes perform poorly due to genomic incompatibilities (Li et al. 1997; Fritz et al. 1998), other hybrids can be at least as fit as the parental taxa (Cruzan and Arnold 1994; Arnold and Hodges 1995; Rieseberg et al. 1996; Burke et al. 1998; Galloway and Fenster 1999; Fritsche and Kaltz 2000; Campbell and Waser 2001). Further, some hybrid genotypes demonstrate novel characters relative to their parents, and may have higher fitness than the parents in particular environments (reviewed in Rieseberg and Ellstrand 1993; Arnold 1997; also Burke et al. 1998; Neuffer et al. 1999; Rosenthal et al. 2002). The potential for natural hybridization to act as a creative force in evolution, such as through the formation of novel evolutionary lineages, depends on the production of such recombinant genotypes that can outperform the parental taxa in at least some habitats (Arnold 1997; Burke and Arnold 2001; Rieseberg et al. 2003). However, we still lack a fundamental understanding of the mechanisms that underlie fitness differences between parental and hybrid plants.

Such fitness differences can arise at any life history stage, and can involve both morphological and physiological characteristics that enable the plant to survive or reproduce better in a particular environment. For plants, photosynthetic traits that govern C acquisition have an integral role in vegetative growth (Lambers et al. 1998; Arntz et al. 2000), and thus may affect individual fitness. Plant photosynthesis will depend, in part, on leaf metabolic capacity, which can be

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partitioned into the activity of the enzyme ribulose-1,5-bisphosphate (RuBP) carboxylase-oxygenase (Rubisco) that catalyzes the first step in C fixation ( $V_{c_{max}}$ ), the rate of RuBP regeneration ( $J_{max}$ ) via electron transport, and other minor components (Wullschlegel 1993). High photosynthetic rates ( $A$ ) achieved by maximizing biochemical capacity for photosynthesis through either of these components could contribute to the success of certain genotypes under stressful conditions (Geber and Dawson 1997; McDowell 2002).

Photosynthesis will also depend upon numerous environmental factors, including light, temperature, and water availability. Because of its dependence on the availability of radiation, plants often show a suite of adaptations to maximize photosynthetic efficiency depending on their light environment (Björkman 1981b; Givnish 1988; Larcher 1995; but see Heschel et al. 2004). Provided sufficient light, temperature is a major controller of leaf CO<sub>2</sub> exchange, and many plants also exhibit temperature-dependent photosynthetic responses that optimize C uptake near their normal growth temperature (Berry and Björkman 1980; Körner and Diemer 1987). Consequently, photosynthetic responses to light and temperature have been used as mechanistic explanations for the distribution of numerous plant species, as they are important in determining physiological tolerances to environmental conditions (Givnish 1988; Bazzaz 1991; Williams and Black 1993; Hamerlynck and Knapp 1996). Similarly, if a hybrid zone occurs where there is habitat heterogeneity, then differences in eco-physiological responses to the abiotic environment should influence the distribution of hybrids and parental species. Such physiological traits are just beginning to receive attention in studies of hybrids between native species as mechanisms of environment-mediated selection, with most effort to date concentrated on photosynthetic traits involved in water relations (McArthur et al. 1998; Johnston et al. 2001; Schwarzbach et al. 2001; Silim et al. 2001; Ludwig et al. 2004).

The present study is part of a series of experiments that investigate the broad question of whether physiological trait expression influences environment-dependent fitness patterns in a natural plant hybrid zone. Previous work suggests that the genetic structure of a hybrid zone between two closely related species of the montane herb *Ipomopsis* is influenced by a mixture of pollinator- and habitat-mediated selection (Campbell and Waser 2001; Campbell et al. 2002). Hybrids and parental species exhibit environment-dependent survival across the hybrid zone (Campbell and Waser 2001), but the critical ecological factors that cause these survival differences are unknown. If divergent ecological pressures are driving the habitat-mediated selection patterns in this hybrid zone, then hybrids and parental plants are expected to have different physiological responses to the abiotic factors that change across the sites. To address this question, a first step is to characterize aspects of photosynthetic physiology that might influence fitness patterns in this hybrid zone. In a parallel

study, we have found that high water-use efficiency (WUE) may help to explain the relatively high survival of hybrids at dry sites within the hybrid zone (Campbell et al. 2005). The study reported here complements that work by examining photosynthetic responses to additional environmental variables shown to vary across the natural hybrid zone.

The objective of this study was to compare the eco-physiological responses of two naturally hybridizing species of *Ipomopsis* (Polemoniaceae) and several types of experimentally produced hybrids to variation in several environmental conditions. First (F1) and second (F2) generation hybrids were included in the study because it is not until the second generation that recombinant nuclear genotypes are formed. Plants were grown from seed in pots under common greenhouse conditions, to examine physiological responses that might be genetically based, rather than a consequence of different germination and growth environments. The main goals of this study were to determine: (1) how photosynthetic light and temperature responses differ among *Ipomopsis* hybrids and their parental species, (2) if the relative responses of parental and hybrid plants to these factors were consistent with their distribution in the natural hybrid zone, and (3) whether *Ipomopsis* hybrids and their parental species differed in their biochemical capacity for photosynthesis.

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## Materials and methods

### Study system

*Ipomopsis aggregata* and *I. tenuituba* are found throughout the western US, and hybridization between the species is often extensive. One such hybrid zone is located along Poverty Gulch, a montane valley draining into the Slate River Valley in Gunnison County, CO that is located 10 km from the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO. Populations of *I. aggregata* are found on the gently sloping, vegetated meadows along the base of the valley up to 2,900 m, while *I. tenuituba* grows on the steep rocky slopes above 3,100 m, and a natural hybrid zone occurs in the intermediate elevations (Campbell et al. 1997). Much of the central region of this contact zone where natural hybrids are located is characterized by steep talus slopes with relatively low vegetative cover. Light intensity is relatively high at sites across Poverty Gulch, typically  $>1,700 \mu\text{mol m}^{-2} \text{s}^{-1}$  at midday (Campbell et al. 2005).

In Poverty Gulch, both species are self-incompatible and monocarpic. Seeds produced in late summer overwinter in the soil and germinate into small vegetative rosettes during the next summer growing season. Individual plants typically spend 2–10 years in this vegetative stage before blooming during a single summer season, and die after seed production (Campbell 1997; Campbell and Waser 2001).

We used a common garden approach to determine the extent to which physiological differences among *Ipomopsis* hybrids and parental species were genetic, rather than environmentally induced. Six genotypic classes of plants were examined: *I. aggregata* (AA), *I. tenuituba* (TT), reciprocal F1 hybrids (F1<sub>A</sub> and F1<sub>T</sub>, where the subscript indicates the maternal cytoplasmic type), and reciprocal F2 hybrids (F2<sub>A</sub> and F2<sub>T</sub>). We included hybrids with both cytoplasmic types in the study, because in this system F1 hybrid survival depends in part on maternal lineage (Campbell and Waser 2001). All plants were generated from hand-pollinating potted plants (see below) and raised from seed in WeatherPort portable mesh greenhouses (Hansen WeatherPort, Olathe, Colorado) at the RMBL, where they were fertilized monthly with a 12–6–6 liquid solution during the summer and watered every 2–3 days as needed. Individual *I. aggregata* and *I. tenuituba* plants were potted in 2000 from pure populations in Poverty Gulch (sites L and A in Campbell et al. 1997) and used as parents for conspecific crosses (*I. aggregata* and *I. tenuituba*) and interspecific crosses to make reciprocal F1 hybrids (*I. aggregata* × *I. tenuituba*). Additional F1 hybrids were generated in 2002 from parental plants raised from seed in pots from crosses in 2000. F2 plants used in this study were part of 13 reciprocal families produced in 2002 from F1s generated in 2000 as part of a separate study. For each set of response curves, all plants in each genotypic class were from independent families to incorporate as much diversity as possible. Raising the parents from seed eliminated the possibility of maternal environmental effects on performance of most of the progeny tested here. However, performance of the AA and TT plants could in principle reflect differences in the field environments of their parents. Such maternal environmental effects are likely small, as previous studies have suggested they are typically mediated through seed mass (Campbell 1997), and plants of the two species that are potted while bolting, as done here, do not differ in seed mass (Campbell and Waser 2001).

#### Characterization of the native environment

In Poverty Gulch, the *Ipomopsis* hybrid zone occurs along an elevational cline. Previous work has found that the region where *I. aggregata* grows has less extreme temperature fluctuations and is more mesic than the center of the hybrid zone (Campbell et al. 2005). To further characterize differences among locations in Poverty Gulch where the parental *Ipomopsis* species and their natural hybrids are found, temperature and relative humidity (RH) were measured every 10 min from 10 July to 30 July 2003 using Hobo data loggers. These sensors were placed in partial shade in *I. aggregata*, natural hybrid, and *I. tenuituba* habitats (sites L, I, and C, respectively, in Campbell et al. 1997) approximately 4 cm above the ground, similar to the height of the vegetative *Ipomopsis* rosettes. Data were downloaded

using BoxCar Pro software and used to determine daily maxima and minima for temperature, RH, and vapor pressure deficit (VPD, following Murray 1967). Environmental conditions across the sites were compared using randomized complete block ANOVA with day as the blocking factor, followed by Tukey a posteriori comparisons (SPSS 11.0.2; SPSS, Chicago, IL) to determine whether sites differed for specific environmental variables.

#### Gas exchange measurements

Rates of photosynthesis ( $A$ , in  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) in relation to varying environmental conditions were measured on fully expanded leaves from potted plants in WeatherPorts at the RMBL during July and August 2004 using a Li-Cor 6400 infrared gas-exchange system (Li-Cor, Lincoln, NE). The order of individuals measured for each response curve was random. Following gas exchange measurements, the leaves were collected in coin envelopes and their areas determined within 1 h using a scanner with ROOTEDGE software (National Soil Tilth Laboratory, Ames, IA). All reported gas exchange parameters have been corrected for leaf area.

#### Light response curves

Measurements were made on four individuals from each genotypic class during the month of July, between 0900 and 1200 hours. Leaf temperature inside the cuvette was  $27 \pm 0.5^\circ\text{C}$  and  $\text{CO}_2$  concentration was 350 ppm, while VPD was allowed to vary with ambient conditions. Averages of two measurements per leaf were obtained at the following levels of photosynthetic photon flux density (PPFD): 400, 800, 1,200, 1,600, 2,000, and 2,400  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ . We were primarily interested in two light response curve parameters: the light-saturated  $A$  ( $A_{\text{lightmax}}$ ), and the light level at which photosynthesis is light saturated (light saturation level). These values were determined for each individual plant using the curve-fitting program Photosyn Assistant v. 1.1 (Dundee Scientific, Scotland), which follows estimation methods of Prioul and Chartier (1977) and Walker (1989) that incorporate a non-rectangular hyperbola model. This model produced  $A_{\text{lightmax}}$  values that were consistent with those based on fitting the Michaelis–Menten model (e.g. Givnish et al. 2004) to our measurements [data not shown; see also Peek et al. (2002) and Heschel et al. (2004) for discussions of other common light curve-fitting models].

Differences in light curve parameters were analyzed using separate one-way ANOVAs in Proc GLM (SAS 8.2; SAS Institute, Cary, NC), with five a priori contrasts to test for differences between species, heterosis (a difference between the F1 and midparent value), hybrid breakdown (difference between the F2 and mean of the F1 and midparent), a difference overall between hybrids

and parentals, and an effect of cross direction in hybrids. The tests for species differences, heterosis, and hybrid breakdown are statistically independent of each other and are the basis for examining the genetic architecture of quantitative traits (Lynch and Walsh 1998). The remaining contrasts were added to test for a more general form of hybrid vigor, that is, if hybrids in general exceeded the midparent value, and if response parameters depended on the cytoplasmic background of the hybrids.

Light levels used in subsequent temperature-response and  $A-c_i$  curves were set above the light saturation levels for all genotypic classes determined here, to minimize the effects of light limitation to photosynthesis.

### Temperature response curves

The temperature response of light-saturated photosynthesis was measured on four plants per genotypic class at a photon flux density of  $1,800 \mu\text{mol m}^{-2} \text{s}^{-1}$ . All temperature response measurements were made before 1030 hours in July and August. During all measurements, VPD was allowed to vary inside the cuvette. Two to three measurements per leaf were made at each of nine temperatures, after leaf temperature had been maintained for 20 min: 15, 17, 20, 22, 25, 27, 30, 32, and 35°C. This range was designed to span most of the midday summer temperatures experienced by *Ipomopsis* in the natural hybrid zone from where these plant lineages originated (Campbell et al. 2005).

The optimal temperature for photosynthesis ( $T_{\text{opt}}$ ) was calculated from the first derivative of the polynomial curve fitted to the temperature response data for each individual plant. Temperature optima and  $A$  achieved at the  $T_{\text{opt}}$  ( $A_{T_{\text{opt}}}$ ) for each genotypic class were compared using ANOVA, with the same five a priori contrasts described above.

### $A-c_i$ curves

Plants may also differ in their biochemical limitations to photosynthesis (von Caemmerer and Farquhar 1981; Sharkey 1985). The biochemical capacity for photosynthesis can be examined using the response curve of photosynthetic  $\text{CO}_2$  assimilation ( $A$ ) to internal leaf  $\text{CO}_2$  concentrations ( $c_i$ ). From this  $A-c_i$  curve, limitations to photosynthetic performance can be partitioned into several major metabolic components, including  $V_{c_{\text{max}}}$ , and  $J_{\text{max}}$  via electron transport (Wullschlegel 1993).

$A-c_i$  curves were measured on five plants from each class. During all measurements, the temperature was  $27 \pm 0.5^\circ\text{C}$ , the photon flux density was  $1,800 \mu\text{mol m}^{-2} \text{s}^{-1}$  and the VPD was  $2.68 \pm 0.20 \text{ kPa}$  inside the cuvette. Three measurements were made on each leaf at each of the following  $\text{CO}_2$  concentrations: 50, 100, 200, 300, 350, 400, 600, and 800 ppm. To generate the  $A-c_i$  curves, measurements were first taken at a

$[\text{CO}_2]$  of 350 ppm, then the  $[\text{CO}_2]$  was increased, decreased, and returned to 350 ppm

These  $A-c_i$  curves were used to examine biochemical photosynthetic parameters of each genotypic class, including  $V_{c_{\text{max}}}$ ,  $J_{\text{max}}$ , and the maximum  $A$  at ambient  $\text{CO}_2$  levels ( $A_{\text{max}}$ ).  $V_{c_{\text{max}}}$  and  $J_{\text{max}}$  were estimated from the  $A-c_i$  curves using a mechanistic model in Photosyn Assistant (Dundee Scientific), based on the photosynthetic model of Farquhar et al. (1980) that was subsequently modified by von Caemmerer and Farquhar (1981), Sharkey (1985), Harley and Sharkey (1991), and Harley et al. (1992). Briefly,  $V_{c_{\text{max}}}$  is estimated from the  $A-c_i$  curve at low  $c_i$  concentrations, where  $A$  is assumed to be limited by the Rubisco-limited rate of carboxylation. At high  $c_i$  concentrations, however,  $A$  is instead limited by the light-saturated rate of electron transport ( $J_{\text{max}}$ ) that regenerates RuBP (Lambers et al. 1998). These parameters are calculated by a minimization routine that produces values that represent the best fit for the  $A-c_i$  curve data. Variation in  $A_{\text{max}}$ ,  $V_{c_{\text{max}}}$ , and  $J_{\text{max}}$  was analyzed using three separate one-way ANOVAs, followed again by five a priori contrasts to examine how the genotypic classes differed for these biochemical parameters.

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## Results

### Environmental conditions in the natural hybrid zone

Maximum daily temperatures were consistently higher at the *I. tenuituba* site than at the *I. aggregata* site, and significantly higher yet at the natural hybrid site, reaching temperatures on average over  $37^\circ\text{C}$  (Table 1). Minimum nighttime temperatures were also warmest at the hybrid site, and coolest at the *I. aggregata* site (Table 1). Daily RH was lowest at the hybrid site, followed by the *I. tenuituba* and *I. aggregata* sites (Table 1). Similarly, daily maximum and minimum VPDs were greater at the hybrid site than either of the parental sites (Table 1).

Overall, conditions were warmer and drier at the *I. tenuituba* site than at the *I. aggregata* site, and the natural hybrid site was even warmer and drier than either parental location. These differences are likely due to the higher vegetative cover at the *I. aggregata* sites, relative to the greater amount of exposed rocky substrate and steeper slope at the *I. tenuituba* and hybrid sites, and the more southerly aspect at the center compared to either end of the hybrid zone.

### Photosynthetic light responses

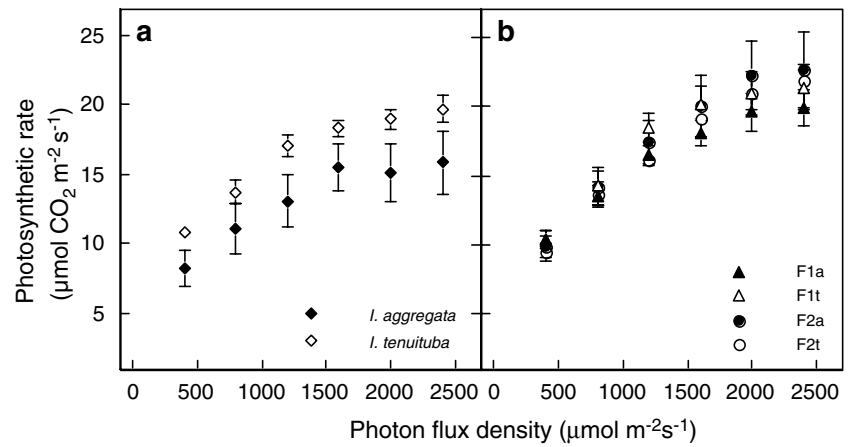
As expected, photosynthetic C gain increased with increasing PPFD (Fig. 1), and light saturation was reached between ca.  $1,000\text{--}1,600 \mu\text{mol m}^{-2} \text{s}^{-1}$  for all genotypes (Table 2), below the  $1,800 \mu\text{mol m}^{-2} \text{s}^{-1}$  used in the other photosynthetic response curves measured in

**Table 1** Environmental conditions at vegetative rosette height (4 cm above the ground) at natural *Ipomopsis aggregata*, hybrid, and *I. tenuituba* sites in Poverty Gulch, CO. Different letters

	<i>I. aggregata</i> site		Hybrid site		<i>I. tenuituba</i> site	
	Mean	SE	Mean	SE	Mean	SE
Max <i>T</i> (°C)	31.5a	0.7	37.2b	0.5	32.5c	0.9
Min <i>T</i> (°C)	5.3a	0.3	8.6b	0.3	7.8c	0.3
Max RH (%)	94.3a	2.5	79.4b	4.7	85.2c	3.9
Min RH (%)	23.2a	2.6	13.8b	1.7	20.3a	1.8
Max VPD (kPa)	3.87a	0.3	5.52b	0.24	3.66a	0.21
Min VPD (kPa)	0.05a	0.02	0.27b	0.07	0.19c	0.05

*T* Temperature, *RH* relative humidity, *VPD* vapor pressure deficit are average daily maximum and minimum values from 10 July to 30 July 2003 (*n* = 20 days)

**Fig. 1** Photosynthetic light response curves for **a** parental species *I. aggregata* and *I. tenuituba* and **b** reciprocal F1 and F2 hybrids [mean values  $\pm$  SE (*n* = 4)]. Open symbols are genotypes with *tenuituba* cytoplasmic background, and closed symbols are genotypes with *aggregata* cytoplasmic background



this study. The parental species and F1 hybrids had similar light saturation levels (Table 3), while the F2 hybrids saturated at significantly higher light levels ( $P = 0.0029$ ), which also resulted in hybrids as a whole having higher light saturation levels than the parents ( $P = 0.0172$ ; Table 3).

*I. tenuituba* reached higher  $A_{\text{lightmax}}$  than *I. aggregata* ( $P = 0.0532$ ; Table 2). Overall, hybrids had  $A_{\text{lightmax}}$  values that were *tenuituba*-like (*I. tenuituba* mean = 25.7) to positively transgressive (mean for hybrid cross types = 21.7–30.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). While there was no evidence for heterosis in  $A_{\text{lightmax}}$  ( $P = 0.8057$ ), F2

hybrids had significantly higher  $A_{\text{lightmax}}$  than the average of the F1 and parental types ( $P = 0.0050$ ), driving the trend for hybrids in general to have higher  $A_{\text{lightmax}}$  than the parental types ( $P = 0.0863$ ; Table 3).

#### Photosynthetic temperature responses

All genotypes showed an initial increase in  $A$  as leaf temperature was raised above 15°C (Fig. 2).  $A$  continued to increase to a  $T_{\text{opt}}$  of 33.2°C in *I. tenuituba*, while leaves from *I. aggregata* reached  $T_{\text{opt}}$  at 21.7°C ( $P = 0.0006$ ). In

**Table 2** Mean values ( $\pm 1$  SE) for physiological traits determined from photosynthetic response curves to short-term stresses for the parental *Ipomopsis* species and reciprocal F1 and F2 hybrids grown in pots at the Rocky Mountain Biological Laboratory (RMBL) in

	<i>I. aggregata</i>	<i>I. tenuituba</i>	F1 <sub>A</sub>	F1 <sub>T</sub>	F2 <sub>A</sub>	F2 <sub>T</sub>
Light response						
$A_{\text{lightmax}}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	17.2 $\pm$ 0.4	25.7 $\pm$ 1.5	21.7 $\pm$ 1.1	22.7 $\pm$ 1.7	28.9 $\pm$ 2.1	30.7 $\pm$ 1.3
Light saturation level (PPFD)	1,013 $\pm$ 40	1,045 $\pm$ 93	1,118 $\pm$ 195	1,324 $\pm$ 126	1,659 $\pm$ 250	1,686 $\pm$ 275
Temperature response						
$T_{\text{opt}}$ (°C)	21.7 $\pm$ 1.32	33.2 $\pm$ 0.57	29.5 $\pm$ 0.94	32.4 $\pm$ 1.48	26.9 $\pm$ 1.67	26.4 $\pm$ 3.17
$A_{T_{\text{opt}}}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	20.0 $\pm$ 0.2	27.7 $\pm$ 0.9	18.0 $\pm$ 2.8	25.2 $\pm$ 1.3	17.7 $\pm$ 0.5	22.6 $\pm$ 1.9

Statistical comparisons of these traits are provided in Tables 3 and 4

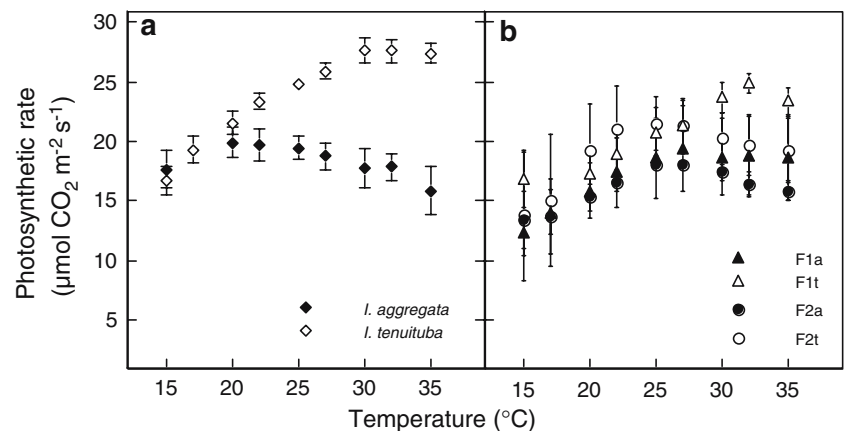
Gothic, CO.  $A_{\text{lightmax}}$  Light-saturated rate of photosynthesis,  $PPFD$  photosynthetic photon flux density,  $T_{\text{opt}}$  optimal temperature for photosynthesis

**Table 3** ANOVA comparing photosynthetic light response curve parameters for experimentally produced parental *Ipomopsis* species and reciprocal F1 and F2 hybrids grown in pots at the RMBL. Five a priori contrasts were specified using the coefficients in parentheses, with genetic classes ordered alphabetically: *I. aggregata* (AA), F1<sub>A</sub> (AT), F2<sub>A</sub>, F2<sub>T</sub>, F1<sub>T</sub> (TA), *I. tenuituba* (TT)

Source	df	SS	F	P
Character: $A_{\text{lightmax}}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )				
Model	5	501.1	2.97	0.0400*
AA vs. TT (1 0 0 0 0 -1)	1	144.5	4.28	0.0532
Heterosis (-0.5 0.5 0 0 0.5 -0.5)	1	2.1	0.06	0.8057
Breakdown (0.25 0.25 -0.5 -0.5 0.25 0.25)	1	345.6	10.24	0.0050*
Hybrids extreme (-0.5 0.25 0.25 0.25 0.25 -0.5)	1	111.3	3.30	0.0860
Cytotype (0 0.5 0.5 -0.5 -0.5 0)	1	8.3	0.25	0.6266
Error	18	607.2		
Character: light saturation level ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )				
Model	5	1,834,420.7	2.72	0.0535
AA vs. TT (1 0 0 0 0 -1)	1	2,145.1	0.02	0.9011
Heterosis (-0.5 0.5 0 0 0.5 -0.5)	1	146,880.6	1.09	0.3109
Breakdown (0.25 0.25 -0.5 -0.5 0.25 0.25)	1	1,599,065.0	11.84	0.0029*
Hybrids extreme (-0.5 0.25 0.25 0.25 0.25 -0.5)	1	929,633.3	6.88	0.0172*
Cytotype (0 0.5 0.5 -0.5 -0.5 0)	1	54,289.0	0.40	0.5341
Error	18	2,431,736.3		

\* $P < 0.05$

**Fig. 2** Photosynthetic temperature response curves for **a** parental species *I. aggregata* and *I. tenuituba* and **b** reciprocal F1 and F2 hybrids [mean values  $\pm$  SE ( $n=4$ )]. Open symbols are genotypes with *tenuituba* cytoplasmic background, and closed symbols are genotypes with *aggregata* cytoplasmic background



general, hybrids had intermediate  $T_{\text{opt}}$  values relative to the parental species ( $P=0.4251$ ), but they were typically greater than that for *I. aggregata* (Table 4). The  $T_{\text{opt}}$  did not show evidence for heterosis ( $P=0.0880$ ), nor was there evidence for hybrid breakdown in the F2s, or an effect of cytotype in the hybrids (Table 4). Nonetheless, some individual hybrids had  $T_{\text{opt}}$  values higher than either parental species.

Interestingly, there was a strong effect of cytotype on  $A_{T_{\text{opt}}}$  in the hybrids, with plants having the *I. tenuituba* cytoplasmic background reaching significantly higher  $A_{\text{max}}$  at their temperature optima, compared to hybrids with the *I. aggregata* cytoplasm ( $P=0.0009$ ; Table 4). This mirrors the pattern found in the parental species, where  $A_{T_{\text{opt}}}$  in *I. tenuituba* ( $A_{T_{\text{opt}}}$  mean =  $27.7 \pm 0.9$ ) significantly exceeded that for *I. aggregata* ( $20.0 \pm 0.2$ ,  $P=0.0022$ ). As a whole, hybrids reached higher  $A_{T_{\text{opt}}}$  than the average of the parents ( $P=0.0362$ ), again largely driven by the F1<sub>T</sub> and F2<sub>T</sub> hybrid classes (Table 2).

#### $A-c_i$ curves

None of the  $A-c_i$  curves for these plants showed strong evidence for reaching an asymptote within the range of  $c_i$

measured here (Fig. 3). This lack of asymptote in the  $A-c_i$  curve is not unprecedented (Körner and Diemer 1987; Geber and Dawson 1997) and should not affect estimates of  $A_{\text{max}}$  or  $V_{c_{\text{max}}}$ , but values of  $J_{\text{max}}$  will likely be underestimated.

The  $A-c_i$  curves indicated that *I. tenuituba* has a higher biochemical capacity for photosynthesis than *I. aggregata* and achieved higher  $A$  under ambient  $\text{CO}_2$  levels ( $P=0.0002$ ; Fig. 4), while hybrids were intermediate to the parental types (Table 5). The higher  $A_{\text{max}}$  in *I. tenuituba* was supported by both greater  $V_{c_{\text{max}}}$  and  $J_{\text{max}}$  (Table 5), and in general genotypes with high Rubisco activity also had high electron transport capacity (Fig. 4), suggesting that photosynthesis was co-limited by  $V_{c_{\text{max}}}$  and  $J_{\text{max}}$  in all six genotypic classes. Hybrid breakdown in the F2 was detected for both  $V_{c_{\text{max}}}$  and  $J_{\text{max}}$  (Fig. 4; Table 5), while F1 hybrids showed heterosis for  $J_{\text{max}}$  (Table 5). Nonetheless, no hybrid class showed reduced  $V_{c_{\text{max}}}$  or  $J_{\text{max}}$  relative to both parents (Fig. 4).

#### Discussion

Ecological divergence between hybrids and their progenitors may enable hybrids to colonize intermediate

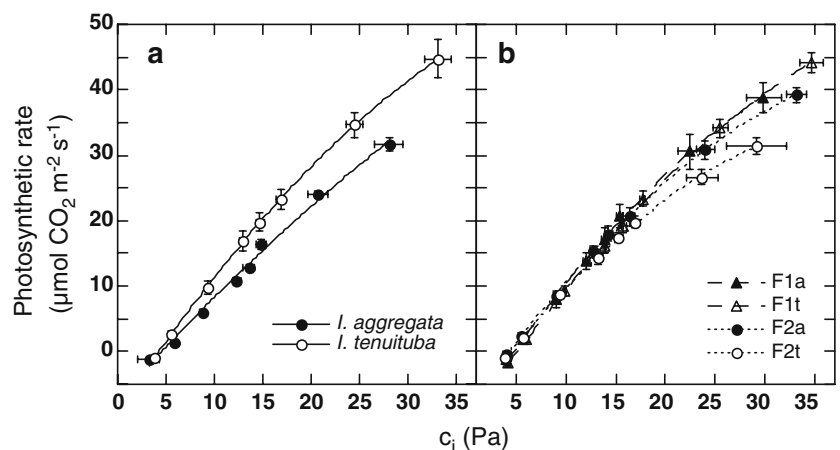
**Table 4** ANOVA for the photosynthetic temperature response curve parameters, optimal temperature for photosynthesis ( $T_{opt}$ ) and maximum  $A$  at  $T_{opt}$  ( $A_{T_{opt}}$ ) for experimentally produced parental *Ipomopsis* species and reciprocal F1 and F2 hybrids grown in pots at the RMBL. For abbreviations, see Tables 2 and 3

Source	df	SS	F	P
Character: $T_{opt}$ (°C)				
Model	5	362.61	4.75	0.0061*
AA vs. TT	1	262.21	17.17	0.0006*
Heterosis	1	49.70	3.25	0.0880
Breakdown	1	34.00	2.23	0.1530
Hybrids extreme	1	10.18	0.67	0.4251
Cytotype	1	5.64	0.37	0.5510
Error	18	637.54		
Character: $A_{T_{opt}}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )				
Model	5	323.78	7.00	0.0009*
AA vs. TT	1	117.81	12.73	0.0022*
Heterosis	1	20.25	2.19	0.1564
Breakdown	1	35.71	3.86	0.0651
Hybrids extreme	1	47.40	5.12	0.0362*
Cytotype	1	144.60	15.62	0.0009*
Error	18	166.61		

\* $P < 0.05$

habitats (Anderson 1948) or environments that are novel to those of the parental species (Rieseberg et al. 2003). If so, these hybrids should differ from their parents for traits that enable them to occupy the divergent habitats. In this study, sites where *I. aggregata*, *I. tenuituba*, and their hybrids occur along a natural hybrid zone in central Colorado were found to differ in several abiotic parameters. Further, the ecophysiological traits expressed in these plants were generally consistent with their natural habitat, even when grown under common greenhouse conditions. In general, hybrids had ecophysiological characters that were intermediate or equivalent to the parental species, but certain individual hybrids were extreme for some photosynthetic traits. This suggests that hybridization in this system has the potential to produce hybrids capable of outperforming the parents in certain environments.

**Fig. 3**  $A-c_i$  curves for **a** parental *I. aggregata* and *I. tenuituba* and **b** reciprocal F1 and F2 hybrids. Each curve is an average of curves measured from five plants in each genotypic class. The maximum carboxylation ( $V_{c_{max}}$ ) and electron transport rates ( $J_{max}$ ) for each class were calculated from these curves. Error bars = 1 SE

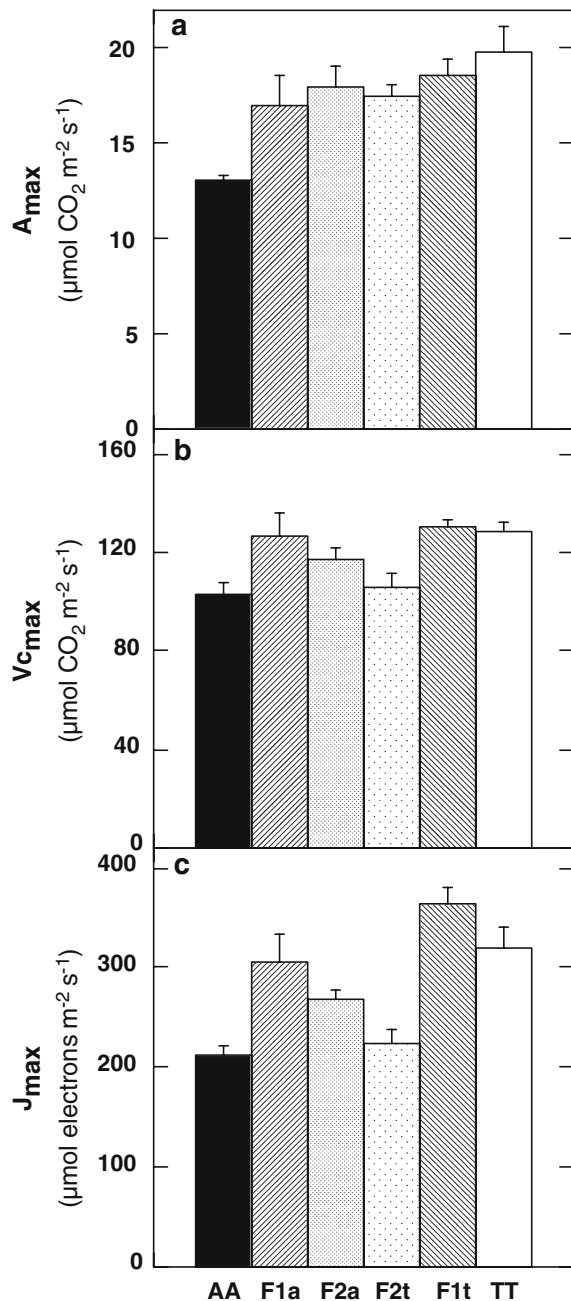


## Photosynthetic capacity for resource capture

As a group, all *Ipomopsis* plants examined in this study shared relatively high rates of  $\text{CO}_2$  assimilation, with  $V_{c_{max}}$  and  $J_{max}$  rates comparable to those found in plants from relatively dry and alpine environments (Körner and Diemer 1987; Wullschlegel 1993). *I. tenuituba* exhibited a greater biochemical capacity for photosynthesis than *I. aggregata*, which was supported by both higher  $V_{c_{max}}$  and  $J_{max}$ . This relatively high photosynthetic capacity was maintained across all  $\text{CO}_2$ , light, and temperature levels considered. The hybrid classes had  $A$  values that were at least as high as those for the parent species, suggesting that hybrids do not suffer from a reduction in C assimilation, as variation in  $V_{c_{max}}$  and  $J_{max}$  appeared to be closely coordinated within each class. Additional leaf biochemical or stomatal limitations to  $A$  may also vary among the genotypic classes (Geber and Dawson 1997), which might help explain the hybrid breakdown in  $V_{c_{max}}$  and  $J_{max}$ , without a similar reduction in  $A_{max}$ . Barring the effects of other confounding stressors, these higher rates of  $A_{max}$  could potentially provide *I. tenuituba* and some hybrids with a greater amount of C to allocate to growth and reproduction relative to *I. aggregata*, at least under the conditions examined here.

## Leaf-level responses to environmental variation

The *Ipomopsis* parent and hybrid plants are found in habitats across the hybrid zone that differ in their light and temperature regimes (Campbell et al. 2005, this study), which was reflected to varying degrees in their photosynthetic responses. In general, species adapted to sunny habitats display higher  $A_{max}$  values and require more light to saturate photosynthetic mechanisms than shade species (Givnish 1988; Givnish et al. 2004). These predictions were illustrated to an extent by the light responses of *I. aggregata* and *I. tenuituba*, which occur in more and less vegetated sites, respectively (Campbell et al. 1997). Photosynthesis per unit leaf area



**Fig. 4** Average maximum **a** photosynthetic rate  $A_{max}$ , **b** carboxylation rate  $V_{c,max}$ , and **c** electron transport rate  $J_{max}$  for parental species *I. aggregata* and *I. tenuituba* and reciprocal F1 and F2 hybrids.  $n=5$  for each genotypic class, and error bars = 1 SE. See Table 5 for statistical comparisons of these traits

in *I. tenuituba* reached higher maximum levels, but saturated at similar light levels, as compared to *I. aggregata*. While hybrids were quite variable with respect to photosynthetic light response parameters, they tended to be *tenuituba*-like (F1s) to positively transgressive (F2s). As such, the recombinant hybrids in particular appear capable of growing quite well at high light levels typical of those on the exposed slopes at the center of the hybrid zone.

**Table 5** ANOVA comparing biochemical photosynthetic parameters derived from  $A-c_i$  curves for parental and hybrid *Ipomopsis* plants (see Materials and methods)

Source	df	SS	F	P
Character: $A_{max}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )				
Model	5	131.63	4.47	0.0051*
AA vs. TT	1	113.37	19.27	0.0002*
Heterosis	1	9.56	1.63	0.2145
Breakdown	1	2.37	0.40	0.5315
Hybrids extreme	1	11.89	2.02	0.1680
Cytoplasm	1	1.38	0.23	0.6328
Error	24	141.20		
Character: $V_{c,max}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )				
Model	5	2,704.30	3.05	0.0294*
AA vs. TT	1	819.03	4.62	0.0423*
Heterosis	1	393.99	2.22	0.1495
Breakdown	1	1,123.29	6.34	0.0192*
Hybrids extreme	1	0.61	<0.01	0.9538
Cytoplasm	1	71.21	0.40	0.5323
Error	24	4,074.25		
Character: $J_{max}$ ( $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$ )				
Model	5	66,897.0	5.98	0.0011*
AA vs. TT	1	13,987.6	6.26	0.0199*
Heterosis	1	12,578.3	5.63	0.0265*
Breakdown	1	25,632.8	11.46	0.0025*
Hybrids extreme	1	362.5	0.16	0.6909
Cytoplasm	1	211.2	0.09	0.7613
Error	24	51,426.0		

\* $P < 0.05$

These exposed slopes also experienced higher daily temperatures and lower RHs than the more vegetated *I. aggregata* sites. Those site differences were largely consistent with an earlier study, except that in July 2002 maximum temperatures in the *I. aggregata* site were lower (average daily maximum 25.5°C) than observed here, producing an even larger contrast between sites (Campbell et al. 2005). Plants from habitats that differ in temperature regimes typically have different temperature response characteristics, such that plants will maximize their photosynthetic rate at temperatures close to their normal growth temperature (Berry and Björkman 1980; Berry and Raison 1981; Cabrera et al. 1998). *I. aggregata* reached its  $T_{opt}$  10°C lower than did *I. tenuituba*; the higher  $T_{opt}$  of *I. tenuituba* and hybrids relative to *I. aggregata* may thus reflect an influence of the larger amount of bare, rocky slopes at the former two sites. Further, hybrids with *tenuituba* cytoplasm were able to maintain higher  $A$  at their  $T_{opt}$  relative to their reciprocal counterparts, suggesting a cytoplasmic component to temperature tolerance in these plants. Similar trends in  $T_{opt}$  with respect to environmental temperature have been reported for herbaceous plants in the Alps (Körner and Diemer 1987) and vegetative rosettes in the Andes (Rada et al. 1992; Cabrera et al. 1998). Although differences in photosynthetic performance are both genetically determined and environmentally induced (Berry and Björkman 1980; Björkman 1981a; Hamerlynck and Knapp 1996; Lambers et al. 1998), this large difference in  $T_{opt}$  among the



genotypic classes was not likely simply due to differences in growth temperatures among these individuals, as the plants used here were raised from seed under similar greenhouse conditions. While it is unknown whether differences in the ability of parental and hybrid *Ipomopsis* plants to acclimate or adapt to different temperature regimes affects growth or survival in this hybrid zone, the large range of  $T_{\text{opt}}$  expressed in the recombinant F2 hybrids relative to both the parental species and F1s suggests that hybridization may generate individuals that are at an advantage with respect to their photosynthetic tolerance to the higher temperatures experienced in the hybrid sites.

### Implications of ecophysiological trait expression

If the ecophysiological characters of parental and hybrid plants reflect adaptations to differing environmental conditions in their respective habitats, then such traits could influence the distribution of these taxa within hybrid zones. Indeed, the putative hybrid sunflower species *Helianthus paradoxus* and *H. anomalus* appear to be mosaics of parental and transgressive phenotypes, which likely enable them to survive in habitats that are extreme relative to those of their parental species (Schwarzach et al. 2001; Rieseberg et al. 2003; Ludwig et al. 2004). Similarly, it is possible that the traits measured for *Ipomopsis* hybrids in this study are adaptive, although in the absence of data relating fitness to individual trait values under field conditions we can only hypothesize about the importance of environment-dependent selection in this hybrid system. Below we describe the broad-scale associations between differences in physiological traits and differences in survival across the genotypic classes that are consistent with adaptive responses, and their implications for the dynamics of this hybrid zone.

In a reciprocal transplant study in the Poverty Gulch hybrid zone, F1 hybrid survival from seed depended on both genotype and environment (Campbell and Waser 2001). On average, F1 hybrids survived as well as their parental species, but those with *I. aggregata* as the maternal parent survived particularly well across all parental sites. However, both F1<sub>A</sub> and F1<sub>T</sub> hybrids had higher survival than *I. aggregata* in the hybrid habitat, with *I. tenuituba* surviving equally as well as the hybrids. The high hybrid survival at the hybrid site could be in part explained by the relatively high photosynthetic temperature optima of hybrids, at least with respect to *I. aggregata*. However, while the *tenuituba*-like photosynthetic temperature responses may contribute to the survival advantage of hybrids relative to *I. aggregata* at hybrid sites, they do not explain the dependency of hybrid survival on the maternal parent at the parental sites. This suggests additional selection is occurring on other traits specifically expressed by hybrids with *aggregata* cytoplasmic background.

While both hybrids and *I. tenuituba* had high survival at hybrid sites (Campbell and Waser 2001), the high survival of *I. tenuituba* may be offset by a reproductive disadvantage relative to hybrids, as pollinator-mediated selection favors the *I. aggregata* floral phenotype across the hybrid zone (Campbell et al. 1997, 1998; Melendez-Ackerman and Campbell 1998). At the same time, the substantially lower  $T_{\text{opt}}$  for *I. aggregata* relative to hybrids and *I. tenuituba* suggests that physiological tolerances may counter the advance of pure *I. aggregata* into the hybrid zone. Consequently, a combination of ecophysiological and morphological traits may help explain the high overall fitness of hybrids in sites within the natural hybrid zone. Further, given the striking survival advantage of *I. tenuituba* over hybrids with the *tenuituba* cytotype at the *tenuituba* site (Campbell and Waser 2001), habitat-mediated selection seems likely to prevent the establishment of hybrids at these locations, thereby limiting the apparent advance of *I. aggregata* nuclear genes towards the higher elevation populations (Wu and Campbell 2005).

In sum, the results of this study indicate some genetic differences in photosynthetic responses among *I. aggregata*, *I. tenuituba*, and their hybrids, with the potential to help explain the overall survival advantage of natural hybrids relative to *I. aggregata* in the relatively hot, dry hybrid sites. There are, however, at least two important caveats about these comparisons. First, in this study we used a relatively small number of experimental hybrids that likely did not encompass the full range of phenotypes found in the natural hybrids. These small sample sizes suggest low statistical power for detecting small differences among the groups. Moreover, while genotypic class averages are useful when examining general trends in hybrid trait expression, some of the phenotypic variation within the hybrid classes is lost. Even studies such as this one that divide hybrids into genealogical classes may group very different genotypes with similarly different traits into a single category (Arnold and Hodges 1995). Consequently, we may not be able to detect the rare individuals with ecologically relevant traits that are able to achieve high fitness in particular environments (Arnold 1997). Further, if habitat-mediated selection acts preferentially on these specific hybrid phenotypes, the range of phenotypes expressed in natural populations may have been narrowed due to strong environmental selection that is absent from the relatively benign greenhouse conditions.

Second, temperature may not be the major limiting factor responsible for the distribution of these plants, and mechanisms other than temperature-dependent photosynthetic responses could more strongly account for the habitat-mediated survival differences across the hybrid zone. Indeed, both experimental and natural hybrids have higher WUE than the parental species (Campbell et al. 2005; Wu 2005). It is possible that the high WUE of hybrids may also facilitate the high survival of hybrids at the dry hybrid sites.

While photosynthetic temperature and light responses alone cannot definitively explain the distributions of *I. aggregata*, *I. tenuituba*, and hybrids, they do provide further support for the role of habitat-mediated selection in hybrid zone dynamics. Even in this relatively limited set of experimental hybrids, the majority of physiological traits measured were *tenuituba*-like, and in some hybrid individuals, exceeded the values for *I. tenuituba*. Many of these traits are consistent with adaptations reported for plants found in warm, dry sites, so they may put certain hybrids at an advantage at the center of the hybrid zone, at least relative to *I. aggregata*. If so, then ecological selection may drive hybrids in the direction of *I. tenuituba* with respect to ecophysiological traits (Campbell et al. 2005, this study), whereas pollinator-mediated selection favors more *aggregata*-like floral traits (Campbell et al. 1997, 1998; Melendez-Ackerman and Campbell 1998). Additional studies such as these that examine the ecophysiology of naturally hybridizing species are an important step towards understanding the relative role of environment-dependent selection in determining the outcome of natural hybridization.

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