Molecular Ecology (2005) **14**, 781–792 doi: 10.1111/j.1365-294X.2005.02441.x

Cytoplasmic and nuclear markers reveal contrasting patterns of spatial genetic structure in a natural *Ipomopsis* hybrid zone

CARRIE A. WU*† and DIANE R. CAMPBELL*†
Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, †Rocky Mountain Biological Laboratory, PO Box 519, Crested Butte, CO 81224

Abstract

Spatial variation in natural selection may play an important role in determining the genetic structure of hybridizing populations. Previous studies have found that F_1 hybrids between naturally hybridizing *Ipomopsis aggregata* and *Ipomopsis tenuituba* in central Colorado differ in fitness depending on both genotype and environment: hybrids had higher survival when *I. aggregata* was the maternal parent, except in the centre of the hybrid zone where both hybrid types had high survival. Here, we developed both maternally (cpDNA PCR-RFLP) and biparentally inherited (nuclear AFLP) species-diagnostic markers to characterize the spatial genetic structure of the natural *Ipomopsis* hybrid zone, and tested the prediction that the majority of natural hybrids have *I. aggregata* cytoplasm, except in areas near the centre of the hybrid zone. Analyses of 352 individuals from across the hybrid zone indicate that cytoplasmic gene flow is bidirectional, but contrary to expectation, most plants in the hybrid zone have *I. tenuituba* cytoplasm. This cytotype distribution is consistent with a hybrid zone in historical transition, with *I. aggregata* nuclear genes advancing into the contact zone. Further, nuclear data show a much more gradual cline than cpDNA markers that is consistent with morphological patterns across the hybrid populations. A mixture of environment- and pollinator-mediated selection may contribute to the current genetic structure of this hybrid system.

Keywords: AFLP, asymmetric hybridization, cpDNA, genetic structure, hybrid zone, *Ipomopsis*

Received 03 August 2004; revision received 29 November 2004; accepted 29 November 2004

Introduction

Natural hybrid zones occur where individuals from genetically distinct populations mate and produce offspring of mixed ancestry. Hybrid zones are of great interest to biologists because they provide natural laboratories in which to study the evolutionary processes that influence interactions between divergent populations (Harrison 1990). The extent of hybridization varies widely among systems, ranging from the production of solely F_1 hybrids (Nason *et al*. 1992; Ferguson *et al*. 1999; Kuehn *et al*. 1999; Milne *et al*. 2003) to the generation of numerous advanced recombinant individuals (Cruzan & Arnold 1993; Rieseberg & Wendel 1993; Sutton *et al*. 1994; Jiggins & Mallet 2000; Arnold *et al*. 2001; Hitt *et al*. 2003). In some systems, the spatial patterns for cytoplasmic and nuclear markers are highly concordant (Nelson *et al*. 1987; Baker *et al*. 1989), but others show pronounced discordance (Lamb & Avise 1986; Harrison 1989; Dorado *et al*. 1992; Brubaker *et al*. 1993) that reflects asymmetric introgression. In fact, cytoplasmic gene flow is frequently observed in plants without evidence of nuclear introgression (reviewed in Arnold 1997; Avise 2004). In theory, the patterns of nuclear and cytoplasmic genetic structure seen in hybrid zones should reflect the combined effects of selection and gene flow (Endler 1977; Loveless & Hamrick 1984; Barton & Hewitt 1985).

Both intrinsic and extrinsic factors can influence selection on hybrids and their parents, though their relative importance remains under debate (Barton & Hewitt 1989; Arnold 1997). Endogenous selection against unfit hybrids

Correspondence: Carrie A. Wu, Fax: 949-824-2181; E-mail: carriewu@uci.edu

is well documented (Dobzhansky 1970; Barton & Hewitt 1989; Coyne & Orr 1998), but may not be as ubiquitous as traditionally thought (Arnold & Hodges 1995; Arnold 1997; Rieseberg & Carney 1998; Avise 2004). Instead, crosses between divergent lineages can produce both unfit and fit hybrid genotypes (Rieseberg & Ellstrand 1993; Cruzan & Arnold 1994; Burke *et al*. 1998; Galloway & Fenster 1999), and their relative fitness can vary depending on the environment (Wang *et al*. 1997; Neuffer *et al*. 1999; Fritsche & Kaltz 2000; Campbell & Waser 2001; Milne *et al*. 2003).

Spatial variation in gene flow can also influence the genetic structure of hybrid zones. In plants, gene flow can occur via seed dispersal or through pollen movement between populations. The pattern and extent of gene exchange between hybridizing taxa will consequently depend on the opportunities for seed and pollen dispersal into the alternate population. Differences in the extent of these two mechanisms for gene flow can lead to asymmetric patterns of cytoplasmic and nuclear introgression (Asmussen *et al*. 1989; Oddou-Muratorio *et al*. 2001). In animal-pollinated plant species, pollinator behaviour can strongly influence pollen movement, and thus the genetic structure of hybrid populations (Campbell *et al*. 1997; Wesselingh & Arnold 2000). Pollinators that show strong fidelity to parental types may limit production of early generation hybrids. Alternatively, if the majority of gene flow occurs between hybrids and a single parent, then the subsequent generations may consist primarily of advanced backcrosses and have genotypes that largely resemble that of the recurrent parent. However, realized gene flow patterns may differ markedly from patterns of pollinator movement if postpollination mechanisms lead to fertilization failure or selective embryo abortion (Carney *et al*. 1994; Cruzan & Arnold 1994; Rieseberg *et al*. 1995; Carney *et al*. 1996; Emms *et al*. 1996; Alarcon & Campbell 2000).

A natural hybrid zone between two species of *Ipomopsis* (Polemoniaceae) presents an unusual opportunity to examine the role of spatial variation in environment-dependent natural selection in determining the genetic structure of natural hybrids. Recent studies have found that experimental F_1 hybrids between naturally hybridizing *Ipomopsis aggregata* (scarlet gilia) and *Ipomopsis tenuituba* in central Colorado differ in fitness depending on genotype and environment: hybrid survival was high in both parental sites if *I. aggregata* was the maternal parent (AT hybrid), but extremely low if the F_1s were mothered by *I. tenuituba* (TA hybrid). Also, AT hybrids set more seed. However, in the centre of the hybrid zone both types of hybrids had relatively high survival (Campbell & Waser 2001). These dramatic differences in the survival of reciprocal F_1 *Ipomopsis* hybrids present two alternative predictions for the cytoplasmic structure of the hybrid populations, based on assumptions of how selection varies spatially across the rest of the hybrid zone. If selection is relatively constant across all of the hybrid sites, then both cytotypes should be present in relatively equal numbers. However, if selection approaches its value in parental populations towards the ends of the hybrid zone, then the *I. aggregata* cytotype should be more common. Either of these outcomes can also be affected by patterns of gene flow. Gene flow through pollen movement can occur in both directions between these *Ipomopsis* species (Campbell *et al*. 1997), with only a slight asymmetry favouring F_1 formation on *I. tenuituba* recipients (Campbell *et al*. 1998; Campbell *et al*. 2002).

The present study was designed to characterize spatial genetic structure of the natural *Ipomopsis* hybrid zone in Poverty Gulch, Colorado, and examine the role of environment-mediated selection in the structuring of the hybrid populations. We developed both maternally (chloroplast DNA polymerase chain reaction–restriction fragment length polymorphisms or cpDNA PCR–RFLP) and biparentally inherited (nuclear amplified fragment length polymorphism or nuclear AFLP) genetic markers to assess the extent and direction of gene flow in the hybrid zone. Our objectives were to determine: (i) whether there is molecular evidence for hybridization; (ii) if both cytotypes are present among natural hybrids; (iii) if patterns of cytoplasmic and nuclear introgression are congruent; and (iv) the extent to which the genetic structure of the natural hybrid zone matches the prediction from selection studies: that half or more of the natural hybrids have *I. aggregata* cytoplasm.

Materials and methods

Study system

Ipomopsis aggregata and *Ipomopsis tenuituba* are herbaceous plants found throughout the western United States, and hybridization between the species is often extensive (Grant & Wilken 1988). One such hybrid zone is located along Poverty Gulch, a montane valley draining into the Slate River Valley in Gunnison County, Colorado, which is located 10 km from the Rocky Mountain Biological Laboratory (RMBL). Populations of *I. aggregata* ssp. *aggregata* are found on the gentle slopes along the base of the valley up to 2900 m above sea level (a.s.l.), while *I. tenuituba* ssp. *tenuituba* grows on the steep rocky slopes above 3100 m a.s.l. (Grant & Wilken 1988; Campbell *et al*. 1997). The parental populations at the ends of this hybrid zone (*I. aggregata* population L and *I. tenuituba* populations A–C in Campbell *et al*. 1997) are separated by 1.93 km, and are linked by a series of discrete hybrid populations (D–K in Campbell *et al*. 1997) that exhibit clinal variation in several floral characters. The range of floral traits expressed in artificially produced F_1 and later-generation hybrids suggests that the natural hybrid population is composed of multiple recombinant genotypes, but this has not been confirmed beyond morphometric analyses.

Flowers of *I. aggregata* have relatively broad, short, red-coloured corollas; *I. tenuituba* plants have pale pink to white flowers with narrow, long corollas. Both species are self-incompatible and monocarpic. At Poverty Gulch, broad-tailed (*Selasphorus platycercus*) and rufous (*Selasphorus rufus*) hummingbirds are the most common pollinators for both species of *Ipomopsis*. These hummingbirds easily transfer pollen between the species of *Ipomopsis*, but preferentially visit *I. aggregata* and hybrid flowers (Campbell *et al*. 1997; Campbell *et al*. 1998). Seeds are released 4–6 weeks after pollination and typically disperse only 0.5–1.0 m from the maternal plant (Waser & Price 1983). Seedlings grow into small vegetative rosettes during the first summer growing season. If rosettes survive until reproduction, they usually produce a single inflorescence in the late spring, flower during the summer, and die after seed production in August and September The median age to flowering is 5 years at 2900 m a.s.l. (Campbell 1997).

Cytoplasmic DNA is transmitted maternally in the vast majority of angiosperms, including these species of *Ipomopsis* (Wolf *et al*. 1993; Wolf *et al*. 1997). Thus, mitochondrial (mtDNA) or chloroplast (cpDNA) markers can be used to determine the cytoplasmic background, and thus the matrilineage, of natural hybrid individuals.

Field sampling and DNA extraction

Leaf material was collected from 20–40 flowering individuals from each of the 12 populations (A–L in Campbell *et al*. 1997) in the Poverty Gulch hybrid zone over a 2-year period. Because these plants are monocarpic (Campbell *et al*. 1997), collecting only from flowering plants prevented resampling the same individual. Leaf tissue was transported to RMBL on ice and promptly freeze-dried. Total genomic DNA was extracted from 10–15 mg freeze-dried tissue using DNeasy Plant Mini Kits (QIAGEN) and quantified using molecular weight standards (Invitrogen). Species-diagnostic markers were developed using genetic material obtained from 10 individuals from each of the parental species, confirmed with the remaining parental individuals sampled from populations A (*I. tenuituba*) and L (*I. aggregata*), and then used to screen the remaining individuals. A total of 352 plants from the hybrid zone was analysed for this study.

Cytoplasmic marker development

To generate cytoplasmic markers, we directly sequenced DNA fragments amplified by polymerase chain reaction (PCR) from 10 individuals from each of the two parental species and surveyed these sequences for base pair polymorphisms located within restriction enzyme recognition sites. PCR amplification was performed using pairs of universal primers from Taberlet *et al*. (1991), Demesure *et al*. (1995), and Dumolin-Lapègue *et al*. (1997), which match regions of the chloroplast or mitochondrial genome (Table 1). Reaction volumes of 25 μL contained 10 mM Tris HCl pH 9.0, 50 mM KCl, 2.0 mM $MgCl_2$, 50 μM of each dNTP, 0.2 μM or 0.5 μM of each primer, 1.25 U *Taq* DNA polymerase (Promega), and 0.5 μL genomic DNA. Hot start PCR amplifications were performed in MJ Research model PTC-100 thermocyclers. An initial 5 min denaturation at 94 °C and 10 min at 72 °C were followed by 35 cycles of 94 °C for 1 min,

Table 1 Universal primer pairs and PCR conditions used to screen Poverty Gulch *Ipomopsis* plants for diagnostic cytoplasmic markers. Primer notation follows that of Taberlet *et al*. (1991), Demesure *et al*. (1995), and Dumolin-Lapègue *et al*. (1997), while reaction conditions have been optimized for *Ipomopsis*

Primer pair	Annealing temp. (°C)*	Elongation time (min)	Restriction endonucleases†	Source‡
Mitochondrial primers				
*nad*1 exon B-*nad*1 exon C	55.0	1.5	—	2
Chloroplast primers				
*trn*L-*trn*F	55.0	1.5	*Nsi*I, *Bst*YI	1
*trn*T-*trn*L	55.0	1.5	—	1
*trn*S-*trn*fM	55.0	1.5	—	2
*trn*K2-*trn*Qr	n/a			3
*trn*T-*psb*Cr	n/a			3
*trn*V-*rbc*Lr	n/a			3
*trn*H-*trn*K1	62.0	2	—	2
*trn*D-*trn*T	54.5	2	*Mbo*I, *Hin*fI	2
*psb*C-*trn*S	57.0	2	*Bsm*AI	2
*psa*A-*trn*Sr2	n/a			2

*n/a indicates primers that did not successfully amplify fragments when tested across a range of annealing temperatures that included optimal values provided in source literature, †Restriction endonucleases with at least one recognition site difference between the species in the amplified fragment, based on sequence data. Dashes (–) indicate that amplified fragments from the two species did not have base pair differences within recognition sites, ‡1, Taberlet *et al*. (1991); 2, Demesure *et al*. (1995); 3, Dumolin-Lapègue *et al*. (1997).

annealing at 54.5–62 °C for 1 min, extension at 72 °C for 1.5–2 min (Table 1) and a final extension for 5 min. Amplification products were electrophoresed in 1.0% agarose gels and visualized under UV after ethidium bromide staining.

Of the 10 chloroplast and one mitochondrial primer pairs assayed, seven pairs consistently produced fragments that could be sequenced (Table 1). These double-stranded PCR fragments were purified with a PSIΨClone PCR96 Kit (Princeton Scientific). Sequencing was conducted using the Sanger method (Sanger *et al*. 1977) with Ampli*Taq* DNA Polymerase and fluorescent-labelled ddNTPs (Promega). Sequencing PCRs were set up following the supplier's recommendations (Perkin-Elmer) and subsequently purified by isopropanol precipitation at room temperature. Automated DNA sequencing was performed on an ABI PRISM 377 automated sequencer. Sequence data were aligned with AutoAssembler 2.0 (Applied Biosystems) and screened for species-diagnostic polymorphisms located within restriction enzyme recognition sites using DNA Strider 1.1 (Marck 1988). PCR products from the parental species were then digested with these restriction enzymes to determine if the digestion patterns for the two species' cytotypes could be distinguished on agarose gels. This directed approach identified enzymes that would yield fragments easily resolvable by gel electrophoresis, so restriction fragment length polymorphism (RFLP) analysis could be used to identify the cytoplasmic background of the remaining individuals from the hybrid zone. All putative species-diagnostic PCR–RFLP markers were verified using tissue samples from the remaining 30 *I. aggregata* and 51 *I. tenuituba* individuals collected from the parental populations, as well as an additional 27 *I. tenuituba* and 19 *I. aggregata* plants that were previously sampled and archived from populations A and L. We also analysed 15 individuals each from *I. tenuituba* and *I. aggregata* populations located 1.15 km and 1.39 km, respectively, from the ends of the clinal hybrid zone.

RFLP analysis of the natural hybrid population

Following the identification of diagnostic markers for *I. aggregata* and *I. tenuituba*, the cytoplasmic backgrounds of the remaining 205 individuals from the hybrid populations D-K were determined by PCR-RFLP analysis. PCR products (5 μL) were digested with the diagnostic restriction endonucleases for 2–6 h at the supplier's recommended reaction conditions (New England Biolabs) to resolve the species-diagnostic haplotypes. The digested products were separated in 1.0% or 2.0% agarose gels containing ethidium bromide and visualized with UV light.

AFLP marker development

The same subset of 20 pure individuals used in the screening for cytoplasmic markers was used to screen for nuclear AFLP markers. AFLP restriction-ligations, preselective and selective amplifications followed the Plant AFLP Kit protocol from Applied Biosystems, with minor modifications: following overnight incubations, restriction-ligation products were verified on 1.0% agarose gels containing ethidium bromide, and subsequent preselective and selective amplification reactions were performed at half (10 μL) of the supplier's recommended reaction volumes (20 μL). Amplified fragments were resolved on an ABI PRISM 377 automated sequencer; up to three PCR products with different fluorescent labels were multiplexed in each lane by combining the reaction products of separate PCRs. Fragment sizes (in bp) were determined with GENESCAN 3.1 software (Applied Biosystems) using an internal size standard (GENESCAN-500 [ROX]; Applied Biosystems) in each lane. Reproducibility was tested by repeating AFLP procedures to verify individual electropherograms and by running PCR products on different gels with identical size standards.

Individuals in the subset of 20 pure individuals were screened with all 128 *Eco*RI/*Mse*I selective primer combinations (+3/+3 and +2/+3) from the ABI Selective Amplification Modules for clear and reproducible bands that showed variation between the two parental species. Prior to screening the natural populations, we tested the inheritance of all putative AFLP markers using 25 experimental F_1 hybrids and their parents. Thirteen of these F_1s had *I. aggregata* mothers, and 12 were mothered by *I. tenuituba*. Because AFLPs are dominant, one of two segregation patterns are expected for nuclear markers that show Mendelian inheritance in F_1 progeny: (i) if the parent is dominant homozygous, the marker will be present in all F_1, or (ii) if the parent is heterozygous, the marker should be present in half of the F_1 offspring, independent of the cross direction. Only markers demonstrating Mendelian nuclear inheritance were used to analyse the genetic structure of the natural population.

AFLP fragment sequencing

Because we found only one species-diagnostic marker with nuclear inheritance (see Results), we sequenced the polymorphic marker fragment to determine if it was homologous to any known gene for floral traits, which are easily used to distinguish the two parental species. To identify the sequence of the AFLP fragment, we cloned PCR product from selective amplifications into the pGEM-T Easy Vector System (Promega), isolated the cloned products using QiaPrep Miniprep kits (QIAGEN), and sequenced the fragment directly from the vector with the ABI Big Dye system on an ABI 377 sequencer, using pUC/M13 sequencing primers. Sequence data were compared to known gene sequences in GenBank using BLAST (Altschul *et al*. 1997).

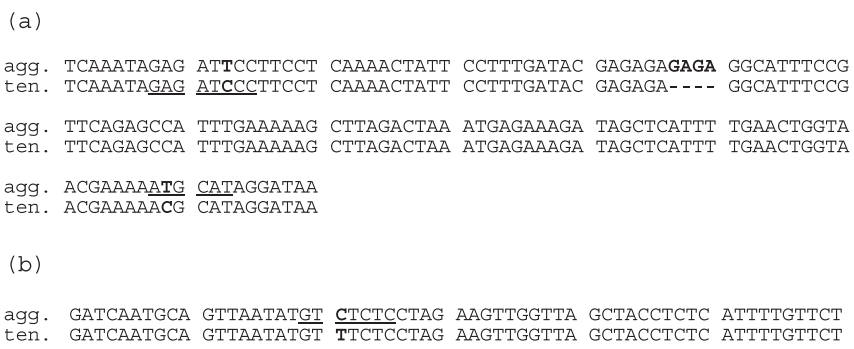

Fig. 1 Comparison of partial (a) *trn*L-*trn*F sequences (bases 350–490) and (b) *psb*C-*trn*S sequences (bases 330–390) of *Ipomopsis aggregata* (first row, agg.) and *Ipomopsis tenuituba* (second row, ten.). (a) Note the two single nucleotide polymorphisms and a two bp repeat difference shown in boldface. The polymorphism at position 363 creates a *Bst*YI site in *I. tenuituba* (underlined) that is absent in *I. aggregata*; the polymorphism at position 479 produces a *Nsi*I site in *I. aggregata* (underlined) that is absent in *I. tenuituba*. (b) A polymorphism at position 351 generates a B*sm*AI site in *I. aggregata* (labelled as in A). These sequences correspond to GenBank Accession nos. AY 701777–AY 701780.

Population	Elevation (m a.s.l.)	*Ipomopsis tenuituba* haplotype	*Ipomopsis aggregata* haplotype
A–C (*tenuituba*)	3145–3240	88	0
D	3172	30	0
E	3151	20	0
F	3047	20	0
G	3100	0	24
H	3013	21	0
I	3010	30	0
J	2925	20	0
K	2905	38	2
L (*aggregata*)	2900	0	59

Table 2 Distribution of cpDNA haplotypes based on *Nsi*I restriction site difference in the *trn*L-*trn*F intergenic region (Taberlet *et al*. 1991). Haplotype assignments are consistent with those determined using *Bst*YI (*trn*L-*trn*F) and B*sm*AI (*psb*C-*trn*S in Demesure *et al*. 1995). D–K are hybrid populations, as in Campbell *et al*. (1997)

Results

Identification of species-diagnostic cytoplasmic markers

We sequenced over 3 kb of cytoplasmic DNA from seven intergenic regions, or approximately 2.2% of the *Ipomopsis* chloroplast genome (Wolf *et al*. 1993). In these regions, we detected 13 single nucleotide polymorphisms (SNPs) and three length mutations. Five SNPs in cpDNA between *I. aggregata* and *I. tenuituba* resulted in restriction site differences between the species, but only digestion with *Nsi*I (*trn*L-*trn*F), *Bst*YI (*trn*L-*trn*F), and *Bsm*AI (*psb*C-*trn*S) yielded banding patterns that were resolvable by agarose gel electrophoresis (Figs 1 and 2). PCR amplification of the *trn*L-*trn*F region from either of these species produces a fragment approximately 1 kb in length. Digestion of the 1.0 kb amplification product with the restriction enzyme *Nsi*I produced distinct patterns depending on the presence (*I. aggregata*) or absence (*I. tenuituba*) of a single restriction site (Fig. 2a). This fragment also contains one (*I. aggregata*) or two (*I. tenuituba*) *Bst*YI recognition sites, which produces two or three fragments, respectively, when digested with this enzyme (Fig. 2b). The *psb*C-*trn*S fragment from *I. aggregata* contains a *Bsm*AI recognition site that is absent in *I. tenuituba*, which results in the cleavage of an 800-bp fragment in *I. tenuituba* into two comigrating 400-bp bands in *I. aggregata* (Fig. 2c).

Distribution of cpDNA haplotypes in the natural hybrid zone

Three hundred fifty-two plants from Poverty Gulch were analysed with these three cpDNA PCR-RFLP markers, including 88 *I. tenuituba* individuals, 59 *I. aggregata* individuals, and 205 natural hybrids. Species-diagnostic haplotypes were consistent for all parental plants. Both parental haplotypes were represented in the hybrid zone, but contrary to expectation, the majority of the natural hybrids had *I. tenuituba* cytoplasm (Table 2; Fig. 3). The *I. aggregata* cytotype was only found in two hybrid populations: two of 40 plants sampled from population K (adjacent to the *I. aggregata* parental population), and all 24 individuals that flowered in population G during the entire sampling period.

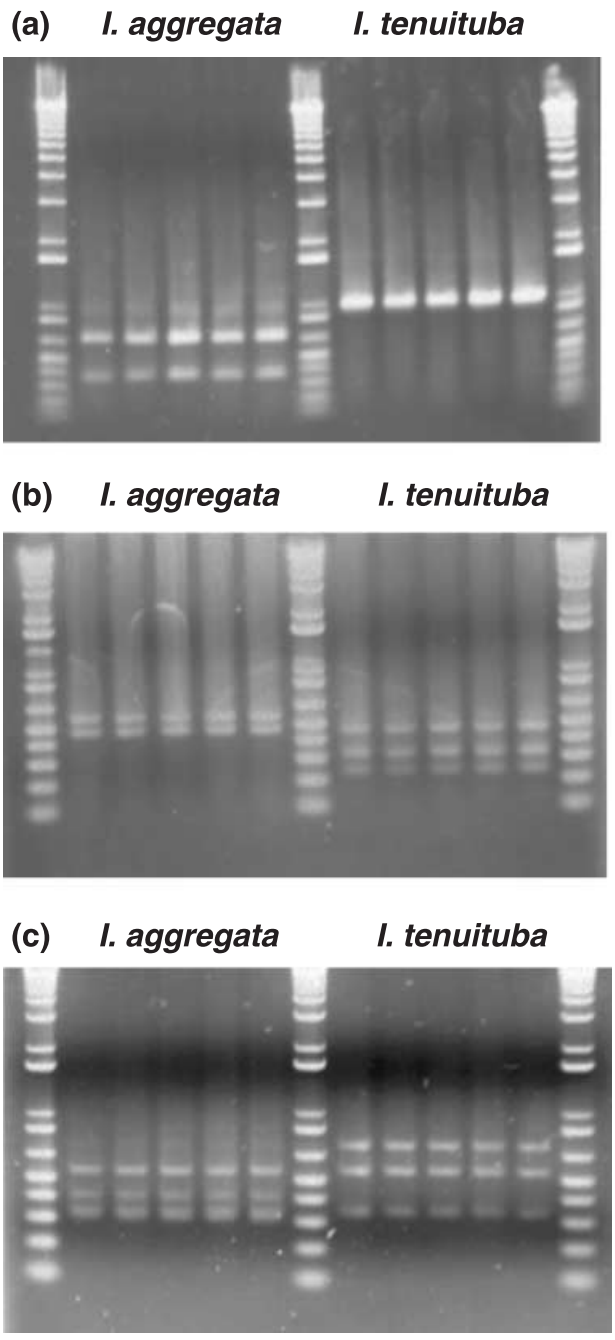

Fig. 2 cpDNA haplotypes for *Ipomopsis aggregata* and *Ipomopsis tenuituba* generated by digesting PCR amplification products with restriction enzymes. *trn*L-*trn*F intron (Demesure *et al*. 1995) digested with (a) *Nsi*I or (b) *Bst*YI, and (c) *psb*C-*trn*S (Taberlet *et al*. 1991) with *Bsm*AI. The size markers in all three gels are 1 kb + ladders (Invitrogen).

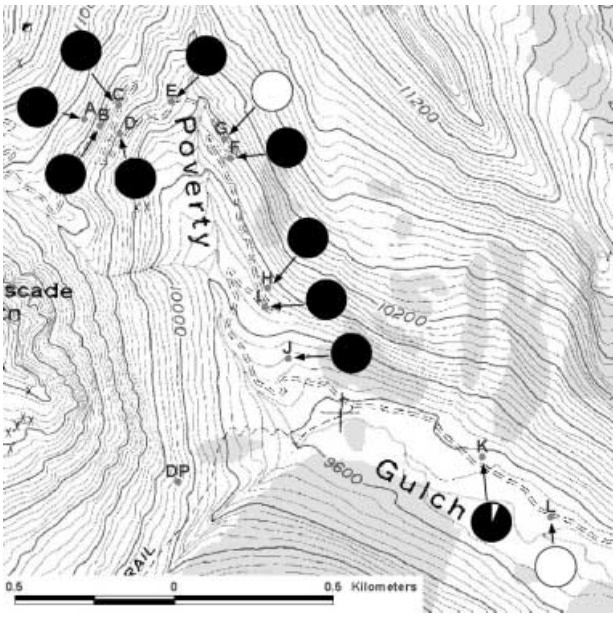

Fig. 3 Distribution of *Ipomopsis aggregata* (white) and *Ipomopsis tenuituba* (black) chloroplast DNA haplotypes in the Poverty Gulch, Colorado hybrid zone. Each circle represents a distinct population, and $N = 20–59$ plants sampled in each population. Daisy Pass (DP) is an *I. tenuituba* population that is not part of the clinal hybrid zone. Source: USGS Oh-Be-Joyful DRG 1 : 24 000.

AFLP markers

In the AFLP analysis of *I. aggregata* and *I. tenuituba*, over 2100 clear and reproducible fragments with lengths between 70 and 400 bp were resolved, including 121 fragments that were variable within a single species. However, we found no variable bands that were shared across the two species, based on the 20 individuals used for screening. From the 128 primer combinations surveyed, only six pairs (*Eco*RI/*Mse*I primer: TT/CTC, AT/CAT, AAG/CTC, ACG/CTG, AAG/CTT, and AGG/CTT) produced a single fragment each that were present in all 10 screened individuals of one species and absent from all 10 individuals of the other species. However, five of these six fragments identified were excluded from subsequent analyses because they were identified as cytoplasmic markers: putative markers for one species were only present in F_1 hybrids with that species as the maternal parent, and were absent from the reciprocal F_1s (Table 3). Consequently, only the primer combination TT/CTC, which generated a single putative diagnostic marker for *I. aggregata* (hereafter *agg*1), was used to assay the natural populations.

Contrary to the pattern revealed by the cytoplasmic markers, within the same 352 individuals from Poverty Gulch, the *agg*1 phenotype showed a smooth, clinal transition across the hybrid zone (Fig. 4), from completely absent in the *I. tenuituba* populations (A–C) to fully present in the *I. aggregata* parental population L, and the adjacent hybrid populations K and J. This fragment is not homologous to any known sequence, floral or otherwise, available in GenBank (Fig. 5; Altschul *et al*. 1997).

Table 3 AFLP selective primer combinations that produced potential species-diagnostic nuclear markers for the parental *Ipomopsis* species. Analysis of 25 F_1 progeny from reciprocal experimental crosses (subscript indicates that maternal parent) indicated that five of the six markers were amplifying cytoplasmic DNA fragments

Primer pair (*Eco*RI/*Mse*I)	Bandsize (bp)	Species with band present	Frequency in F_1 hybrids		Marker inheritance
			F_{1A}	F_{1T}	
AAG/CTC	134	*I. aggregata*	13/13	0/12	maternal
ACG/CTG	75	*I. tenuituba*	0/13	12/12	maternal
AAG/CTT	155	*I. aggregata*	13/13	0/12	maternal
AGG/CTC	134	*I. aggregata*	13/13	0/12	maternal
AT/CAT	211	*I. tenuituba*	0/13	12/12	maternal
TT/CTC	81	*I. aggregata*	13/13	12/12	nuclear

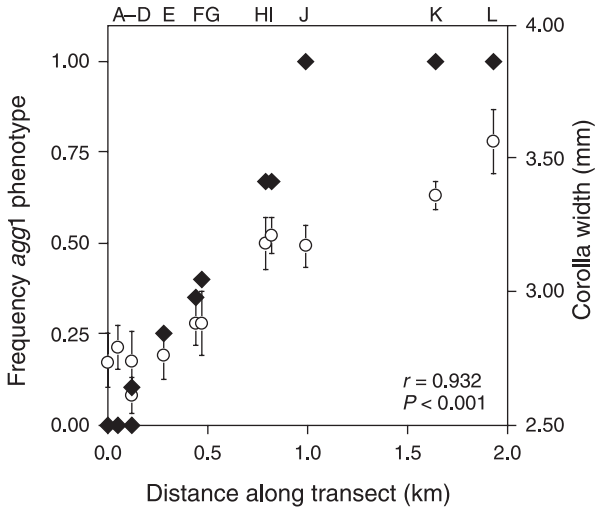

Fig. 4 Frequency clines for the *agg*1 phenotype (◆) and corolla width (○) across the Poverty Gulch, Colorado *Ipomopsis* hybrid zone. The 12 populations labelled A–L across the top of the figure are the same as in Fig. 3. Distance from the highest elevation *Ipomopsis tenuituba* population (A) was determined using a hand-held Garmin GPS unit, and *r* is the Spearman–rank correlation between *agg*1 phenotype frequency in each population and distance along the cline. Floral trait data presented here are modified from Campbell *et al*. (1997); error bars are ± 1 SEM across 10 plants.

Discussion

Ipomopsis aggregata and *Ipomopsis tenuituba* frequently hybridize when the two species occur in sympatry (Grant & Wilken 1988). In the present study, our main objective was to characterize the genetic structure of the natural *Ipomopsis* hybrid zone in Poverty Gulch, a contact site where selection patterns have been extensively studied. To this end, we developed both maternally (cpDNA PCR-RFLP) and biparentally inherited (nuclear AFLP) species-diagnostic markers and analysed over 350 individuals from across the hybrid zone. By sequencing amplified intergenic regions of mtDNA and cpDNA, we identified three useful cpDNA restriction-site polymorphisms between *I. aggregata* and *I. tenuituba*, which were undetected in previous studies (Wolf *et al*. 1993, 1997). These three cpDNA markers produced a single haplotype for each parental species, and we detected no novel haplotypes in the hybrid populations.

In contrast to cpDNA markers, we found little detectable genetic variation between *I. aggregata* and *I. tenuituba* at AFLP loci. In spite of surveying the nuclear genome with 128 primer pairs, we identified only a single species-diagnostic nuclear marker, *agg*1. Five other putative markers showed maternal inheritance, while the remaining fragments were either observed in all individuals from both of the parental species used in the marker development phase, or were variable among members of only one species. The *agg*1 sequence is not homologous to any known plant gene associated with flower colour or morphology (Altschul *et al*. 1997), which are important taxonomic characters in *Ipomopsis*. In fact, a BLAST search failed to locate any sequence homologous to this *I. aggregata* fragment. Although this paucity of differentiation between species is not widespread among studies employing AFLPs, recent work in hybridizing oaks also failed to detect diagnostic markers for the parental species (Ishida *et al*. 2003). Further, just 10 AFLP loci were variable among three sister genera in Araucariaceae (Peakall *et al*. 2003), and only a single AFLP locus was found that distinguished one coral from two other members of the same species complex (Lopez *et al*. 1999). While the genomic distribution of AFLP markers generated by *Eco*RI/*Mse*I restriction digestion appears to be skewed

5′ GACTGCGTAC CAATTC**TT**GA CAGACGAAGA CATTTAGTTT GACTTGAGTA ACCCACCCAC

CTAACCTCAT TAGTTATACA CCAATAAGAT CGAG**GAG**TTA CTCAGGACTC ATC 3′

Fig. 5 Complete sequence of the only species-diagnostic AFLP marker detected for *Ipomopsis aggregata* (GenBank Accession no. AY 701781). Regions corresponding to the AFLP primers are underlined, and the selective bases are indicated in boldface type. This sequence is not homologous to any known plant gene published in GenBank.

towards centromeric regions in plants (Young *et al*. 1999), this bias is unlikely to explain the low levels of variability detected in *Ipomopsis*, as such noncoding regions are likely to have relatively higher rates of marker variability (Young *et al*. 1999). Neither is this lack of diagnostic markers likely a result of a technical problem with our AFLP methods, as five of these AFLP primer combinations detected notably higher levels of polymorphism among four closely related (Wolf *et al*. 1991) congeneric species (*Ipomopsis arizonica*, *Ipomopsis tenuifolia*, *Ipomopsis multifolia*, and *Ipomopsis therberi*; C.A. Wu, unpublished). Rather, this apparent high genetic similarity is consistent with allozymic studies that found no fixed differences between the two species (Wolf *et al*. 1991), and their placement within the same species complex (Grant & Wilken 1988, Wolf *et al*. 1993).

The contact site between these species in Poverty Gulch shows a smooth gradation between parental phenotypes in several floral traits (Grant & Wilken 1988; Campbell *et al*. 1997). In the present study we found molecular evidence to support this morphometric evidence for hybridization. Within hybrid populations (D–K) that exhibit intermediate floral morphology, we detected recombinant individuals that contained the *I. aggregata* nuclear marker, but possessed *I. tenuituba* cpDNA, and vice versa. The presence of both cytotypes in natural hybrids indicates that hybridization between *I. aggregata* and *I. tenuituba* has occurred in both directions. However, this hybridization has occurred with a striking asymmetry. Only *c.* 15% of the natural hybrids surveyed had the *I. aggregata* cytotype, and all but two of these hybrids were found in a small population located near the centre of the hybrid zone (Fig. 3). The high frequency of *I. tenuituba* cpDNA in the hybrid populations suggests that this species predominated as the seed parent in the contact zone. This pervasiveness of *I. tenuituba* cytoplasm is contrary to our expectations based on the survival advantage of hybrids with *I. aggregata* cytoplasm in both parental sites (Campbell & Waser 2001), and suggests that additional factors beyond environment-specific selection in the F_1 generation influence the cytoplasmic genetic structure of the hybrid populations.

Asymmetric hybridization is relatively common in plants (Barton & Hewitt 1985; Brubaker *et al*. 1993; Cruzan & Arnold 1994; Arnold 1997; Rieseberg 1997; Caraway *et al*. 2001) and animals (Avise & Saunders 1984; Barton & Hewitt 1985; Coyne & Orr 1998; Young *et al*. 2001). Mechanisms other than selection in the initial F_1 generation that could explain such asymmetry can be categorized as to whether they influence gene flow (through either seed or pollen movement), reflect a nonequilibrium situation, or involve selection on advanced generation hybrids. Patterns in gene flow could contribute to the observed asymmetry in this *Ipomopsis* hybrid zone, although many specific mechanisms that are typically proposed for plant systems are unlikely to apply here. For example, differences in seed dispersal between hybridizing taxa may skew cytotype representation in a hybrid population, such that the species with greater seed movement will be over-represented (Sutton *et al*. 1994; O'Hanlon *et al*. 1999; Oddou-Muratorio *et al*. 2001). However, neither *I. aggregata* nor *I. tenuituba* have specialized seed dispersal mechanisms; seeds fall extremely close to the maternal parent, and generally lack secondary gravity- or water-mediated dispersal despite the steepness of the terrain (Waser & Price 1983; Campbell & Waser 2001). Breeding systems differences can also cause asymmetric hybridization (Ferguson *et al*. 1999; Hollingsworth *et al*. 1999; Xiang *et al*. 2000), but are not relevant for these *Ipomopsis* because both species are hermaphroditic and self-incompatible.

Gene flow through pollen is more likely to affect the genetic structure of these populations. Such gene flow depends on both dispersal of pollen to stigmas and post-pollination competitive abilities, often partly because of differences in pollen tube growth rates (Carney *et al*. 1994; Emms *et al*. 1996). The latter process is unlikely to contribute to asymmetry in the direction of hybridization in this case, as neither species shows pollen precedence in experimental pollinations. Instead, the frequency of seeds sired by either species is proportional to its representation in the pollen mixture, regardless of whether the recipient plant is *I. aggregata*, *I. tenuituba*, or a natural hybrid (Alarcon & Campbell 2000). Pollen dispersal reflects the extent of overlap in phenology, as well as mating patterns when the species are in simultaneous bloom. The flowering phenologies of the *Ipomopsis* populations in Poverty Gulch overlap considerably so that phenology is unlikely to promote asymmetric gene flow (Grant & Wilken 1988; Melendez-Ackerman 1997) as it does in *Gossypium* (Brubaker *et al*. 1993) and *Spartina* (Anttila *et al*. 2000). However, mating patterns are likely to be a significant factor in the genetic structuring of these hybrid populations. In animal-pollinated plant species such as these *Ipomopsis* species, pollen movement depends on both pollinator behaviour and effectiveness. At this contact site, hawkmoths (*Hyles lineata*) are exceedingly rare during most years, and when present, are often diurnal in this high-elevation location and do not fully restrict their visits to *I. tenuituba* (Wolf & Campbell 1995; Campbell *et al*. 1997). Rather, flowers of *I. tenuituba*, like those of *I. aggregata*, normally receive more visits by hummingbirds than by hawkmoths. Hummingbirds show a preference for *I. aggregata* and hybrids over *I. tenuituba* but do fly between the species in artificial mixed arrays (Campbell *et al*. 1997), and are capable of moving pollen between them, albeit asymmetrically. Hummingbirds transfer pollen more efficiently from *I. aggregata* to *I. tenuituba* than vice versa (Campbell *et al*. 1998), but when the frequency of flights in the two directions is also taken into account, the asymmetry is slight (Campbell *et al*. 2002). So even though interspecific pollen transfer is relatively infrequent (Campbell

et al. 2002), when the parental species are in close proximity, F_1 hybrids may be formed slightly more frequently on *I. tenuituba* recipients, producing hybrids with *I. tenuituba* cpDNA. This asymmetry in pollen transfer efficiency between the parental species may also have helped to restrict the production of large numbers of AT hybrids in the *I. aggregata* habitat, where they otherwise survive well (Campbell & Waser 2001). However, given how slight the difference in pollen transfer efficiency is, it seems unlikely to explain by itself the cytoplasmic structure of the hybrid zone.

The preponderance of hybrids with *I. tenuituba* cytoplasm could alternatively be explained by a hybrid zone that is not in equilibrium, but instead is consistent with an advancing wave model (Fisher 1937; Fisher 1950) in which *I. aggregata* genes have spread into *I. tenuituba* populations by an advantage in pollen movement (Campbell *et al*. 1997). In this scenario, TA hybrids would initially have been formed by dispersal of *I. aggregata* pollen into *I. tenuituba* populations, where the centre of the hybrid zone is currently located. At this site, both *I. tenuituba* and TA hybrids have relatively high survival (Campbell & Waser 2001). However, because of hummingbird preference (Campbell *et al*. 1997), the TA hybrids would be expected to enjoy a higher quality and quantity of pollinator visits than *I. tenuituba* plants (Campbell *et al*. 1998). In this way, *I. aggregata* genes could have introgressed into higher elevation populations.

An equilibrium scenario cannot, however, be ruled out until we know whether or not cytoplasmic background has a similar influence on survival in advanced generation hybrids as it does in the F_1 generation. A study is underway in which we have planted F_2 and backcross hybrids into the field to measure their survival and reproductive success. While lifetime fitness of these plants remains unknown, pollination data show how pollinator-mediated selection might further contribute to the maintenance of *I. tenuituba* cpDNA in advanced hybrids. When F_1 and F_2 hybrids are intermixed with parental species in experimental arrays, pollen receipt by hybrids ranges from intermediate to extreme relative to the parent species (Campbell *et al*. 2002), which suggests that backcrossing may easily proceed with hybrids as the maternal parent. If TA hybrids receive pollen from *I. aggregata*, repeated backcrossing could generate a preponderance of morphologically *aggregata*-like hybrids with *I. tenuituba* cpDNA. This is precisely what is found in population K (Figs 3 and 4), which contains plants that are virtually indistinguishable from *I. aggregata* based on floral characters (Campbell *et al*. 1997). In experimental arrays, backcross pollen delivery was more common to *I. aggregata* flowers than to *I. tenuituba* (Campbell *et al*. 2002). However, when mixed loads of *I. aggregata* and hybrid pollen were applied to the stigmas of *I. aggregata* flowers, hybrids had reduced siring success relative to expectations (Campbell *et al*. 2003). Thus, the formation of backcross progeny by *I. aggregata* mothers may be reduced relative to the high levels predicted by pollen transfer alone (Campbell *et al*. 2002), thereby limiting the movement of *I. tenuituba* genes into *I. aggregata* populations.

There is a strong incongruence between the cytoplasmic and nuclear marker structure in this hybrid zone (Figs 3 and 4). Unlike the cpDNA markers, the *agg*1 phenotype shows a more gradual frequency cline across the hybrid zone that is coincident with changes in floral traits that experience selection, including corolla width and flower colour (Campbell *et al*. 1997; Melendez-Ackerman 1997). An important caveat in drawing conclusions about nuclear gene flow is that we only know what is happening at one region of the nuclear genome, and it is possible that selection is acting differentially on the *agg*1 region relative to other regions, even though the fragment was not homologous to any known candidate gene. Nevertheless, the nuclear DNA cline we observed is consistent with a historical advance of *I. aggregata* nuclear genes into *I. tenuituba* populations via pollen flow. In such a scenario, advanced backcrosses to *I. aggregata* would be expected to occur near the current *I. aggregata* population (e.g. site K), with a greater diversity of hybrid genotypes near the geographical centre of the hybrid zone (sites H and I). Although selection could be involved, the highly divergent genetic patterns between the AFLP and PCR-RLFP markers may be because of the different transmission modes of nuclear and cytoplasmic DNA, consistent with restricted gene flow by seed (Brubaker *et al*. 1993; McCauley 1994; McCauley *et al*. 1996; Tarayre *et al*. 1997) in this contact zone.

While gene flow in this hybrid zone appears to primarily occur via pollen transfer, rare, long distance seed dispersal followed by pollen transfer may also occur. This may explain the cluster of individuals with *I. aggregata* cytoplasm in population G (Fig. 3), a small population located at the end of a trail switchback, that may have formed from an isolated founder event as one or a few *I. aggregata* seeds were transported up-valley. A newly founded population at that location would likely experience an influx of pollen from nearby *I. tenuituba* or *tenuituba*-like hybrids in the absence of seed movement (Waser & Price 1983). The resulting hybrids would possess *I. aggregata* cpDNA, but *I. tenuituba*-like nuclear genotypes and morphologies that are consistent with neighbouring hybrid populations.

The contemporary genetic structure of this *Ipomopsis* hybrid zone appears to have been primarily affected by a combination of pollinator behaviour (Campbell *et al*. 1997; Campbell *et al*. 1998; Campbell *et al*. 2002) and habitat selection on hybrids and the parental species (Campbell & Waser 2001). Together, the nuclear and cytoplasmic data presented here are consistent with a hybrid zone that has been in transition (Paige *et al*. 1991), with *I. aggregata* pollen advancing into hybrid populations as the initial F_1 hybrids with *I. tenuituba* cpDNA backcross with *I. aggregata* and other hybrids, generating a hybrid swarm composed primarily of individuals with *I. tenuituba* cytoplasm. Could the advance

of this hybrid swarm towards the higher elevations continue until the *I. tenuituba* populations are swamped by hybrid genotypes? Given the striking survival advantage of *I. tenuituba* over TA plants at the *I. tenuituba* site (Campbell & Waser 2001), habitat-mediated selection seems likely to prevent the establishment of hybrids in these locations, thereby limiting the advance of *I. aggregata* genes into higher elevation *I. tenuituba* populations. It is unclear at this point what the critical ecological factors are that cause these survival differences between parental and hybrid plants. We are currently examining differences in physiology that may influence the fitness of these *Ipomopsis* species under natural conditions.

Hybrid zones will continue to provide natural laboratories in which to study the evolutionary processes that influence interactions between divergent populations (Harrison 1990). While an increasing number of studies are finding that hybrid fitness can be environment-dependent (Emms & Arnold 1997; Wang *et al*. 1997; Fritsche & Kaltz 2000), we are still largely ignorant as to the mechanisms of this exogenous selection, and their relative importance to one another in shaping the genetic structure of hybrid zones. Studies that examine the distribution of genetic variation within natural populations, combined with measurements of fitness differences among hybrids and their parental species, can provide a more complete assessment of the evolutionary mechanisms responsible for the dynamics and maintenance of hybrid zones.

Acknowledgements

We are grateful to Katie Estes for assistance with PCR-RFLP analyses and Rebecca Gaut for assistance with DNA sequencing. Brandon Gaut at UC Irvine generously provided use of the ABI 377 automated sequencer, and Elizabeth Friar at the Rancho Santa Ana Botanic Garden offered useful suggestions for chloroplast marker development and tissue from other *Ipomopsis* species. Brandon Gaut, Steve Weller, Eugene Wu, Deborah Charlesworth, and two anonymous reviewers provided helpful comments on the manuscript. This work was supported by the University of California, Irvine, the Lee R.G. Snyder Memorial Award, and National Science Foundation grants DEB-9806447 to D. Campbell and DEB-0308772 to C. Wu and D. Campbell.

References

Alarcon R, Campbell DR (2000) Absence of conspecific pollen advantage in the dynamics of an *Ipomopsis* (Polemoniaceae) hybrid zone. *American Journal of Botany*, **87**, 819–824.

Altschul SF, Madden TL, Schaffer AA *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, **25**, 3389–3402.

Anttila CK, King RA, Ferris C, Ayres DR, Strong DR (2000) Reciprocal hybrid formation of *Spartina* in San Francisco Bay. *Molecular Ecology*, **9**, 765–770.

Arnold ML (1997) *Natural Hybridization and Evolution*. Oxford University Press, New York.

Arnold ML, Hodges SA (1995) Are natural hybrids fit or unfit relative to their parents? *Trends in Ecology and Evolution*, **10**, 67–71.

Arnold ML, Kentner EM, Johnston JA, Cornman S, Bouck AC (2001) Natural hybridisation and fitness. *Taxon*, **50**, 93–104.

Asmussen MA, Arnold ML, Avise JC (1989) The effects of assortative mating and migration on cytonuclear associations in hybrid zones. *Genetics*, **122**, 923–934.

Avise JC (2004) *Molecular Markers, Natural History, and Evolution*, 2nd edn. Sinauer Associates, Sunderland, MA.

Avise JC, Saunders NC (1984) Hybridization and introgression among species of sunfish (*Lepomis*): analysis by mitochondrial DNA and allozyme markers. *Genetics*, **108**, 237–255.

Baker JD, Davis SK, Bradley RD, Hamilton MJ (1989) Ribosomal-DNA, mitochondrial-DNA, chromosomal, and allozymic studies on a contact zone in the pocket gopher, *Geomys*. *Evolution*, **43**, 63–75.

Barton NH, Hewitt GM (1985) Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, **16**, 113–148.

Barton NH, Hewitt GM (1989) Adaptation, speciation, and hybrid zones. *Nature*, **341**, 497–503.

Brubaker CL, Koontz JA, Wendel JF (1993) Bidirectional cytoplasmic and nuclear introgression in the New World cottons, *Gossypium barbadense* and *G. hirsutum* (Malvaceae). *American Journal of Botany*, **80**, 1203–1208.

Burke JM, Voss TJ, Arnold ML (1998) Genetic interactions and natural selection in Louisiana iris hybrids. *Evolution*, **52**, 1304–1310.

Campbell DR (1997) Genetic and environmental variation in life-history traits of a monocarpic perennial: a decade-long field experiment. *Evolution*, **51**, 373–382.

Campbell DR, Waser NM (2001) Genotype-by-environment interaction and the fitness of plant hybrids in the wild. *Evolution*, **55**, 669–676.

Campbell DR, Waser NM, Melendez-Ackerman EJ (1997) Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. *American Naturalist*, **149**, 295–315.

Campbell DR, Waser NM, Wolf PG (1998) Pollen transfer by natural hybrids and parental species in an *Ipomopsis* hybrid zone. *Evolution*, **52**, 1602–1611.

Campbell DR, Waser NM, Pederson GT (2002) Predicting patterns of mating and rates of hybridization from pollinator behavior. *American Naturalist*, **159**, 438–450.

Campbell DR, Alarcon R, Wu CA (2003) Reproductive isolation and hybrid pollen disadvantage in *Ipomopsis*. *Journal of Evolutionary Biology*, **16**, 536–540.

Caraway V, Carr GD, Morden CW (2001) Assessment of hybridization and introgression in lava-colonizing Hawaiian *Dubautia* (Asteraceae: Madiinae) using RAPD markers. *American Journal of Botany*, **88**, 1688–1694.

Carney SC, Cruzan MB, Arnold ML (1994) Reproductive interactions between hybridizing irises: analyses of pollen-tube growth and fertilization success. *American Journal of Botany*, **81**, 1169–1175.

Carney SC, Hodges SA, Arnold ML (1996) Effects of differential pollen-tube growth on hybridization in the Louisiana irises. *Evolution*, **50**, 1871–1878.

Coyne JA, Orr HA (1998) The evolutionary genetics of speciation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **353**, 287–305.

Cruzan MB, Arnold ML (1993) Ecological and genetic associations in an *Iris* hybrid zone. *Evolution*, **47**, 1432–1445.

Cruzan MB, Arnold ML (1994) Assortative mating and natural selection in an *Iris* hybrid zone. *Evolution*, **48**, 1946–1958.

Demesure B, Sodzi N, Petit RJ (1995) A set of universal primers for amplification of polymorphic noncoding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology*, **4**, 129–131.

Dobzhansky T (1970) *Genetics and the Evolutionary Process*. Columbia University Press, New York.

Dorado O, Rieseberg LH, Arias DM (1992) Chloroplast DNA introgression in Southern California sunflowers. *Evolution*, **46**, 566–572.

Dumolin-Lapègue S, Pemonge M-H, Petit RJ (1997) An enlarged set of consensus primers for the study of organelle DNA in plants. *Molecular Ecology*, **6**, 393–397.

Emms SK, Arnold ML (1997) The effect of habitat on parental and hybrid fitness: transplant experiments with Louisiana irises. *Evolution*, **51**, 1112–1119.

Emms SK, Hodges SA, Arnold ML (1996) Pollen tube competition, siring success, and consistent asymmetric hybridization in Louisiana irises. *Evolution*, **50**, 2201–2206.

Endler JA (1977) *Geographic Variation, Speciation and Clines*. Princeton University Press, Princeton, New Jersey.

Ferguson CJ, Levin DA, Jansen RK (1999) Natural hybridization between an outcrossing and a selfing *Phlox* (Polemoniaceae): the maternal species of F_1 hybrids. *Plant Systematics and Evolution*, **218**, 153–158.

Fisher RA (1937) The wave of advance of advantageous genes. *Annals of Eugenics*, **7**, 355–369.

Fisher RA (1950) Gene frequencies in a cline determined by selection and diffusion. *Biometrics*, **6**, 353–361.

Fritsche F, Kaltz O (2000) Is the *Prunella* (Lamiaceae) hybrid zone structured by an environmental gradient? Evidence from a reciprocal transplant experiment. *American Journal of Botany*, **87**, 995–1003.

Galloway LF, Fenster CB (1999) The effect of nuclear and cytoplasmic genes on fitness and local adaptation in an annual legume, *Chamaecrista fasciculata*. *Evolution*, **53**, 1734–1743.

Grant V, Wilken DH (1986) Taxonomy of the *Ipomopsis aggregate* group (polemoniaceae).

Grant V, Wilken DH (1988) Natural hybridization between *Ipomopsis aggregata* and *I. tenuituba* (Polemoniaceae). *Botanical Gazette*, **149**, 213–221.

Harrison RG (1989) Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology and Evolution*, **4**, 6–11.

Harrison RG (1990) Hybrid zones: windows on evolutionary process. In: *Oxford Surveys in Evolutionary Biology* (eds Futuyma D, Antonovics J). Oxford University Press, Oxford, UK.

Hitt NP, Frissell CA, Muhlfeld CC, Allendorf FW (2003) Spread of hybridization between native west slope cutthroat trout, *Oncorhynchus clarki lewisi*, and non-native rainbow trout, *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Science*, **60**, 1440–1451.

Hollingsworth ML, Bailey JP, Hollingsworth PM, Ferris C (1999) Chloroplast DNA variation and hybridization between invasive populations of Japanese knotweed and giant knotweed (*Fallopia*, Polygonaceae). *Botanical Journal of the Linnean Society*, **129**, 139–154.

Ishida TA, Hattori K, Sato H, Kimura MT (2003) Differentiation and hybridization between *Quercus crispula* and *Q. dentata* (Fagaceae): insights from morphological traits, amplified fragment length polymorphism markers, and leafminer composition. *American Journal of Botany*, **90**, 769–776.

Jiggins CD, Mallet J (2000) Bimodal hybrid zones and speciation. *Trends in Ecology and Evolution*, **15**, 250–255.

Kuehn MM, Minor JE, White BN (1999) An examination of hybridization between the cattail species *Typha latifolia* and *Typha angustifolia* using random amplified polymorphic DNA and chloroplast DNA markers. *Molecular Ecology*, **8**, 1981–1990.

Lamb T, Avise JC (1986) Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behavior. *Proceedings of the National Academy of Sciences of the United States of America*, **83**, 2526–2530.

Lopez JV, Kersanach R, Rehner SA, Knowlton N (1999) Molecular determination of species boundaries in corals: genetic analysis of the *Montastraea annularis* complex using amplified fragment length polymorphisms and a microsatellite marker. *The Biological Bulletin*, **196**, 80–93.

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics*, **15**, 65–95.

Marck C (1988) 'DNA strider': a 'C' program for the fast analysis of DNA and protein sequences on the Apple Macintosh family of computers. *Nucleic Acids Research*, **16**, 1829–1836.

McCauley DE (1994) Contrasting the distribution of chloroplast DNA and allozyme polymorphism among local populations of *Silene alba*: Implications for studies of gene flow in plants. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 8127–8131.

McCauley DE, Stevens JE, Peroni PA, Raveill JA (1996) The spatial distribution of chloroplast DNA and allozyme polymorphisms within a population of *Silene alba* (Caryophyllaceae). *American Journal of Botany*, **83**, 727–731.

Melendez-Ackerman EJ (1997) Patterns of color and nectar variation across an *Ipomopsis* (Polemoniaceae) hybrid zone. *American Journal of Botany*, **84**, 41–47.

Milne RI, Terzioglu S, Abbott RJ (2003) A hybrid zone dominated by fertile F_1s: maintenance of species barriers in *Rhododendron*. *Molecular Ecology*, **12**, 2719–2729.

Nason JD, Ellstrand NC, Arnold ML (1992) Patterns of hybridization and introgression in populations of oaks, manzanitas, and irises. *American Journal of Botany*, **79**, 101–111.

Nelson K, Baker JD, Honeycutt RL (1987) Mitochondrial DNA and protein differentiation between hybridizing cytotypes of the white-footed mouse, *Peromyscus leucopus*. *Evolution*, **41**, 864–872.

Neuffer B, Auge H, Mesch H, Amarell U, Brandl R (1999) Spread of violets in polluted pine forests: morphological and molecular evidence for the ecological importance of interspecific hybridization. *Molecular Ecology*, **8**, 365–377.

O'Hanlon PC, Peakall R, Briese DT (1999) Amplified fragment length polymorphism (AFLP) reveals introgression in weedy *Onopordum* thistles: hybridization and invasion. *Molecular Ecology*, **8**, 1239–1246.

Oddou-Muratorio S, Petit RJ, Le Guerroue B, Guesnet D, Demesure B (2001) Pollen- versus seed-mediated gene flow in a scattered forest tree species. *Evolution*, **55**, 1123–1135.

Paige KN, Capman WC, Jennetten P (1991) Mitochondrial inheritance patterns across a cottonwood hybrid zone: cytonuclear disequilibria and hybrid zone dynamics. *Evolution*, **45**, 1360–1369.

Peakall R, Ebert D, Scott LJ, Meagher PF, Offord CA (2003) Comparative genetic study confirms exceptionally low genetic variation in the ancient and endangered relictual conifer, *Wollemia nobilis* (Araucariaceae). *Molecular Ecology*, **12**, 2331–2343.

Rieseberg LH (1997) Hybrid origins of plant species. *Annual Review of Ecology and Systematics*, **28**, 359–389.

Rieseberg LH, Carney SC (1998) Tansley review, 102: plant hybridization. *New Phytologist*, **140**, 599–624.

Rieseberg LH, Ellstrand NC (1993) What can molecular and morphological markers tell us about plant hybridization? *Critical Reviews in Plant Science*, **12**, 213–241.

Rieseberg LH, Wendel JF (1993) Introgression and its consequences in plants. In: *Hybrid Zones and the Evolutionary Process* (ed. Harrison RG). Oxford University Press, Oxford.

Rieseberg LH, Desrochers AM, Youn SJ (1995) Interspecific pollen competition as a reproductive barrier between sympatric species of *Helianthus* (Asteraceae). *American Journal of Botany*, **82**, 515–519.

Sanger F, Air GM, Barrell BG *et al.* (1977) Nucleotide-sequence of a bacteriophage PhiChi174 DNA. *Nature*, **265**, 687–695.

Sutton BCS, Pritchard SC, Gawley JR, Newton CH, Kiss GK (1994) Analysis of Sitka spruce – interior spruce introgression in British Columbia using cytoplasmic and nuclear DNA probes. *Canadian Journal of Forest Research*, **24**, 278–285.

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, **17**, 1105–1109.

Tarayre M, Saumitou-Laprade P, Cuguen J, Couvet D, Thompson JD (1997) The spatial genetic structure of cytoplasmic (cpDNA) and nuclear (allozyme) markers within and among populations of the gynodioecious *Thymus vulgaris* (Labiatae) in southern France. *American Journal of Botany*, **84**, 1675–1684.

Wang H, McArthur ED, Sanderson SC, Graham JH, Freeman DC (1997) Narrow hybrid zone between two subspecies of big sagebrush (*Artemesia tridentata*: Asteraceae). IV. Reciprocal transplant experiments. *Evolution*, **51**, 95–102.

Waser NM, Price MV (1983) Optimal and actual outcrossing in plants, and the nature of plant–pollinator interaction. In: *Handbook of Experimental Pollination Biology* (eds Jones C, Little R), pp. 341–359. Scientific and Academic Editions, New York.

Wesselingh RA, Arnold ML (2000) Pollinator behaviour and the evolution of Louisiana iris hybrid zones. *Journal of Evolutionary Biology*, **13**, 171–180.

Wolf PG, Campbell DR (1995) Hierarchical analysis of allozymic and morphometric variation in a montane herb, *Ipomopsis aggregata* (Polemoniaceae). *Journal of Heredity*, **86**, 386–394.

Wolf PG, Soltis PS, Soltis DE (1991) Genetic relationships and patterns of allozymic divergence in the *Ipomopsis aggregata* complex and related species (Polemoniaceae). *American Journal of Botany*, **78**, 515–526.

Wolf PG, Soltis PS, Soltis DE (1993) Phylogenetic significance of chloroplast DNA restriction site variation in the *Ipomopsis aggregata* complex and related species (Polemoniaceae). *Systematic Botany*, **18**, 652–662.

Wolf PG, Murray RA, Sipes SD (1997) Species-independent, geographical structuring of chloroplast DNA haplotypes in a montane herb *Ipomopsis* (Polemoniaceae). *Molecular Ecology*, **6**, 283–291.

Xiang L, Werth CR, Emery SN, McCauley DE (2000) Population-specific gender-biased hybridization between *Dryopteris intermedia* and *D. carthusiana*: evidence from chloroplast DNA. *American Journal of Botany*, **87**, 1175–1180.

Young WP, Schupp JM, Keim P (1999) DNA methylation and AFLP marker distribution in the soybean genome. *Theoretical and Applied Genetics*, **99**, 785–790.

Young WP, Ostberg CO, Keim P, Thorgaard GH (2001) Genetic characterization of hybridization and introgression between anadromous rainbow trout (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O. clarki clarkii*). *Molecular Ecology*, **10**, 921–930.

The authors are broadly interested in the mechanisms of evolution in natural populations. This study is part of Carrie Wu's PhD dissertation on the genetic and ecophysiological consequences of hybridization in *Ipomopsis*. Diane Campbell has been conducting long-term studies of natural hybridization, using *Ipomopsis* as a model system, that combine field experiments, computer modeling, and molecular genetic approaches.
