

Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeat (ISSR) bands

ANDREA D. WOLFE,* QIU-YUN XIANG*‡ and SUSAN R. KEPHART†

*Department of Plant Biology, The Ohio State University 1735 Neil Avenue, Columbus, OH 43210–1293, USA, †Department of Biology, Willamette University, Salem, OR 97301, USA

Abstract

Inferences regarding hybridization rely on genetic markers to differentiate parental taxa from one another. Intersimple sequence repeat (ISSR) markers are based on single-primer PCR reactions where the primer sequence is derived from di- and trinucleotide repeats. These markers have successfully been used to assay genetic variability among cultivated plants, but have not yet been tested in natural populations. We used genetic markers generated from eight ISSR primers to examine patterns of hybridization and purported examples of hybrid speciation in *Penstemon* (Scrophulariaceae) in a hybrid complex involving *P. centranthifolius*, *P. grinnellii*, *P. spectabilis* and *P. clevelandii*. This hybrid complex has previously been studied using three molecular data sets (allozymes, and restriction-site variation of nuclear rDNA and chloroplast DNA). These studies revealed patterns of introgression involving *P. centranthifolius*, but were unsuccessful in determining whether gene flow occurs among the other species, and support for hypotheses of diploid hybrid speciation was also lacking. In this study, we were able to fingerprint each DNA accession sampled with one to three ISSR primers and most accessions could be identified with a single primer. We found population- and species-specific markers for each taxon surveyed. Our results: (i) do not support the hybrid origin of *P. spectabilis*; (ii) do support the hypothesis that *P. clevelandii* is a diploid hybrid species derived from *P. centranthifolius* and *P. spectabilis*; and (iii) demonstrate that pollen-mediated gene flow via hummingbird vectors is prevalent in the hybrid complex.

Keywords: diploid hybrid speciation, genetic marker, introgression, ISSR, *Penstemon*, pollen-mediated gene flow

Received 24 November 1997; revision received 23 February 1998; accepted 23 February 1998

Introduction

The importance of hybridization in plants has been a subject of intense scrutiny for most of the 20th century. Edgar Anderson (1948; Anderson & Stebbins 1954), Charles B. Heiser Jr. (1949b), and G. Ledyard Stebbins (1950, 1971) argued that hybridization could serve as a source of increased genetic variation, ultimately leading to speciation. In a widely cited opposing opinion, W. H. Wagner Jr. (1970) argued that hybridization produces evolutionary

noise by posing the question: 'In the broad picture of evolution, may these phenomena not merely be trivial aberrations and defects in the biology of plants, produced by simple genetic transformations or chance fertilizations, which plants can tolerate, but which have little or no importance to the big picture?'

Recently, molecular studies have demonstrated that hybridization does play a significant role in the evolution of plants (reviewed in Rieseberg & Ellstrand 1993; Arnold 1997). For example, chloroplast capture has been inferred in numerous independent lineages from phylogenetic studies (Rieseberg & Soltis 1991; Brubaker *et al.* 1993; Mason-Gamer *et al.* 1995; Soltis & Kuzoff 1995; Wolfe & Elisens 1995; Schilling & Panero 1996; Soltis *et al.* 1996), gene flow within and beyond obvious

Correspondence: A. D. Wolfe. Fax: +1-614-292-6345; E-mail: wolfe.205@osu.edu

‡Present address: Department of Biological Sciences, Idaho State University, Pocatello, ID 83209–8007, USA.

hybrid zones has been documented (Heiser 1973; reviewed in Rieseberg & Wendel 1993; Arnold 1997), and new species are known to arise as polyploid hybrids (reviewed in Soltis & Soltis 1993). Yet while polyploid hybrid speciation has long been recognized as an important mechanism of evolution in plants (Stebbins 1950; Barrington *et al.* 1989; Phipps *et al.* 1991; Haufler *et al.* 1995; Soltis *et al.* 1995; Graham 1997), the importance of hybridization in the formation of diploid species is less clear (or much less certain), with only a handful of known examples (*Stephanomeria diegensis*, Gallez & Gottlieb 1982; three species of *Helianthus*, Rieseberg 1991; Rieseberg *et al.* 1991; *Iris nelsonii*, Arnold *et al.* 1991; *Paeonia*, Sang *et al.* 1995; *Encelia virginensis*, Allan *et al.* 1997).

Documentation of hybridization initially depended on morphological characters. Hybrid indices were used to demonstrate intermediacy between parental types to infer that individuals under consideration either were or were not of hybrid origin (Anderson 1948, 1949; Heiser 1949a; reviewed in Grant 1981). The complexity of using morphological characters was compounded with the elucidation that not all characters are additive and that many morphological features are under complex genetic control (Gottlieb 1972; reviewed in Meyerowitz 1997). Flavonoid and allozyme studies offered some help in terms of deciphering patterns of hybridization, but not always with complete success (Smith & Levin 1963; Levin 1967; Levy & Levin 1971; Levy 1976; King 1979; some case studies in Rieseberg & Wendel 1993). Recent developments in systematics include the use of molecular characters such as nuclear rDNA and chloroplast DNA (cpDNA) restriction-site variation, nucleotide sequencing, and PCR-generated markers (e.g. RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism)). Molecular markers have clearly been useful in many studies of hybridization, but are often too invariable to differentiate among closely related species, or may be technically cumbersome or expensive for many applications.

Here we report the use of hypervariable nuclear markers generated from intersimple sequence repeat (ISSR) PCR reactions to examine patterns of hybridization in natural populations of a known hybrid complex in the genus *Penstemon* (Scrophulariaceae). We chose a group of plants that have previously been examined using three other molecular data sets (allozymes, and rDNA and cpDNA restriction-site variation; Wolfe & Elisens 1993, 1994, 1995) to assess hypotheses of diploid hybrid speciation and introgressive hybridization. We then compare the results of the present study with previous results to assess the effectiveness of ISSR banding patterns for elucidating patterns of hybridization and diploid hybrid speciation.

Materials and methods

Background on Penstemon hybrid complex

Two examples of diploid hybrid speciation proposed by Straw (1955a,b, 1956a,b) involved four species of *Penstemon* with distributions in southern California. The first purported hybrid species, *P. spectabilis*, was derived from *P. centranthifolius* and *P. grinnellii*. Ethological isolation was proposed as the factor stabilizing the wasp-pollinated *P. spectabilis* from the hummingbird- and carpenter bee-pollinated parental species (*P. centranthifolius* and *P. grinnellii*, respectively). The second putative hybrid species, *P. clevelandii*, was derived from *P. centranthifolius* and *P. spectabilis*. *P. clevelandii* is pollinated by solitary bees as well as hummingbirds. Ecological isolation was invoked as the primary stabilizing factor because *P. clevelandii* occurs in habitats that are different from either of the purported parental species. Natural hybrids have been found between each pair of purported parental species (*P. X dubius* on at least two occasions with either *P. centranthifolius* or *P. grinnellii*; and *P. X parishii* is commonly found wherever *P. centranthifolius* and *P. spectabilis* are in sympatry).

Straw's hypotheses were based on morphological and artificial crossing studies as well as studies of pollinator behaviour, and there was good evidence that the proposed scenarios were probable. In a series of studies using molecular markers, Wolfe & Elisens (1993, 1994, 1995) found: (i) that pollen-mediated gene flow was apparent between *P. centranthifolius* and the insect-pollinated species in the hybrid complex with most of the gene flow coming from *P. centranthifolius* into the other species; (ii) very few genetic markers to differentiate the closely related insect-pollinated species; (iii) no support for hybrid speciation, but no solid evidence to negate the hypotheses due to a lack of genetic markers; and (iv) chloroplast capture events in the lineages of the hybrid complex. Wolfe & Elisens (1995) concluded that the evidence from their molecular studies was more consistent with a hypothesis of secondary contact and introgression than hybrid speciation. The evidence used for inferences of introgression pointed towards patterns involving nuclear markers of *P. centranthifolius* compared to each of the insect-pollinated species, but there was little information with which to determine whether hybridization or hybrid speciation had occurred involving the latter because of the lack of markers among them.

Background on ISSR markers

Intersimple sequence repeat markers are generated from single-primer PCR reactions where the primer is designed from di- or trinucleotide repeat motifs with a 5' or 3' anchoring sequence of one to three nucleotides (Gupta *et al.* 1994; Zietkiewicz *et al.* 1994). Incorporation of

anchoring sequences eliminates strand-slippage artefacts that may otherwise be encountered. Anchoring sequences are generally random and may include redundant bases (e.g. RY). Different anchoring sequences used on a common microsatellite motif produce unique banding profiles. The amplified regions represent the nucleotide sequence between two SSR priming sites orientated on opposite DNA strands (Fig. 1). The premise is that SSR regions are scattered evenly throughout the genome (Hamada & Kakunaga 1982; Tautz & Renz 1984; Condit & Hubbell 1991) and the chances of amplifying between two adjacent regions within the limits of *Taq* polymerase processivity is high enough that a large number of polymorphic bands should be generated.

ISSR markers are inherited in a dominant or codominant Mendelian fashion (Gupta *et al.* 1994; Tsumura *et al.* 1996). They are interpreted as dominant markers similar to RAPD data and are scored as diallelic with 'band present' or 'band absent' (Fig. 1). The absence of a band is interpreted as primer divergence or loss of a locus through the deletion of the SSR site or chromosomal rearrangement (Wolfe & Liston 1998). The use of ISSR markers has been restricted to cultivated species (Kantety *et al.* 1995; Salimath *et al.* 1995; Sharma *et al.* 1995; Wolff *et al.* 1995; de Oliveira *et al.* 1996; Tsumura *et al.* 1996; Yang *et al.* 1996; Fang & Roose 1997; Fang *et al.* 1997; Godwin *et al.* 1997; Nagaoka & Ogihara 1997; Parsons *et al.* 1997), but the use of these new markers in natural populations shows their potential for a range of applications from population-level to interspecific studies (Robinson *et al.* 1997; Wolfe & Liston 1998; this study; A. Liston personal communication; unpublished data).

PCR reactions

A total of 134 DNA accessions representing 11 taxa of *Penstemon* section *Gentianoides* and section *Spectabiles* was included in the study (Table 1). The DNAs were the same accessions used in previous studies employing nuclear rDNA and chloroplast restriction-site variation (Wolfe & Elisens 1994, 1995).

ISSR Schematic (5' anchor)

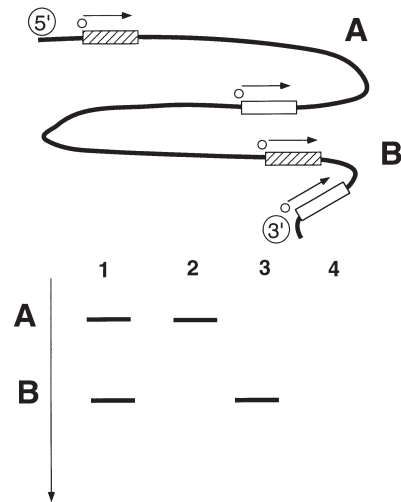


Fig. 1 Diagram of ISSR primer annealing and possible banding patterns. A and B refer to intersimple sequence repeat regions that are amplified if primer sequences anchored on the 5' end of microsatellite regions are intact. Boxes with hash marks represent primer sequences orientated in the 5' direction and clear boxes represent primer sequences on the complementary strand. If all primer sites are present, two bands will result as in lane 1. If one or more primer sites are absent, one or both bands may be absent as in lanes 2–4.

Eight ISSR primers were used in single-primer reactions: 7: (CT)₈-RG; 814.1: (CT)₈-TG; 17898: (CA)₆-RY; 17899: (CA)₆-RG; 17901: (GT)₆-YR; 17902: (GT)₆-AY; AW3: (GT)₆-RG; and M1: CAA-(GA)₅. Optimization reactions were run on a 1 °C temperature gradient (40–47 °C) with a matrix of different MgCl₂ and *Taq* polymerase concentrations. After optimization, standard reaction conditions were 0.4 μM primer, 1× *Taq* polymerase buffer, 0.2 μM dNTPs, 0.25–0.5 U *Taq* polymerase (Gibco BRL), 3 mM MgCl₂, and 0.5 μL of DNA in a 25 μL volume. We used a Stratagene Robocycler with the thermocycler program set

Population No.	Collection No.	Location	No. of Accessions	cpDNA haplotype	rDNA type
Section <i>Gentianoides</i>					
<i>P. centranthifolius</i>					
1	Wolfe 445	Glenn Co.	5	CE1	CE
2	Wolfe 337	San Benito Co.	2	CE3	CE
3	Wolfe 339	San Benito Co.	1	CE3	CE
4	Wolfe 453	San Benito Co.	1	CE2	CE
5	Wolfe 450	Monterey Co.	1	CE2	CE
6	Wolfe 452	Monterey Co.	2	CE2	CE
7	Wolfe 305	Los Angeles Co.	2	CE3	CE

Table 1 Collection and DNA accession information for taxa in the *Penstemon* hybrid complex. cpDNA haplotype and rDNA type were determined in Wolfe & Elisens (1994, 1995)

Population No.	Collection No.	Location	No. of Accessions	cpDNA haplotype	rDNA type
8*	Wolfe 327	San Bernardino Co.	2	CE3	CE, SP
9	Wolfe 308	Riverside Co.	6	CE3	CE
10*	Wolfe 309	Riverside Co.	5	SP2	CE
11	Wolfe 314	Riverside Co.	2	CE3	CE
12	Wolfe 199	San Diego Co.	2	CE3	CE
13	Wolfe 209	San Diego Co.	7	CE3	CE
14	Wolfe 217	San Diego Co.	1	CE3	CE
15†	Wolfe 221	San Diego Co.	1	CE3	CE
16*	Wolfe 346	San Diego Co.	6	CE3	CE
<u>Natural hybrids</u>					
<i>P. X parishii</i>					
17‡	Wolfe 222	San Diego Co.	4	SP2	CE, SP
18*‡	Wolfe 224	San Diego Co.	2	SP2	CE, SP
<u>Section <i>Spectabiles</i></u>					
<i>P. clevelandii</i> var. <i>clevelandii</i>					
19	Wolfe 186	San Diego Co.	3	CL2	SP
20	Wolfe 198	San Diego Co.	1	CL2	SP
21	Wolfe 206	San Diego Co.	1	CL2	SP
22‡	Wolfe 207	San Diego Co.	1	CL2	CE, SP
<i>P. clevelandii</i> var. <i>connatus</i>					
23	Wolfe 313	Riverside Co.	7	CL1	CE, SP
<i>P. spectabilis</i> var. <i>spectabilis</i>					
24‡	Wolfe 310	Riverside Co.	1	SP2	CE, SP
25	Wolfe 319	Riverside Co.	4	SP2	CE, SP
26‡	Wolfe 210	San Diego Co.	2	SP2	CE, SP
27	Wolfe 212	San Diego Co.	2	SP2	CE, SP
28	Wolfe 218	San Diego Co.	2	SP2	CE, SP
29	Wolfe 223	San Diego Co.	8	SP2	CE, SP
30‡	Wolfe 347	San Diego Co.	2	SP2	SP
<i>P. spectabilis</i> var. <i>subviscosus</i>					
31†‡	Wolfe 323	San Bernardino Co.	8	SP1	CE, SP
32‡	Wolfe 326	San Bernardino Co.	2	SP1	CE, SP
<i>P. grinnellii</i> var. <i>grinnellii</i>					
33	Wolfe 306	Los Angeles Co.	5	GR	GR
34	Wolfe 331	Los Angeles Co.	2	CE3, GR	CE, GR
35	Wolfe 332	Los Angeles Co.	2	GR	GR
36	Wolfe 333	Los Angeles Co.	1	GR	GR
<i>P. grinnellii</i> var. <i>scrophularioides</i>					
37	Wolfe 299	Kern Co.	8	GR	CE, GR
38	Wolfe 303	Kern Co.	4	GR	CE, GR
39	Wolfe 464	Kern Co.	4	GR	CE, GR
<i>P. incertus</i>					
40	Wolfe 459	Kern Co.	1	IN	IN
41	Wolfe 465	Kern Co.	2	IN	IN
<i>P. palmeri</i> var. <i>palmeri</i>					
42	Wolfe 291	Utah: Washington Co.	2	PA2	PA
43	Wolfe 292	Nevada: Clark Co.	3	PA3	PA
44	Wolfe 293	Nevada: Clark Co.	1	PA3	PA
<i>P. palmeri</i> var. <i>eglandulosa</i>					
45	Wolfe 282	Arizona: Mohave Co.	2	PA1	PA
46	Wolfe 283	Arizona: Mohave Co.	1	PA1	PA

Table 1 continued.

*Population sympatric with *P. spectabilis*.

†Population sympatric with *P. X parishii*.

‡Population sympatric with *P. centranthifolius*.

Abbreviations: CE = *P. centranthifolius*, CL = *P. clevelandii*, XP = *P. X parishii*, SP = *P. spectabilis*,

GR = *P. grinnellii*.

at 1.5 min at 94 °C; 35 cycles of 40 s at 94 °C, 45 s at 44 °C or 45 °C, 1.5 min at 72 °C; 45 s at 94 °C, 45 s at 44 °C, 5 min at 72 °C; 6 °C soak. Replicate accessions were assayed in separate experiments to verify repeatability of results. Negative controls where all reagents but DNA were added to the reaction mix were run with each experiment.

PCR reactions were characterized on 1.5% agarose gels in 1× TAE buffer by loading entire reaction volumes into prepared wells. Gels were run until the bromophenol blue markers ran 10 cm (5 h at 80 mA) and then were stained with ethidium bromide (EtBr). ISSR bands were visualized on a UV transilluminator and were documented digitally using an Alpha Innotech imaging system (Alpha Innotech Corporation). The digital image files were transferred in a TIFF format to a PowerMac 7500 and analysed using the BioMax 1D image analysis software (Eastman Kodak Company). Fragment sizes were estimated based on 1-kb ladder size standards (Gibco BRL) according to the algorithm provided in the BioMax 1D software. Fragment sizes were used to assign loci for each primer. Bands were scored as diallelic for each assigned locus (1 = band present; 0 = band absent).

Data analysis

We calculated the total number of bands and distribution of bands across taxa, number of polymorphic bands, average number of bands per primer for each taxon, and bands shared among taxa. Several categories of marker bands were defined: (i) bands present in at least 25% of all populations and 25% of all DNA accessions of a single taxon were designated as marker bands for that taxon if those bands were found in only a few populations and / or individuals of the other species in the hybrid complex; (ii) bands present in at least 25% of all populations but not in 25% of all DNA accessions of a single taxon were identi-

fied as potential markers for that taxon, but not used for calculations reserved for marker bands; (iii) bands present in more populations or accessions of one taxon compared to the others assayed were identified, but not used in calculations to estimate levels of gene flow. Eight populations included in this survey reflected sympatric distributions between *P. centranthifolius* and *P. spectabilis*, and both populations of *P. X parishii* are sympatric with one or both parental species (Table 1). Because sympatry between *P. centranthifolius* and *P. spectabilis* is common and hybridization has been consistently observed between sympatric populations (Straw 1955a; Wolfe & Elisens 1993, 1994, 1995), we assayed the distribution of markers among populations as a whole and with sympatric populations excluded. One population of *P. clevelandii* was sympatric with *P. centranthifolius* (Table 1) and the distribution of markers from *P. centranthifolius* was calculated in the same fashion.

A distance matrix was generated from the raw data matrix of 1s and 0s by using a computer program written by Vera Ford (unpublished; University of California, Davis). Pairwise average similarity comparisons between groups of DNA accessions representing specific taxa were also calculated. Vera Ford's program only considers band matches in the estimation of distance and similarity values. UPGMA trees were generated for both the distance and similarity matrices using PHYLIP (Felsenstein 1995).

Results

A total of 286 ISSR bands was scored with a range of bands per taxon of 60–184 (Table 2). ISSR banding profiles were capable of fingerprinting each DNA accession in this study with one to three primers. Most accessions could be identified using a single primer. Some accessions within the same population required three primers for assigning a

Table 2 Summary of ISSR bands scored. Abbreviations are as shown in Table 1; numbers in parentheses represent the number of DNA accessions contributing to the average number of bands per primer for each taxon (*)

Primer	Primer sequence	Total no. of bands	Size range of bands (kb)	CE (46)	SP (31)	GR (26)	CL (13)	PA (9)	XP (6)	IN (3)
7	(CT) ₈ -RG	26	553–3000	4.2*	4.5*	4.0*	4.3*	1.4*	5.2*	1.3*
814.1	(CT) ₈ -TG	24	446–2491	3.3	3.3	2.8	4.5	3.4	4.2	1.3
17898	(CA) ₆ -RY	28	539–2117	3.6	2.9	3.4	3.3	4.0	4.8	2.3
17899	(CA) ₆ -RG	51	300–3300	6.8	6.5	6.3	9.0	12.5	9.5	10.3
17901	(GT) ₆ -YR	46	497–3000	4.5	4.5	5.5	5.7	6.1	4.7	3.7
17902	(GT) ₆ -AY	35	479–2600	5.2	5.9	6.0	7.2	7.1	5.7	6.0
AW3	(GT) ₆ -RG	31	373–2900	5.2	4.1	5.4	5.3	4.0	4.7	3.3
M1	CAA-(GA) ₅	45	471–3600	8.3	7.2	7.8	7.5	9.0	6.7	10.3
	Average	35.8	457–2876	5.1	4.8	5.2	5.8	5.9	5.7	4.8
			Total no. of bands	184	164	149	140	105	108	60
			Per cent bands polymorphic	95	90	88	89	91	72	83

genotype. There was an apparent correlation of the number of DNA accessions with the number of bands scored: the more accessions surveyed per taxon, the higher the number of bands within taxa (Table 2). The percentage of polymorphic bands ranged from 72 to 95 with no apparent trend with regard to the number of accessions surveyed (Table 2). Only one band was found in all populations (absent from only three DNA accessions), and two additional bands were nearly monomorphic across all taxa. The average number of bands per primer ranged from 1.3 to 12.5 with the average number of bands across all primers ranging from 4.8 to 5.9 (Table 2). Among the 3'-anchored SSR primers, the CA- and GT-repeats yielded a higher average number of bands per primer than the CT-repeats. However, the 5'-anchored GA-repeat primer (M1) had approximately the same average number of bands/primer as one of the CA-repeat primers (17899).

All ISSR bands scored ranged from 300 to 3600 bp (Table 2). Bands running faster than 300 bp migrated off the gel matrix before EtBr staining and were not scored. The majority of bands scored were in the range of 500 to 2000 bp. We observed no major discrepancies among replicate experiments in that bands scored from one set of reactions were identical to subsequent reactions. This pattern has held through trials with several other genera (A.

D. Wolfe *et al.* unpublished; A. Liston *et al.* unpublished). In addition, negative controls had no amplification products.

Taxon-specific ISSR bands were found for each species (Table 3). *Penstemon centranthifolius* had the most Category I and II marker bands (47) plus eight private bands (those restricted to a single taxon; data not shown) that could be classified as Category I or II markers; *P. spectabilis* had nine Category I or II marker bands; *P. grinnellii* had eight Category I or II marker bands plus seven private bands of that classification; *P. cleveandii* had three Category I or II marker bands plus one additional private band that could be classified as Category I. Marker bands were not assayed for *P. incertus* and *P. palmeri* because they are not part of the hybrid complex.

P. cleveandii and *P. X parishii* had an additive pattern of Category I and II markers from *P. centranthifolius* and *P. spectabilis* (Table 3). When considering the distribution of markers as a whole and those that can be inferred as the result of introgression (e.g. from sympatric populations or where *P. X parishii* shares bands with one or both parental species), most of the marker bands from *P. centranthifolius* found in *P. cleveandii* result from an additive pattern and not from introgression, whereas the majority of *P. centranthifolius* bands found in DNA accessions from *P. spectabilis*

Table 3 Distribution of ISSR bands among taxa in the hybrid complex arranged by groups of taxa and in alphanumerical order of primer names and loci. Abbreviations are as shown in Table 1

Bands shared among taxa	ISSR locus*	Marker*	CE 16 (46)†	CL 5 (13)†	XP 2 (6)†	SP 9 (31) †	GR 7 (26) †
CE/CL	814–1770	CE	5 (10) 1 (1)	1(1)	1(1)	0 (0)	0 (0)
	17898–1110	CE	10 (17) 3 (3)	1 (1)	0 (0)	0 (0)	0 (0)
	17899–300	ce/cl	1 (1)	1 (1)	0 (0)	0 (0)	0 (0)
	17899–617	ce	3 (4)	2 (2)	0 (0)	0 (0)	0 (0)
	17899–647	ce	3 (4) 2 (2)	2 (2) 1 (1)	1 (1)	0 (0)	0 (0)
	17898–900	ce	3 (5)	1 (1)	0 (0)	0 (0)	0 (0)
	17899–1110	CE	5 (9) 2 (4)	1 (1)	0 (0)	0 (0)	0 (0)
	17899–1524	CE	4 (8) 1 (5)	1 (2)	1 (1)	0 (0)	0 (0)
	17899–1658	ce	2 (2)	1 (1)	0 (0)	0 (0)	0 (0)
	17899–2092	cl	1 (1) 1 (1)	1 (2)	0 (0)	0 (0)	0 (0)
	17901–2200	CL	2 (4) 2 (4)	3 (5)	1 (1)	0 (0)	0 (0)
	17902–1166	CE	4 (6) 2 (4)	1 (3)	0 (0)	0 (0)	0 (0)
	17902–1325	ce/cl	2 (2) 1 (1)	2 (2)	0 (0)	0 (0)	0 (0)
	M1–1000	CE	7 (12) 1 (2)	1 (1)	1 (1)	0 (0)	0 (0)
	M1–2800	CE	6 (16) 2 (10)	4 (8)	1 (1)	0 (0)	0 (0)

Table 3 Continued

Bands shared among taxa	ISSR locus*	Marker*	CE 16 (46)†	CL 5 (13)†	XP 2 (6)†	SP 9 (31) †	GR 7 (26) †	
CE/XP	17898–1175	ce	4 (4) 2 (2)	0 (0)	1 (1)	0 (0)	0 (0)	
	17899–2503	ce	2 (3) 1 (1)	0 (0)	2 (4)	0 (0)	0 (0)	
	17901–2100	CE	4 (13) 2 (3)	0 (0)	1 (1)	0 (0)	0 (0)	
	17902–918	ce	1 (1)	0 (0)	1 (1)	0 (0)	0 (0)	
	M1–630	CE	7 (15) 2 (6)	0 (0)	1 (2)	0 (0)	0 (0)	
CL/XP	814–1699	cl	0 (0)	1 (1)	1 (1)	0 (0)	0 (0)	
CL/SP	7–681	sp	0 (0)	1 (1)	0 (0)	1 (2) <u>1 (2)</u>	0 (0)	
	814–712	SP/CL	0 (0)	3 (9)	1 (1)	5 (14) <u>2 (5)</u>	0 (0)	
	814–743	sp	0 (0)	2 (2)	0 (0)	3 (3) 2 (2) <u>1 (1)</u>	0 (0)	
	17901–1785	sp	0 (0)	1 (1)	1 (1)	2 (2) 1 (1)	0 (0)	
	17901–2700	SP	0 (0)	1 (1)	1 (1)	3 (5) <u>1 (1)</u>	0 (0)	
	17902–1850	SP/CL	0 (0)	3 (6) 1 (1)	1 (1)	7 (14) 2 (3) <u>2 (3)</u>	0 (0)	
	AW3–703	sp	0 (0)	1 (1) 1 (1)	0 (0)	2 (5) <u>1 (2)</u>	0 (0)	
	AW3–1230	CL	0 (0)	4 (8) 1 (1)	1 (2)	2 (4)	0 (0)	
	XP/SP	7–3000	sp	0 (0)	0 (0)	1 (2)	1 (1) <u>1 (1)</u>	0 (0)
		17898–1531	sp	0 (0)	0 (0)	1 (1)	1 (1) <u>1 (1)</u>	0 (0)
		17901–1411	SP	0 (0)	0 (0)	1 (2)	3 (4) <u>1 (1)</u>	0 (0)
	CE/CL/SP	7–1040	CE	5 (5) 1 (1)	2 (3) 1 (1)	1 (1)	1 (1)	0 (0)
		7–1170	CE	8 (12) 1 (1)	1 (3)	0 (0)	1 (2)	0 (0)
		17899–422	CL	1 (1) 1 (1)	3 (3) 1 (1)	0 (0)	1 (1) <u>1 (1)</u>	0 (0)
		17899–1146	CE/CL	4 (5) 1 (1)	3 (7)	1 (1)	1 (2)	0 (0)
17899–1357		CE	11 (19) 3 (5)	1 (1)	2 (2)	3 (3) 1 (1) <u>1 (1)</u>	0 (0)	
17899–1843		CE	6 (12) 1 (2)	2 (2)	0 (0)	4 (5) 2 (2) 1 (2)	0 (0)	
17902–720		CE	12 (34) 3 (11)	3 (4) 1 (1)	2 (3)	2 (3) 1 (1)	0 (0)	
17902–2000		CE	15 (25) 3 (10)	2 (2)	1 (1)	2 (2) 1 (1)	0 (0)	
17902–2300		CE	7 (14) 2 (2)	2 (3) 1 (1)	0 (0)	1 (1)	0 (0)	
M1–471		sp	1 (1)	1 (1)	1 (2)	3 (3) 2 (2)	0 (0)	

Table 3 Continued

Bands shared among taxa	ISSR locus*	Marker*	CE 16 (46)†	CL 5 (13)†	XP 2 (6)†	SP 9 (31) †	GR 7 (26) †
CE/SP (nonintrogressive)‡	17899-569	ce/sp	1 (1)	0 (0)	0 (0)	1 (1)	0 (0)
CE/SP (introgressive)‡	7-2300	CE	4 (7) 2 (3)	0 (0)	1 (1)	1 (1)	0 (0)
	814-913	CE	10 (27) 3 (12)	0 (0)	1 (2)	1 (2)	0 (0)
	17899-451	CE/SP	15 (31) 3 (8)	0 (0)	0 (0)	8 (17) 3 (5) <u>1 (5)</u>	0 (0)
	17901-543	CE	4 (7) 1 (3)	0 (0)	1 (1)	1 (1) 1 (1)	0 (0)
	17901-805	CE	10 (21) 3 (6)	0 (0)	1 (4)	1 (2)	0 (0)
	17901-1240	CE	4 (4) 1 (1)	0 (0)	1 (3)	2 (3) 1 (1)	0 (0)
	17901-1370	CE/SP	7 (16) 2 (4)	0 (0)	0 (0)	4 (9) 2 (4) <u>1 (3)</u>	0 (0)
	17901-1765	sp	1 (1) 1 (1)	0 (0)	0 (0)	2 (3) <u>1 (2)</u>	0 (0)
	17902-866	ce/sp	2 (3) 1 (2)	0 (0)	0 (0)	3 (3) 2 (2)	0 (0)
	AW3-680	CE/SP	4 (9) 3 (7)	0 (0)	0 (0)	4 (5) 2 (2) <u>1 (1)</u>	0 (0)
	AW3-880	CE	16 (43) 3 (13)	0 (0)	2 (4)	2 (2) 1 (1)	0 (0)
	M1-900	ce	2 (3)	0 (0)	0 (0)	1 (2) 1 (2)	0 (0)
	M1-2125	sp	1 (1)	0 (0)	0 (0)	2 (2) <u>1 (1)</u>	0 (0)
SP/GR	7-1600	sp	0 (0)	0 (0)	0 (0)	2 (4) <u>1 (1)</u>	1 (1)
	17899-950	GR	0 (0)	0 (0)	0 (0)	2 (6)	5 (18)
	17899-1804	SP	0 (0)	0 (0)	0 (0)	3 (6) <u>2 (4)</u>	2 (2)
	17901-1334	SP	0 (0)	0 (0)	0 (0)	3 (5) <u>1 (2)</u>	1 (1)
	17901-1700	GR	0 (0)	0 (0)	0 (0)	1 (1) 1 (1)	4 (9)
	AW3-478	sp/gr	0 (0)	0 (0)	0 (0)	1 (1) 1 (1)	1 (1)
	M1-743	GR	0 (0)	0 (0)	0 (0)	2 (3) <u>1 (1)</u>	4 (9)
	M1-2659	SP	0 (0)	0 (0)	0 (0)	5 (7) 1 (1) <u>1 (2)</u>	1 (1)
CE/SP/GR	17898-1030	CE	7 (10) 1 (1)	0 (0)	1 (2)	1 (1) <u>1 (1)</u>	1 (2)
	17898-1150	CE	7 (9) 3 (4)	0 (0)	0 (0)	3 (4) 1 (1) <u>1 (2)</u>	1 (1)
	17899-520	spec	1 (1)	0 (0)	0 (0)	3 (3) 1 (1)	4 (6)

Table 3 Continued

Bands shared among taxa	ISSR locus*	Marker*	CE 16 (46)†	CL 5 (13)†	XP 2 (6)†	SP 9 (31) †	GR 7 (26) †
	17899–1730	CE	6 (13) 2 (4)	0 (0)	1 (1)	1 (1) 1 (1)	1 (1)
	17899–2477	CE	7 (19) 2 (5)	0 (0)	1 (1)	5 (7) 2 (2) <u>1 (3)</u>	1 (2)
	17901–1100	CE	4 (4) 1 (1)	0 (0)	1 (1)	1 (1) <u>1 (1)</u>	2 (2)
	17902–567	SP	4 (7) 1 (2)	0 (0)	1 (1)	8 (16) 2 (3) <u>2 (5)</u>	2 (2)
	17902–673	GR	1 (1)	0 (0)	0 (0)	2 (2) 2 (2)	4 (6)
	AW3–593	CE	10 (17) 2 (3)	0 (0)	0 (0)	2 (2) 1 (1)	1 (1)
	AW3–1100	GR	2 (6) 2 (6)	0 (0)	0 (0)	1 (1)	6 (9)
	M1–2078	GR	3 (3) 1 (1)	0 (0)	0 (0)	1 (1)	5 (8)
CE/GR	17898–866	GR	1 (1) 1 (1)	0 (0)	0 (0)	0 (0)	2 (7)
	17901–1219	CE/GR	4 (8) 1 (1)	0 (0)	1 (1)	0 (0)	3 (11)
	17901–1846	ce	1 (1)	0 (0)	1 (2)	0 (0)	1 (1)
	17902–941	CE/GR	4 (7) 1 (2)	0 (0)	0 (0)	0 (0)	4 (4)
	AW3–439	(gr)	1 (1)	0 (0)	0 (0)	0 (0)	2 (5)
	AW3–1423	ce/gr	3 (5) 1 (3)	0 (0)	0 (0)	0 (0)	2 (7)
	M1–1900	CE	6 (16)	0 (0)	0 (0)	0 (0)	1 (1)
	M1–1950	CE	13 (30) 3 (8)	0 (0)	1 (3)	0 (0)	2 (2)
CL/GR							
XP/GR							
CL/XP/GR	17901–1173	cl/gr	0 (0)	1 (1)	0 (0)	0 (0)	1 (1)
	17901–1450	cl/gr	0 (0)	1 (2)	0 (0)	0 (0)	3 (3)
	17901–1669	cl/gr	0 (0)	2 (2)	0 (0)	0 (0)	3 (6)
	17901–1867	cl/gr	0 (0)	2 (2)	0 (0)	0 (0)	2 (3)
	17902–1368	cl/gr	0 (0)	2 (3)	1 (1)	0 (0)	2 (2)
	17902–1723	gr	0 (0)	0 (0)	1 (1)	0 (0)	1 (1)
	AW3–933	GR	0 (0)	2 (2)	0 (0)	0 (0)	7 (24)
SPEC	7–617	SPEC	0 (0)	4 (7)	1 (2)	9 (27) 3 (4) <u>2 (10)</u>	5 (9)
	7–721	SPEC	0 (0)	2 (2)	1 (1)	2 (2) 1 (1)	3 (7)
	7–805	spec	0 (0)	1 (1)	0 (0)	2 (3) 1 (1) <u>1 (2)</u>	1 (1)
	814–1600	SP	0 (0)	1 (1)	0 (0)	5 (8) 2 (2) <u>1 (4)</u>	1 (2)
	17898–555	SPEC	0 (0)	5 (12) 1 (1)	2 (5)	9 (29) 3 (5) <u>2 (9)</u>	6 (15)
	17898–1200	SPEC	0 (0)	2 (6)	2 (3)	2 (3) 1 (1) <u>1 (2)</u>	6 (15)

Table 3 Continued

Bands shared among taxa	ISSR locus*	Marker*	CE 16 (46)†	CL 5 (13)†	XP 2 (6)†	SP 9 (31) †	GR 7 (26) †
	17899-686	SPEC	0 (0)	2 (2)	0 (0)	3 (3) 2 (2)	1 (1)
	17899-829	SPEC	0 (0)	5 (10) 1 (1)	2 (5)	5 (7) 1 (1) <u>2 (3)</u>	4 (11)
	17899-911	SPEC	0 (0)	1 (1)	0 (0)	2 (2) 1 (1) <u>1 (1)</u>	4 (8)
	17899-1461	spec	0 (0)	1 (2)	0 (0)	2 (4) 1 (1) <u>1 (1)</u>	2 (3)
	17899-1676	SPEC	0 (0)	2 (6)	2 (4)	6 (11) 3 (3)	6 (8)
	17899-1922	SPEC	0 (0)	4 (8)	2 (5)	1 (3)	6 (14)
	17899-2252	SPEC	0 (0)	3 (4) 1 (1)	1 (1)	1 (1) 1 (1)	4 (13)
	17899-2800	SPEC	0 (0)	4 (8) 1 (1)	1 (1)	4 (4) 1 (1)	1 (1)
	17901-670	SPEC	0 (0)	1 (1)	0 (0)	2 (2) <u>1 (1)</u>	3 (6)
	17901-1042	SPEC	0 (0)	4 (4) 1 (1)	0 (0)	7 (16) 3 (5) <u>1 (5)</u>	7 (20)
	17902-810	SPEC	0 (0)	4 (11)	1 (1)	9 (24) 3 (4) <u>2 (10)</u>	7 (22)
	17902-1400	SPEC	0 (0)	4 (6) 1 (1)	2 (5)	5 (6)	6 (14)
	AW3-373	SPEC	0 (0)	4 (10) 1 (1)	2 (4)	9 (31) 3 (5) <u>2 (10)</u>	6 (14)
	AW3-837	SPEC	0 (0)	5 (11) 1 (1)	1 (1)	9 (24) 3 (4) <u>2 (8)</u>	7 (22)
	AW3-1358	SPEC	0 (0)	5 (13) 1 (1)	2 (3)	9 (26) 3 (4) <u>2 (8)</u>	7 (23)
	M1-1809	spec	0 (0)	1 (1)	0 (0)	1 (1)	1 (1)
	M1-1850	SPEC	0 (0)	5 (10) 1 (1)	2 (2)	9 (30) 3 (5) <u>2 (10)</u>	6 (15)
	M1-2330	SPEC	0 (0)	1 (2)	0 (0)	3 (3) 1 (1) <u>1 (1)</u>	1 (1)
CE/SPEC	7-1670	CE	14 (41) 2 (11)	2 (3) 1 (1)	2 (3)	1 (1)	1 (1)
	814-1020	CE	14 (27) 3 (7)	1 (1)	2 (3)	3 (4) 1 (1)	3 (4)
	17898-1330	CE	16 (44) 3 (13)	2 (3) 1 (1)	1 (4)	6 (10) 2 (2) <u>1 (1)</u>	2 (6)
	17898-1600	CE	10 (20) 2 (3)	3 (4) 1 (1)	2 (5)	4 (6) 1 (1) <u>1 (2)</u>	2 (5)
	17899-715	SP	2 (2)	3 (7) 1 (1)	2 (5)	7 (21) 2 (3) <u>2 (8)</u>	0 (0)

Table 3 Continued

Bands shared among taxa	ISSR locus*	Marker*	CE 16 (46)†	CL 5 (13)†	XP 2 (6)†	SP 9 (31) †	GR 7 (26) †
	17899–1234	SPEC	2 (4) 1 (3)	3 (5)	1 (2)	5 (12) 2 (3)	2 (4)
	17899–1315	CE	3 (6) 1 (2)	1 (2)	0 (0)	1 (1)	1 (1)
	17901–885	SPEC	1 (1)	4 (7)	1 (1)	4 (11) <u>2 (5)</u>	6 (9)
	17901–1475	CE	10 (16) 3 (6)	1 (2)	1 (1)	3 (4) 1 (1) <u>1 (1)</u>	1 (3)
	17901–2400	SP	1 (1) 1 (1)	3 (3) 1 (1)	1 (1)	2 (4)	4 (7)
	17902–1220	CE	6 (10) 2 (2)	0 (0)	2 (2)	1 (1)	3 (5)
	17902–2200	SPEC	2 (2) 2 (2)	3 (7) 1 (1)	1 (2)	9 (24) 3 (5) <u>2 (9)</u>	7 (20)
	AW3–640	CE	12 (28) 1 (4)	5 (9) 1 (1)	2 (6)	4 (4) 1 (1) <u>1 (1)</u>	1 (1)
	AW3–1575	CE	8 (20) 3 (6)	1 (1)	1 (1)	1 (1)	1 (2)
	AW3–1800	SPEC	1 (1)	4 (5)	0 (0)	6 (7) 2 (2) <u>1 (2)</u>	2 (2)
	AW3–2000	CE	16 (43) 3 (12)	1 (2)	1 (3)	0 (0)	1 (1)
	M1–880	SPEC	1 (1)	5 (12) 1 (1)	1 (1)	8 (24) 3 (4) <u>2 (8)</u>	7 (22)
	M1–1060	CE	6 (9) 2 (5)	2 (2)	0 (0)	0 (0)	1 (1)
	M1–1132	CE	7 (7) 1 (1)	1 (2)	0 (0)	2 (2) 1 (1)	1 (2)
	M1–1220	SPEC	1 (1)	1 (3)	0 (0)	2 (2) 1 (1) <u>1 (1)</u>	7 (20)
	M1–1600	CE	14 (26) 3 (6)	1 (1)	0 (0)	2 (2) <u>1 (1)</u>	4 (5)
	M1–1750	CE	10 (17) 2 (4)	1 (2)	0 (0)	2 (3) <u>1 (1)</u>	3 (6)
	M1–2460	CE	10 (19) 3 (7)	2 (5)	0 (0)	2 (4) <u>1 (1)</u>	1 (1)
	M1–2900	CE	6 (9) 1 (1)	2 (3)	1 (2)	2 (2) 1 (1)	1 (1)
	M1–3400	CE	6 (11)	2 (2) 2 (5)	1 (3)	4 (5) 2 (2)	1 (1)

*Loci and taxa in bold upper-case letters denote Category I marker bands where the band is present in at least 25% of all populations and individuals of taxon indicated; taxa in lower-case letters denote Category II bands present in at least 25% of populations indicated, but in less than 25% of all individuals; taxa in upper-case letters (not bold) denote Category III bands where bands are present in more populations and/or individuals of that taxon compared to others in the hybrid complex.

†Number of populations (number of individuals); boldface shows distribution of markers exclusively shared by pairs of taxa or pairs with *P. X parishii* included. Italics indicate distribution of ISSR bands where populations of *P. centranthifolius* are sympatric with either *P. spectabilis* or *P. clevelandii* (refer to Table 1). Numbers underlined indicate number of populations (number of individuals) of *P. spectabilis* ssp. *subviscosus*, which has the chloroplast genome of *P. grinnellii* (Wolfe *et al.* 1995).

‡Introgressive bands shared inferred from distribution of bands in sympatric populations or where bands also occur in the F_1 hybrid between *P. centranthifolius* and *P. spectabilis*.

can be attributed to introgression (Tables 3, 5). This same pattern holds for total bands (Table 4).

One other clear pattern is apparent from the distribution of marker bands among taxa. The proportion of marker bands from the hummingbird-pollinated *P. centranthifolius* in populations of the insect-pollinated species of section *Spectabiles* is nearly twice that of marker alleles from species of section *Spectabiles* into populations of *P. centranthifolius* (Table 5). The distribution of markers among the insect-pollinated species is also asymmetrical with a higher proportion of *P. spectabilis* markers appearing in populations of *P. clevelandii* and *P. grinnellii* than vice versa. There is also a higher similarity between *P. centranthifolius* or *P. spectabilis* with either *P. X parishii* or *P. clevelandii* (Table 5), and between *P. X parishii* and *P. clevelandii* than there is between the two purported parental taxa *P. centranthifolius* and *P. spectabilis*. One anomalous result is the occurrence of *P. grinnellii* markers in *P. clevelandii* (0.400). This result is from a single ISSR band (AW3-933) appearing in two individuals from two populations of *P. clevelandii* (Table 3) and may represent convergence or retention of an ancestral polymorphism because

the distributions of *P. clevelandii* and *P. grinnellii* are largely allopatric.

Private bands (not shared among species, but may have appeared in the F1 hybrid *P. X parishii*) were distributed as follows: *P. centranthifolius* = 37 (plus five additional shared only with *P. X parishii*); *P. clevelandii* = 9 (plus one additional band shared only with *P. X parishii*); *P. X parishii* = 2; *P. spectabilis* = 12 (plus three additional shared only with *P. X parishii*); *P. grinnellii* = 16; *P. incertus* = 1; *P. palmeri* = 13. Private bands are taxon specific, but these were not included in calculations performed in assessing potential introgression.

The UPGMA tree from the distance matrix was able to resolve all DNA accessions into species groups with the exception of one accession each from *P. spectabilis* and *P. grinnellii* (Fig. 2). Where multiple DNA accessions were available, ISSR data were also able to resolve seven populations of *P. centranthifolius*, both populations of *P. X parishii*, one population each of *P. spectabilis*, *P. incertus*, and *P. palmeri*, and three population groups of *P. grinnellii* based on geographical proximity. In addition, northern and southern lineages of *P. centranthifolius* were resolved

Table 4 Distribution of total (marker and nonmarker) ISSR bands from *Penstemon centranthifolius* shared among populations of *P. clevelandii* and *P. spectabilis*.

<i>P. centranthifolius</i> ISSR band type*	Total no. of CE ISSR bands	Total no. of CE Bands in SP	Proportion not associated with CE/SP sympatry†	Total no. of CE Bands in CL	Proportion not associated with CE/CL sympatry‡
CE1	131	86	22%	66	70%
CE2	53	25	8%	25	84%
Total	184	111	19%	91	74%

*CE1 = ISSR bands present in populations of *P. centranthifolius* where some populations are sympatric with *P. spectabilis*; CE2 = ISSR bands present in populations of *P. centranthifolius* where no populations are sympatric with *P. spectabilis*. †Proportion of bands not associated with sympatry represents proportion CE1 or CE2 bands found in nonsympatric populations of either *P. spectabilis* or *P. clevelandii*. Abbreviations are as shown in Table 1.

	CE†	CL†	XP	SP†	GR
CE	–	0.370‡ (0.219)* 0.310 (0.196)	0.477 (0.280)	0.288 (0.125) 0.142 (0.063)	0.232 (0.101)
CL	0.125 (0.087)	–	0.500 (0.250)	0.222 (0.129)	0.000 (0.000)
XP	0.000 (0.000)	0.000 (0.000)	–	0.000 (0.000)	0.000 (0.000)
SP	0.188 (0.098) 0.156 (0.076)	0.500 (0.423)	0.667 (0.389)	–	0.214 (0.077)
GR	0.125 (0.075)	0.400 (0.154)	0.000 (0.000)	0.155 (0.077)	–

Table 5 Proportion of Category I ISSR marker bands (refer to Table 3) distributed among taxa.

Proportion = number of bands observed / maximum possible number of bands.

† Where two sets of numbers appear in table, the top set is for all populations and the lower set is the proportion distributed among populations where sympatry between *Penstemon centranthifolius* and either *P. clevelandii* or *P. spectabilis* is absent.

‡ Proportion for †populations * (individuals).

and concurred with the same grouping observed with cpDNA restriction site data (Fig. 2; Wolfe & Elisens 1995).

One population of *P. X parishii* clustered with *P. centranthifolius*, whereas the other population clustered with a single DNA accession of *P. clevelandii* in a cluster containing *P. spectabilis* accessions (Fig. 2). All DNA accessions of *P. clevelandii* are clustered within the larger *P. spectabilis* group. Accessions of *P. palmeri* form a cluster that is outside the hybrid complex, but *P. incertus* clusters with several populations of *P. grinnellii*.

The UPGMA trees based on distance (Fig. 2) and average similarity values between groups (Fig. 3) show topologies that are more highly resolved than phylogenetic reconstructions based on rDNA and cpDNA restriction-site mapping (Wolfe & Elisens 1994, 1995), and greater resolution of species groups than UPGMA trees based on allozyme identity coefficients (Wolfe & Elisens 1993). Sister relationships are inferred between *P. grinnellii*/*P.*

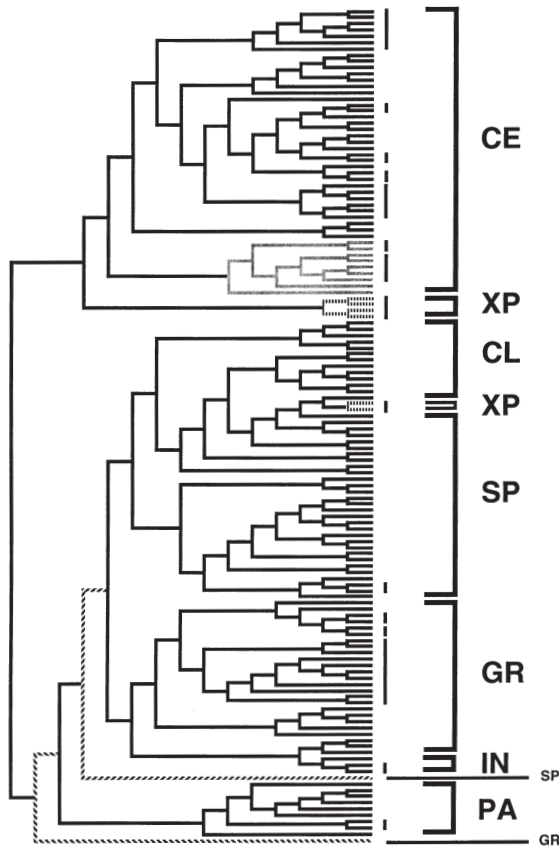


Fig. 2 UPGMA tree generated from distance matrix. Populations of all species are resolved into discrete clusters except for two accessions as illustrated. The light grey cluster represents the northern populations of *Penstemon centranthifolius*, which have the CE1 and CE2 chloroplast haplotypes (Table 1). Clusters depicted by hashmarks represent the two populations of *P. X parishii* included in the study.

UPGMA OF AVERAGE SIMILARITY BETWEEN GROUPS

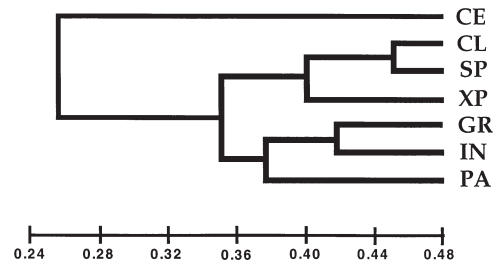


Fig. 3 UPGMA tree generated from average similarity values of taxon groups.

incertus and *P. spectabilis*/*P. clevelandii*. *Penstemon centranthifolius* is more distantly related to the insect-pollinated species in agreement with all previous analyses (Wolfe & Elisens 1993, 1994, 1995). In addition, *P. X parishii* clusters with *P. spectabilis* and *P. clevelandii* in the average similarity tree (Fig. 3), but shows affinities with *P. clevelandii* and *P. centranthifolius* in the distance tree (Fig. 2).

Discussion

In contrast to previous molecular studies of the hybrid complex (Wolfe & Elisens 1993, 1994, 1995), we were able to differentiate all species and all DNA accessions using ISSR markers (Fig. 2). *Penstemon centranthifolius* was the most clearly demarcated species, in agreement with previous studies, but each of the insect-pollinated species of section *Spectabiles* was also identified with species-specific ISSR bands (Table 3) and populations of each species cluster as a discrete unit (Fig. 2). This fine resolution enabled us to address the hypotheses of diploid hybrid speciation (Straw 1955b, 1956a,b) as well as the questions of whether gene flow occurs among the species of section *Spectabiles*.

Hybrid speciation

The criteria needed to support a hypothesis of diploid hybrid speciation include an additive profile of parental nuclear genetic markers and the organelle genome of at least one of the parental taxa (Wolfe & Elisens 1995). These criteria differ slightly for ancient vs. recent hybrid species (e.g. population level patterns, and the accumulation of novel markers in both the nuclear and organellar genomes) and depend entirely on being able to differentiate the parental taxa from one another (Wolfe & Elisens 1995).

The classic example of diploid hybrid speciation resulting from ethological isolating factors involved the forma-

tion of *P. spectabilis* (Straw 1955b, 1956a,b). Several authors used this scenario to illustrate rapid speciation (Baker 1963; Stebbins 1971; Grant 1981). Straw's hypothesis was based on morphological evidence, field observations of pollinator behaviour, and the results from artificial crossing studies involving *P. centranthifolius* and *P. grinnellii*. The morphological evidence was compelling in that artificial hybrids between the parental taxa strongly resembled the morphological appearance of *P. spectabilis*. The observation that floral isolating mechanisms were extremely strong was also good support for the hybrid speciation hypothesis. For example, carpenter bees are unable to enter the corolla of *P. centranthifolius* and *P. spectabilis*, hummingbirds are apparently inefficient pollinators of *P. spectabilis* and *P. grinnellii* due to the positioning of the stamens and stigma, and wasps are observed only in *P. spectabilis* flowers. Only two historical collections are known of the natural F₁ hybrid between *P. centranthifolius* and *P. grinnellii*, *P. X dubius*. However, the rarity of this taxon does not necessarily mean that it was not more abundant in the past when speciation leading to *P. spectabilis* occurred. Introgression of genes from *P. centranthifolius* into *P. grinnellii* has been observed with allozyme and rDNA restriction-site data in addition to documentation of chloroplast capture events involving these species (Wolfe & Elisens 1993, 1994, 1995); therefore it is obvious that the opportunity for hybrid speciation has historically been available.

Allozyme and rDNA restriction-site patterns revealed no additive pattern of markers from the purported parental species, *P. centranthifolius* and *P. grinnellii* in the hypothesized hybrid (Wolfe & Elisens 1993, 1994). In the current study, we had many additional markers for both parental species and there were also no additive patterns observed here (Tables 3, 5). In addition, *P. spectabilis* had a well-differentiated chloroplast haplotype, with the exception of one subspecies that has apparently undergone a chloroplast capture event in the past and is more closely related to the *P. grinnellii* cpDNA haplotype than to its sister subspecies (e.g. the SP1 haplotype has only two restriction-site differences compared to the GR haplotype: Table 1; Wolfe & Elisens 1995). We conclude therefore that *P. spectabilis* is not a diploid hybrid species.

The other hypothesized diploid hybrid species in Straw's (1955b, 1956a,b) hybrid complex is *P. clevelandii*. This hypothesis was based on the strong morphological resemblance of *P. clevelandii* to the natural hybrid between *P. centranthifolius* and *P. spectabilis*, *P. X parishii*. *P. clevelandii* and *P. X parishii* share numerous floral and leaf characteristics (e.g. magenta corollas of intermediate shape and size compared to the parental taxa; Hall 1902; Keck 1937; Straw 1955b). *P. centranthifolius* and *P. spectabilis* occur in sympatry throughout the range of *P. spectabilis* and *P. X parishii* is found in most populations where

sympatry occurs. The evidence for historical and current introgression of genes from *P. centranthifolius* into populations of *P. spectabilis* and vice versa is also compelling (Tables 3, 4, 5; Wolfe & Elisens 1993, 1994, 1995).

There is strong support for the diploid hybrid origin of *P. clevelandii* in that this species has an additive profile of ISSR markers from both its purported parental species (*P. centranthifolius* and *P. spectabilis*; Tables 3, 5) and has a higher genetic similarity to *P. X parishii* than the latter has to the same parental taxa (Wolfe *et al.* 1998). *P. clevelandii* also shares a larger proportion of ISSR marker bands with *P. X parishii* than *P. X parishii* does with *P. centranthifolius* and has the highest proportion of marker bands shared between *P. centranthifolius* and *P. spectabilis* than any other pair of taxa surveyed except for the known hybrid, *P. X parishii*. (Tables 3, 5; Wolfe *et al.* 1998).

P. clevelandii has consistently appeared as a sister species to *P. spectabilis* in phylogenetic reconstructions based on molecular data (Wolfe & Elisens 1994, 1995). The same pattern holds with ISSR data (Figs 2, 3). In addition, *P. X parishii* accessions cluster with either *P. centranthifolius* or *P. clevelandii* in the UPGMA analysis (Fig. 2). *P. clevelandii* has many unique allozyme alleles and private or marker ISSR bands as well as distinct cpDNA haplotypes (Wolfe & Elisens 1993, 1995). One alternative hypothesis that should be considered is whether hybridization occurs between *P. clevelandii* and *P. spectabilis* or between *P. clevelandii* and *P. X parishii*. Three ISSR band loci show a potential for hybridization involving these taxa (CL: 901–2200; CL AW3–1230; and cl 814–1699; Table 3). At 901–2200 and AW3–1230, either *P. centranthifolius* or *P. spectabilis* also have bands for these alleles. In the case of 814–1699, the band is exclusively shared between *P. clevelandii* and *P. X parishii*. Eighteen marker bands from *P. centranthifolius* occur in *P. X parishii* as well as *P. clevelandii* and four marker bands from *P. spectabilis* occur in both. Given the pattern of these latter markers, it is clear that gene flow is occurring between these taxa and it is most likely that the ISSR bands shared between *P. clevelandii* and *P. X parishii* represent historical or current hybridization between *P. centranthifolius* and *P. spectabilis*. The current results taken together with previous molecular studies support a hypothesis that *P. clevelandii* is a diploid hybrid species of ancient origin.

Penstemon *X parishii*

The F₁ hybrid between *P. centranthifolius* and *P. spectabilis* had a primarily additive pattern of ISSR bands for both parental species (Table 3). In addition, *P. X parishii* had two private alleles and bands shared exclusively with *P. clevelandii* and *P. grinnellii*. Most molecular data sets reveal completely additive patterns of hybrid taxa with no unique markers. Our results are consistent with the

hypervariable nature of ISSR bands and probably reflect genetic recombination artefacts.

Patterns of introgression

Sympatric distributions account for most of the gene flow observed between *P. centranthifolius* and *P. spectabilis* (Tables 3, 4, 5). As noted above, natural hybrids are observed in these populations. However, there is a lack of hybrid swarm formation and apparent later-generation backcrosses (Straw 1955a; A. D. Wolfe, personal observation). Introgression of genes from *P. centranthifolius* into populations of *P. grinnellii* also occurs outside the range of their distributional overlap (Wolfe & Elisens 1993, 1994, 1995). The pattern of introgression was asymmetrical with more alleles from the hummingbird-pollinated *P. centranthifolius* appearing in all insect-pollinated species of section *Spectabiles* surveyed. Wolfe & Elisens (1995) proposed that pollen-mediated gene flow via hummingbirds explained the patterns observed.

With the more-sensitive assay offered using ISSR markers, we were able to address the pollen-mediated gene flow hypothesis and to also examine whether evidence for hybridization among the insect-pollinated species exists. Each of the insect-pollinated species in the hybrid complex had ISSR marker bands (Table 3). We also designated bands that were markers for species of section *Spectabiles* as a whole compared to *P. centranthifolius*. The distribution of ISSR markers from *P. centranthifolius* into each of the insect-pollinated species considered individually and as a whole, and vice versa, strongly support Wolfe & Elisens's (1995) hypothesis of pollen-mediated gene flow. For example, 13 out of 47 marker bands specific to species of section *Spectabiles* (considered from individual species or from markers designated as SPEC) are found in populations of *P. centranthifolius*, whereas 45 out of 47 marker bands specific to *P. centranthifolius* are found in species of section *Spectabiles* distributed in one, two or three of the species (16, 15, and 14, respectively; Table 3). The proportion of markers bands is also weighted toward distribution of bands from *P. centranthifolius* into section *Spectabiles* rather than the converse distribution (Table 5).

With regard to the question of gene flow between species in section *Spectabiles*, the pattern that emerges from our study is that more ISSR markers from *P. spectabilis* occur in populations of *P. clevelandii*, *P. X parishii*, and *P. grinnellii* than the converse arrangement (Table 5). In addition, there is one ISSR marker from *P. grinnellii* exclusively shared with *P. clevelandii*, but no markers from *P. clevelandii* apparent in populations of *P. grinnellii* (Table 3). Because there is very little distributional overlap between the latter two species, it is most likely that the shared band results from retention of an ancestral polymorphism rather than hybridization. In

contrast, the range overlap of *P. spectabilis* with *P. clevelandii* is extensive throughout their distributions, and there is considerable overlap between *P. spectabilis* and *P. grinnellii* in the transverse mountain ranges near Los Angeles.

It is possible that much of the ISSR marker distribution among species of section *Spectabiles* results from retention of ancestral polymorphisms. However, the possibility also exists for gene flow given the natural history of the group. For example, although wasp pollination and visitation are restricted to *P. spectabilis*, hummingbirds visit all species in the hybrid complex. The pattern of pollen-mediated gene flow discussed previously (Wolfe & Elisens 1995) matches hummingbird migrational patterns throughout southern California (Grant & Grant 1968), which are largely along an elevational gradient as the flowering season progresses. The elevational ranges of the species of section *Spectabiles* are 185–1400 m, 500–1800 m, and 800–2900 m for *P. spectabilis*, *P. clevelandii*, and *P. grinnellii*, respectively. The elevational range of *P. centranthifolius* is 10–2000 m. Given the geographical and elevational distribution ranges of all species in the complex, it seems likely that hummingbirds are important vectors of gene exchange among these species of *Penstemon*.

Straw (1956a) hypothesized that floral isolating mechanisms were strong among these species because of the adaptations to different pollinators. It is also evident that floral isolating mechanisms are sufficiently strong to maintain species identity even with the limited amounts of gene flow observed here and in previous studies. For example, no hybrid swarms have been observed among any pairs of taxa where sympatry has been observed and introgression patterns observed from morphological observations are minimal. How are species boundaries maintained given the patterns of introgression observed here and in previous studies? Grant (1993) outlined three scenarios relating to floral isolation when hybridization is involved between ornithophilous and sphingophilous species: (i) if hummingbirds dominate pollination of the hybrid population, selection would be skewed toward ornithophilous characters; (ii) if hawkmoths dominate, sphingophilous characters would dominate the selection filter; and (iii) if visits from both pollinators were equivalent, selection would be for flowers with intermediate characteristics. In the *Penstemon* hybrid complex, hummingbirds are the common vector for each species and apparently cause relatively small amounts of pollen-mediated gene flow. However, the dominant and most effective pollinators for each of the species of section *Spectabiles* are the 'legitimate' insect pollinators. Floral isolating mechanisms and species identities are maintained in this hybrid complex because the selection pressure for insect pollination exceeds selection pressure for hummingbird floral syndromes in section *Spectabiles*.

Utility of ISSR bands in natural populations

Wolfe & Liston (1998) reviewed the literature on ISSR applications and found that ISSR marker studies have previously been restricted to cultivated species. The ability of ISSR markers to resolve relationships among cultivars was of prime interest in designing a test of the utility of the method for use in natural populations. Our study group was chosen specifically because three other molecular data sets were available for comparison. One possible drawback to the hypervariability of these markers was that they might prove to be unusable for studies of natural populations or for species-level studies. Our results clearly show the utility of ISSR markers for addressing questions of hybridization and diploid hybrid speciation. Furthermore, we have demonstrated the increased resolution to be gained in assessing relationships among species and for testing hypotheses of hybridization by using ISSR markers compared to other molecular methods. The ability to fingerprint individuals in a population via a method that is technically feasible and cost-effective should prove extremely useful for applications ranging from population genetics to ecological and systematics studies.

Tests of the utility of the method for population-level studies have demonstrated that ISSR bands may be effective in identification of clonal vs. sexual reproduction in *Festuca idahoensis* (Robinson *et al.* 1997), identification of ramets and clusters of siblings in an endangered orchid, *Cypripedium fasciculatum* (A. Liston, personal communication), and for estimating diversity among populations of rare and endangered plants (A. D. Wolfe *et al.* unpublished). The high degree of polymorphic bands obtained by using ISSR markers may prove useful where applications of RAPD markers have been ineffective (Wolfe & Liston 1998). One feature of using these markers for examination of hybridization was the lack of resolution of all populations into discrete clusters in the UPGMA tree (Fig. 2). It is probable that the lack of population resolution in our study results from introgression of 'foreign' ISSR markers into populations. It remains to be seen whether ISSR bands will be effective in examining biogeographic patterns among populations of a single species that is not subject to hybridization.

Although ISSR markers have great potential use in population- and species-level studies, it is not yet clear as to whether ISSR bands represent nuclear markers or a combination of nuclear and organellar markers. We can probably eliminate the plastid genome as a source of ISSR bands because only mononucleotide SSRs have been found in vascular plant plastid DNA sequences, with the exception of a single (AT)₁₀ repeat found in the genome of the holoparasite, *Epifagus virginiana* (Powell *et al.* 1995a, b). No information is available as to whether di- or trinucleotide SSRs are found in mitochondrial DNA.

However, in one study where inheritance patterns of ISSR markers have been examined, three ISSR bands were found to behave in a non-Mendelian fashion (e.g. *Pseudotsuga menziesii*; Tsumura *et al.* 1996).

Recommendations for use of ISSR markers

ISSR markers offer many improvements over other available techniques including: (i) small amounts of DNA may be used; (ii) small reaction volumes and amounts of enzyme are needed for PCR; (iii) the hypervariability of banding patterns; (iv) fresh or large quantities of material for DNA extraction are not required; (v) no specialized apparatus (e.g. automated sequencer, autorad development, etc.) or kits required other than those needed for standard PCR techniques; and (vi) banding patterns are easily scorable. Primers should be selected that will not yield overlapping results. For example, the primers (CA)₈-RY and (CA)₈-AT will have some bands at the same loci and scoring these bands separately will weight identical data points. In addition, the higher annealing temperatures used for ISSR reactions may reduce the amount of template-primer mismatch artefacts than may be encountered with RAPD markers, which generally rely on lower annealing temperatures. Many researchers who have compared RAPD and ISSR methods have found that ISSR markers exhibit higher levels of polymorphism and/or reproducibility compared to RAPD markers (Yang *et al.* 1996; Nagaoka & Ogihara 1997; Parsons *et al.* 1997). In addition, where a direct cost comparison was made among RFLP (restriction fragment length polymorphism), RAPD, and ISSR techniques, the latter was found to be the most economical per polymorphism observed (\$29, \$57, and \$10, respectively; Yang *et al.* 1996).

A consistent protocol for scoring bands should be implemented at the beginning of data analysis and maintained throughout the study. We found digital imaging and band scoring far superior to our attempts to score bands manually, and digital techniques eliminated a source of potential bias during data analysis. In the absence of digital technology, we recommend the elimination of questionable bands from the analysis. With the hypervariability of ISSR markers, it is unlikely that the elimination of a subset of the data will have a major impact on the results.

It is probable that switching from agarose to a polyacrylamide gel matrix will improve resolution of banding patterns and fragment sizing. Several of the bright bands scored in our analysis may represent more than one locus and could be of potential use in resolution of population patterns. In addition, switching from EtBr staining to radioactive labelling of the amplicons may also increase resolution in that more scorable bands may be produced

compared to analysis of EtBr-stained agarose gels (Godwin *et al.* 1997).

Limitations of the technique are similar to those encountered in the use of RAPD markers: (i) clean DNA template and similar concentrations among accessions are required for standardization of reactions; (ii) optimization of initial reactions is needed; (iii) bands are scored as dominant markers; and (iv) genetic diversity estimates are based on diallelic characters. Even with these few limitations, we believe that ISSR markers will provide an attractive alternative to RAPD markers and a technique that is much more easily implemented than amplified fragment length polymorphisms (AFLP; reviewed in Wolfe & Liston 1998). Our examination of hybridization and tests of hybrid speciation hypotheses have demonstrated that ISSR markers offer a much higher degree of resolution of species relationships and patterns of introgression than available with conventional molecular methods. ISSR markers clearly have great potential in studies of natural plant populations in addition to their demonstrable utility in analysis of cultivated species.

Acknowledgements

We thank A. Liston and D. Crawford for many helpful discussions about the utility of ISSR bands for studies of natural populations, and E. Esselman for consultation on data analysis. Funding for this research was obtained through set-up funds to A. D. Wolfe from The Ohio State University and NSF grants DEB-9123080 and DEB-9632675.

References

- Allan GJ, Clark C, Rieseberg LH (1997) Distribution of parental DNA markers in *Encelia virginensis* (Asteraceae: Heliantheae), a diploid species of putative hybrid origin. *Plant Systematics and Evolution*, **205**, 205–221.
- Anderson E (1948) Hybridization of the habitat. *Evolution*, **2**, 1–9.
- Anderson E (1949) *Introgressive Hybridization*. John Wiley & Sons, New York.
- Anderson E, Stebbins GL Jr (1954) Hybridization as an evolutionary stimulus. *Evolution*, **8**, 378–388.
- Arnold ML (1997) *Natural Hybridization and Evolution*. Oxford University Press, New York.
- Arnold ML, Buckner CM, Robinson JJ (1991) Pollen-mediated introgression and hybrid speciation in Louisiana irises. *Proceedings of the National Academy of Sciences USA*, **88**, 1398–1402.
- Baker HG (1963) Evolutionary mechanisms in pollination biology. *Science*, **139**, 877–883.
- Barrington DS, Haufler CH, Werth CR (1989) Hybridization, reticulation, and species concepts. *American Fern Journal*, **79**, 55–64.
- 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.
- Condit R, Hubbell SP (1991) Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. *Genome*, **34**, 66–71.
- Fang DQ, Roose ML (1997) Identification of closely related citrus cultivars with inter-simple sequence repeat markers. *Theoretical and Applied Genetics*, **95**, 408–417.
- Fang DQ, Roose ML, Krueger RR, Federici CT (1997) Fingerprinting trifoliolate orange germ plasm accessions with isozymes, RFLPs and inter-simple sequence repeat markers. *Theoretical and Applied Genetics*, **95**, 211–219.
- Felsenstein J (1995) *PHYLIP (Phylogeny Inference Package) version 3.57c*. Department of Genetics, University of Washington, Seattle, Washington.
- Gallez GP, Gottlieb LD (1982) Genetic evidence for the hybrid origin of the diploid plant *Stephanomeria diegensis*. *Evolution*, **36**, 1158–1167.
- Godwin ID, Aitken EAB, Smith LW (1997) Application of inter-simple sequence repeat (ISSR) markers to plant genetics. *Electrophoresis*, **18**, 1524–1528.
- Gottlieb LD (1972) Levels of confidence in the analysis of hybridization in plants. *Annals of the Missouri Botanical Garden*, **59**, 435–446.
- Graham A (1997) Neotropical plant dynamics during the Cenozoic – Diversification, and the ordering of evolutionary and speciation processes. *Systematic Botany*, **22**, 139–150.
- Grant V (1981) *Plant Speciation*. Columbia University Press, New York.
- Grant V (1993) Effects of hybridization and selection on floral isolation. *Proceedings of the National Academy Sciences USA*, **90**, 990–993.
- Grant KA, Grant V (1968) *Hummingbirds and their Flowers*. New York, Columbia University Press.
- Gupta M, Chyi Y-S, Romero-Severson J, Owen JL (1994) Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theoretical and Applied Genetics*, **89**, 998–1006.
- Hall HM (1902) A botanical survey of San Jacinto mountain. *University of California Publications in Botany*, **1**, 1–140.
- Hamada H, Kakunaga T (1982) Potential Z-DNA forming sequences are highly dispersed in the human genome. *Nature*, **298**, 396–398.
- Haufler CH, Windham MD, Rabe EW (1995) Reticulate evolution in the *Polypodium vulgare* complex. *Systematic Botany*, **20**, 89–109.
- Heiser CB Jr (1949a) Study in the evolution of the sunflower species *Helianthus annuus* and *H. bolanderi*. *University of California Publications in Botany*, **23**, 157–208.
- Heiser CB Jr (1949b) Natural hybridization with particular reference to introgression. *The Botanical Review*, **15**, 645–687.
- Heiser CB Jr (1973) Introgression re-examined. *The Botanical Review*, **39**, 347–366.
- Kantety RV, Zeng XP, Bennetzen JL, Zehr BE (1995) Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. *Molecular Breeding*, **1**, 365–373.
- Keck DD (1937) Studies in *Penstemon* V. The section *Peltanthera*. *American Midland Naturalist*, **18**, 790–829.
- King BL (1979) Flavonoid analysis of hybridization in *Rhododendron* sect. *Pentanthera* (Ericaceae). *Systematic Botany*, **2**, 14–27.
- Levin DA (1967) Hybridization between annual species of *Phlox*: population structure. *American Journal of Botany*, **54**, 1122–1130.

- Levy M (1976) Altered glycoflavone expression in individual autotetraploids of *Phlox drummondii*. *Biochemical Systematics and Ecology*, **4**, 249–254.
- Levy M, Levin DA (1971) The origin of novel flavonoids in *Phlox* allotetraploids. *Proceedings of the National Academy of Sciences USA*, **68**, 1627–1630.
- Mason-Gamer RJ, Holsinger KE, Jansen RK (1995) Chloroplast DNA haplotype variation within and among populations of *Coreopsis grandiflora* (Asteraceae). *Molecular Biology and Evolution*, **12**, 371–381.
- Meyerowitz EM (1997) Genetic control of cell division patterns in developing plants. *Cell*, **88**, 299–308.
- Nagaoka T, Ogihara Y (1997) Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theoretical and Applied Genetics*, **94**, 597–602.
- de Oliveira AC, Richter T, Bennetzen JL (1996) Regional and racial specificities in sorghum germplasm assessed with DNA markers. *Genome*, **39**, 579–587.
- Parsons BJ, Newbury HJ, Jackson MT, FordLloyd BV (1997) Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. *Molecular Breeding*, **3**, 115–125.
- Phipps JB, Robertson KR, Rohrer JR, Smith PG (1991) Origins and evolution of subfamily Maloideae (Rosaceae). *Systematic Botany*, **16**, 303–332.
- Powell W, Morgante M, Andre C *et al.* (1995a) Hypervariable microsatellites provide a general source of polymorphic DNA markers for the chloroplast genome. *Current Biology*, **5**, 1023–1029.
- Powell W, Morgante M, McDevitt R, Vendramin GG, Rafalski JA (1995b) Polymorphic simple sequence repeat regions in chloroplast genomes: Applications to the population genetics of pines. *Proceedings of the National Academy of Sciences USA*, **92**, 7759–7763.
- Rieseberg LH (1991) Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. *American Journal of Botany*, **78**, 1218–1237.
- Rieseberg LH, Soltis DE (1991) Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants*, **5**, 65–84.
- Rieseberg LH, Ellstrand NC (1993) What can molecular and morphological markers tell us about hybridization? *Critical Reviews in Plant Sciences*, **12**, 213–241.
- Rieseberg LH, Wendel JF (1993) Introgression and its consequences in plants. In: *Hybrid Zones and the Evolutionary Process* (ed. Harrison R), pp. 70–109. Oxford University Press, Oxford.
- Rieseberg LH, Beckstrom-Sternberg SM, Liston A, Arias DM (1991) Phylogenetic and systematic inferences from chloroplast DNA and isozyme variation in *Helianthus* sect. *Helianthus* (Asteraceae). *Systematic Botany*, **16**, 50–76.
- Robinson WA, Liston A, Doescher PS, and Svejcar T (1997) Using ISSR markers to quantify clonal vs sexual reproduction in *Festuca idahoensis* (Poaceae). *American Journal of Botany*, **84**, (supplement), **89** (Abstract).
- Salimath SS, de Oliveira AC, Godwin ID, Bennetzen JL (1995) Assessment of genome origins and genetic diversity in the genus *Eleusine* with DNA markers. *Genome*, **38**, 757–763.
- Sang T, Crawford DJ, Stuessy TF (1995) Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. *Proceedings of the National Academy of Sciences USA*, **92**, 6813–6817.
- Schilling EE, Panero JL (1996) Phylogenetic reticulation in subtribe Helianthinae. *American Journal of Botany*, **83**, 939–948.
- Sharma PC, Hüttel B, Winter P, Kahl G, Gardner RC, Weising K (1995) The potential of microsatellites for hybridization- and polymerase chain reaction-based DNA fingerprinting of chickpea. *Electrophoresis*, **16**, 1755–1761.
- Smith DM, Levin DA (1963) A chromatographic study of reticulate evolution in the Appalachian *Asplenium* complex. *American Journal of Botany*, **50**, 952–958.
- Soltis DE, Kuzoff RK (1995) Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae). *Evolution*, **49**, 727–742.
- Soltis DE, Soltis PS (1993) Molecular data and the dynamic nature of polyploidy. *Critical Reviews in Plant Science*, **12**, 243–273.
- Soltis PS, Plunkett GM, Novak SJ, Soltis DE (1995) Genetic variation in *Tragopogon* species: additional origins of the allotetraploids *T. mirus* and *T. miscellus* (Compositae). *American Journal of Botany*, **82**, 1329–1341.
- Soltis DE, Johnson LA, Looney C (1996) Discordance between ITS and chloroplast topologies in the Boykinia group (Saxifragaceae). *Systematic Botany*, **21**, 169–185.
- Stebbins GL Jr (1950) *Variation and Evolution in Plants*. Columbia University Press, New York.
- Stebbins GL Jr (1971) The role of hybridization in evolution. In: *Processes of Organic Evolution*, pp. 116–135. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
- Straw RM (1955a) *Floral ecology and evolution in Penstemon*. Graduate School, Claremont University, Claremont, CA.
- Straw RM (1955b) Hybridization, homogamy, and sympatric speciation. *Evolution*, **9**, 441–444.
- Straw RM (1956a) Floral isolation in *Penstemon*. *American Naturalist*, **90**, 47–53.
- Straw RM (1956b) Adaptive morphology of the *Penstemon* flower. *Phytomorphology*, **6**, 112–119.
- Tautz D, Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Research*, **12**, 4127–4138.
- Tsumura Y, Ohba K, Strauss SH (1996) Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). *Theoretical and Applied Genetics*, **92**, 40–45.
- Wagner WH Jr (1970) Biosystematics and evolutionary noise. *Taxon*, **19**, 146–151.
- Wolfe AD, Elisens WJ (1993) Diploid hybrid speciation in *Penstemon* (Scrophulariaceae) revisited. *American Journal of Botany*, **80**, 1082–1094.
- Wolfe AD, Elisens WJ (1994) Nuclear ribosomal DNA restriction-site variation in *Penstemon* section *Peltanthera* (Scrophulariaceae): an evaluation of diploid hybrid speciation and evidence for introgression. *American Journal of Botany*, **81**, 1627–1635.
- Wolfe AD, Elisens WJ (1995) Evidence of chloroplast capture and pollen-mediated gene flow in *Penstemon* sect. *Peltanthera* (Scrophulariaceae). *Systematic Botany*, **20**, 395–412.
- Wolfe AD, Liston A (1998) Contributions of PCR-based methods to plant systematics and evolutionary biology. In: *Plant Molecular Systematics II* (eds Soltis DE, Soltis PS, Doyle JJ), pp. 43–86. Chapman Hall, New York.
- Wolfe AD, Kephart SR, Xiang Q-Y (1998) Diploid hybrid specia-

- tion in *Penstemon* (Scrophulariaceae). *Proceedings of the National Academy of Science USA*, **95**, 5112–5115.
- Wolff K, Zietkiewicz E, Hofstra H (1995) Identification of chrysanthemum cultivars and stability of DNA fingerprint patterns. *Theoretical and Applied Genetics*, **91**, 439–447.
- Yang W, de Oliveira AC, Godwin I, Schertz K, Bennetzen JL (1996) Comparison of DNA marker technologies in characterizing plant genome diversity: variability in Chinese sorghums. *Crop Science*, **36**, 1669–1676.
- Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprint-

ing by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, **20**, 176–183.

The Wolfe laboratory is using molecular markers to study patterns of hybridization, species divergence, and the genetic diversity of rare and endangered plants. Susan Kephart was a visiting professor and Jenny (Qiu-Yun) Xiang was a postdoctoral research associate through the duration of this project.
