To the Graduate Council:

I am submitting herewith a thesis written by Lori Jean Martin entitled "Patterns of Hybridization in the *Piriqueta caroliniana* complex in Central Florida." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Botany.

Mitchell B. Cruzan, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and
Dean of the Graduate School

Patterns of Hybridization in the *Piriqueta caroliniana* complex in Central Florida

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Lori Jean Martin

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Dedication

This thesis is dedicated to my parents

Edward Royal Martin

and

Doris Newman Martin

whose continuing love and support have

made this achievement possible.

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Abstract

I have identified a broad zone of hybridization between *P. caroliniana* and *P. viridis* in Florida that extends from the vicinity of Lake Okeechobee north to that of Gainesville. I examine the distribution patterns of diagnostic morphological characters and nuclear genetic markers to assess the extent of introgression in this system. I also analyze soil characteristics to investigate whether environmental parameters influence the distribution of morphological and genetic characters in the hybrid zone. The results are consistent with hybrid zone movement in which the alleles of *P. viridis* are introgressing northward into populations of *P. caroliniana.* Populations at the southern end of the hybrid zone are morphologically intermediate to the parental taxa, but display low levels of phenotypic variability within populations. In the northern portion of the hybrid zone there are higher proportions of fixed alleles, higher levels of variation for the genotypic hybrid index, and higher levels of morphological variation within populations. In addition to these broad patterns of variation, correlations between morphological characters and soil environments within the hybrid zone suggest that environmental selection is influencing the distribution of hybrid phenotypes. The uniformity of hybrid phenotypes in older hybrid populations suggests that they may represent an intermediate stage of hybrid speciation.

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Chapter I

Introduction

Background on Hybrid Zone Theory and Models

Numerous studies suggest that hybridization in plants is frequent in extant species (reviewed in: Harrison 1990; Arnold 1992), and has been important throughout the evolutionary history of the angiosperms (Grant 1981; Whittman et al. 1991). Hybrid zones have been defined as "interactions between genetically distinct groups of individuals resulting in at least some offspring of mixed ancestry," (Harrison 1993) and referred to as "windows on evolutionary process" (Harrison 1990). While hybrid zones have been viewed as transient, resulting in either the fusion of the parental genomes or the strengthening of barriers to gene flow, historical evidence has revealed that many hybrid zones are stable over long periods of time (Stebbins 1959; Grant 1981; Arnold 1992; Harrison 1993). Thus, recent studies have been directed toward understanding the evolutionary roles significant in the maintenance of hybrid zones. This growing body of research will ultimately allow better predictions to be made regarding the evolutionary fate of hybrid zones and the role they play in the process of speciation.

A majority of studies have used clinal analysis to examine hybridization, where a cline is the frequency distribution of parental characters across the hybrid zone (Endler 1977). When parental populations divergent in allele frequencies establish contact through hybridization, alleles that are neutral or favored by selection may be readily transferred between them. Over time, the clines for these alleles will flatten in shape, reflecting their introgression from one parental population to the other via hybrid

populations. Clinal patterns for alleles that do not introgress so readily, however, will maintain an abrupt sigmoidal shape. This shape is indicative of a dispersal barrier, which may include selection acting against the introgression of alleles. Models have been developed that describe specifically how dispersal and selection may act to maintain these clinal patterns (Endler 1977; Barton and Hewitt 1985).

One model that has been proposed to explain the maintenance of hybrid zones between species assumes that clines indicate the presence of a tension zone of unfit hybrids. According to the tension zone model (Key 1968; Barton and Hewitt 1985), hybrid zones are maintained by a balance between the dispersal of parental alleles into the hybrid zone and intrinsic selection (i.e., negative epistasis between alleles from different coadapted gene complexes) against hybrid individuals. Hybrid individuals are assumed to have reduced fitness as a result of incompatibilities between the parental genomes. Extrinsic selection, or selection in response to the environment, is negligible in this model, and therefore, hybrid zones are not fixed relative to the environment. Specifically, hybrid zones may move along a population density gradient. If hybrid populations represent a population density trough however, which is likely if they are experiencing strong selection, the hybrid zone may persist without movement for long periods of time.

The development of the tension zone model was influenced by the observed concordance of clines for multiple characters present in many hybrid zone studies. Barton and Hewitt (1985) argue that it is unlikely that extrinsic selection will act identically upon many independent traits. They offer the tension zone model as an alternative explanation for these cases involving concordant clines. In a hybrid zone between the fire-bellied

toads *Bombina bombina* and *B. variegata* in southern Poland, Szymura and Barton (1986) support the tension zone model, highlighting the concordance of several morphological, allozyme, and mtDNA clines, along with evidence of increased aberrations and mortality in hybrids. The clines are indicative of a strong dispersal barrier within the hybrid zone, and based on specific clinal shape, the authors conclude that hybrid populations must have substantially reduced fitness, whereby selection is acting intrinsically against hybrids rather than favoring certain genotypes in certain environments.

Extrinsic selection is an important component of other models of hybrid zone maintenance. According to the bounded hybrid superiority model (Moore 1977), hybrids have higher fitness than either parent in transitional habitats or ecotones, and are therefore able to outcompete parental genotypes in these environments. Moore (1977) supports the bounded superiority model with references to botanical literature where systematic botanists have often recognized that plant hybrids are restricted to disturbed environments. The implication is that the hybrid phenotypes have higher fitness in these novel habitats. But as Harrison (1993) points out, the explanation for these observations may be that hybrids are only produced in circumstances where there is disturbance. Moore (1977) also cites examples of many narrow vertebrate hybrid zones that occur in ecotonal regions intermediate to the environments occupied by the parental taxa. He proposes that selection favoring hybrids in intermediate habitats is responsible for the maintenance of such zones over long periods of time. In the case of a hybrid zone that has been present historically in the United States central Great Plains between the red- and yellow-shafted flickers, Moore and Buchanan (1985) favor the bounded hybrid

superiority model over the tension zone model of hybrid zone maintenance . Although the Great Plains represents a density trough in flicker populations due to its paucity of arboreal vegetation, this does not explain the variance in position and width of the hybrid zone, which occurs narrowly across most of the United States and Canada. Several indirect lines of evidence, including this region's climate and its association with other ecological transition zones and avian hybrid zones, support this being a hybrid zone that is maintained by selection in response to the environment.

Extrinsic selection is an important factor in the mosaic hybrid zone model as well (Harrison 1986; Howard 1986; Rand and Harrison 1989). According to this model, hybrid zones may exhibit patchy distributions of genotypes, which reflect the patchy nature of the environment. Rand and Harrison (1989) support this model in a study of two species of field crickets in Connecticut, *Gryllus pennsylvanicus* and *G. firmus*. The former species occurs inland and prefers loamy soils, while the latter is distributed along the Atlantic coast and prefers sandy soils. The authors postulate that if gene flow can counteract local adaptation in the zone of overlap, paired populations occupying different soil types within the hybrid zone should be more similar than those which are geographically separated. They found, however, that populations from the two soil types within the hybrid zone maintain distinct morphological characters and allele frequency differences. This provides strong evidence that extrinsic selection is shaping and maintaining this hybrid zone.

Some recent studies in which the above models have been examined have concluded that hybrid zones may exhibit characteristics of more than one model

simultaneously. Bert and Arnold (1995) found evidence of both intrinsic and extrinsic selection acting within a hybrid zone of two hard-clam species, *Mercenaria mercenaria* and *M. campechiensis*. In a study of a hybrid zone between the fire ants *Solenopsis invicta* and *S. richteri*, Shoemaker et al. (1996) found clinal patterns showing allele frequency reversals indicative of a mosaic hybrid zone, as well as gradual clinal transitions and the concordance of several clines, both of which resemble patterns predicted by the tension zone model. In the present study, I also find patterns consistent with different aspects of different models, including associations between morphological characters and the environment as well as hybrid zone movement. It appears that this and other studies are revealing that hybrid zones are areas often maintained by several interacting evolutionary forces, and they may require components from more than one of the existing models of hybrid zone maintenance to be explained sufficiently.

***Piriqueta*: A Unique Look at Hybridization**

With the concentration on hybrid zone stability in recent studies, there have been few studies that examine systems in which there is weak reproductive isolation and weak selection resulting in extensive introgression of the parental genomes (but see Shoemaker et al. 1996). Presumably cycles of partial divergence and introgression among populations within species have been common throughout evolutionary history, and may represent a potent force that has shaped extant taxa. However, the transient nature of fusion events between divergent populations makes them relatively less common than stable hybrid zones and intrinsically more difficult to study. The *Piriqueta* hybrid zone in central Florida may represent a unique opportunity to examine the fusion between

weakly-isolated divergent forms. Information an the patterns and consequences of fusion between previously-isolated populations may provide a better understanding of the maintenance of hybrid zones and the factors responsible for the delineation of species.

One type of evidence for weak reproductive isolation and selection would be movement of a hybrid zone, which is not often observed (Shaw et al. 1985; Shoemaker et al. 1996). In a moving hybrid zone, one would expect differences in levels of variation in different portions of the hybrid zone that correspond to hybrid population age. When two taxa first come into contact, early hybrid generations (i.e., F_2, F_3, B_2, B_3, etc.) will display high levels of variability as a result of segregation and recombination between the two parental genomes. In older hybrid populations, however, much of this variability may be lost as a result of selection and drift. Hence, in moving hybrid zones, there should be a region of higher variability corresponding to the advancing front of introgressing alleles. In addition to differences in levels of variation among hybrid populations, moving hybrid zones may display correlations among diagnostic neutral markers that result from the simultaneous dispersal of multiple alleles from the introgressing taxon (Shoemaker et al. 1996)

In this study I examine patterns of introgression and hybridization between two taxa in the *Piriqueta caroliniana* complex in central Florida, *P. caroliniana* Urban and *P. viridis* Small. Previous studies suggest that these taxa hybridize naturally and that reproductive isolation barriers between these taxa are relatively weak (Ornduff 1970; Wang and Cruzan 1997). However, each taxon is unique with respect to its morphology and ecological affinities. Moreover, these differences are maintained under greenhouse

conditions and are consistent across allopatric populations that span broad geographic areas (pers. obs.). I examine the distribution of morphological characters and nuclear genetic markers to assess patterns and the extent of introgression between these taxa. In particular, I find patterns of variation in diagnostic morphological and genetic markers that are consistent with the hypothesis that *P. viridis* alleles are introgressing north into populations of *P. caroliniana.*

Chapter II

Materials and Methods

Study System

Piriqueta (Turneraceae) is widely distributed throughout Central and South America, the West Indies, and the southeastern United States. Based on vegetative morphology, Small (1933) recognized four species of *Piriqueta*, which have distinct habitat associations in the United States. *P. caroliniana* has both hirsute and stellate hairs and grows in deep, sandy soils of pinelands from North Florida to south Georgia, *P. tomentosa* has only stellate hairs and grows in dry pinelands on hardrock limestone soils in south Florida and the Keys, *P. viridis* has glabrous foliage and grows in the poorly-drained soils of southern pine flatwoods in south Florida, and *P. glabrescens* has glabrous foliage and grows in short-hydroperiod marshes of the everglades. Long and Lakela (1971) only recognized two varieties of *P. caroliniana* occurring in the United States. Their classification is based on broader environmental associations and more general vegetative morphology than Small's classification and groups together the species he described. They describe *P. caroliniana* var. *tomentosa* as having tomentose to hirsute foliage and occurring in drier sites with sandy soil from Florida to North Carolina and *P. caroliniana* var. *glabra* as having glabrous stems and leaves and occurring in low ground in south Florida and the Keys. In the most recent taxonomic treatment of this genus, Arbo (1995) noted a lack of reproductive characters separating these taxa and grouped all *Piriqueta* occurring in the United States into *P. cistoides* spp. *caroliniana*. In my field observations I have been able to recognize the four distinct morphological types described

by Small (1933). While these forms may dot represent distinct species, for convenience I will be using Small's (1933) system of classification to describe a hybrid zone occurring between *P. caroliniana* and *P. viridis* in central Florida.

Both *P. caroliniana* and *P. viridis* are herbaceous perennials found primarily in open areas exposed to sunlight throughout most of the day. Both have orange-yellow, insect-pollinated flowers that are 2 to 4 cm. in diameter with five petals and stamens and three separate styles that join at top of the ovary. Ovaries contain twenty to forty ovules, and seed set ranges from five to more than thirty seeds. Plants in Florida flower from early spring through late fall with each flower lasting a single day. Fruits are dry capsules with three sutures that open in approximately twenty days. Seeds are thought to be primarily gravity dispersed, but the presence of an eliasome is indicative of ant dispersal. Seeds germinate readily in the greenhouse and appear to lack dormancy.

Individuals of both taxa may reproduce both sexually and vegetatively. Their sexual mating system is distylous with individuals bearing flowers of one of two morphs, short or long-styled, and like morphs are incompatible (Ornduff and Perry 1964). Interspecific greenhouse crosses indicate that the two taxa are intercompatible, and interspecific matings produce fertile F_1 progeny (Ornduff 1970; Wang and Cruzan 1997). Both species are diploid with $n = 7$ (Lewis et al. 1962; Ornduff 1970).

P. caroliniana occurs primarily in north Florida and south Georgia, but has been reported as far north as North Carolina (Long and Lakela 1971). It can be found in northern pine flatwoods but grows primarily in the excessively drained sandhill communities of Florida's high pine, which is essentially a high elevation extension of the

flatwoods distributed along the panhandle and south through the peninsula to Highlands County. Associated vegetation includes longleaf pine (*Pines palustris*), typical slash pine (Pinus elliottii var. elliottii), turkey oak (*Quercus laevis*), bluejack oak (*Quercus marilandica*), and wiregrass (*Aristida stricta*). In the southern end of the Lake Wales ridge in Highlands County, longleaf pine is replaced by south Florida slash pine (*Pinus elliottii* var. *densa*: Myers 1990). *P. viridis* occurs in the lower elevations of pine flatwoods in south Florida. The soils are light-textured, fine sands imperfectly to poorly-drained. These regions may be inundated with water for extended periods of time during the wet season. The overstory consists primarily of south Florida slash pine, while the understory varies widely but often includes wiregrass and shrubs such as saw palmetto (*Serenoa repens*) and gallberry (*Ilex glabra*) (Abrahamson and Hartnett 1990).

P. caroliniana has elliptic leaves that are covered with both stellate and hirsute hairs while *P. viridis* has linear to lanceolate leaves and is completely glabrous. Progeny of allopatric populations maintain these morphological character differences when grown in a common greenhouse environment. Putative hybrid populations in central Florida consist of individuals that vary in leaf shape and pubescence characters and appear intermediate between plants in allopatric populations.

Sampling Techniques

Nine populations of *P. caroliniana* have been identified in northern Florida and southern Georgia, four populations of *P. viridis* in south Florida, and twenty-seven putative hybrid populations in central Florida (Table 1, Fig. 1). During the summers of 1995 and 1996, I collected leaves in the field from one allopatric *P. caroliniana*

Table 1. Locations of *Piriqueta* populations.

Population	Type	County	Location
GA1	C	Effingham Co., GA	Junction of RT 119 and RT 119C
GA2	C	Long Co., GA	RT 57, 3.2 km SE of Ludowici
GA3	C	Wayne Co., GA	RT 84, 1.8 km N of RT 203
GA4	C	Wayne Co., GA	RT 84, 4.8 km S of RT 203
GA5	C	Lanier Co., GA	RT 129, 3.7 km N of RT 84
GA7	C	Decatur Co., GA	RT 84, 10.4 km E of Bainbridge City Limit
P2	H	Pasco Co., FL	RT 471, 7.4 km N of US 98
P4*	H	Lake Co., FL	RT 44, 13.6 km W of RT 42
P5*	H	Polk Co., FL	RT 54, 3.4 km E of US 27
P6	H	Polk Co., FL	US 27A, 5.4 km N of Waverly Rd.
P7	H	Polk Co., FL	US 27A, 4.5 km S of RT 640
P8	H	Highlands Co., FL	RT 17 just N of Sebring at Lake Letta Dr.
P9*	H	Highlands Co., FL	RT 17, 2.2 km N of US 98
P10*	V	Collier Co., FL	US 41, 5.6 km SE of RT 951
P14	H	Highlands Co., FL	Junction of US 27 and RT 66
P15	H	Highlands Co., FL	1 km W of junction of US 27 and RT 66
P16	H	Polk Co., FL	US 27, 0.5 km S of US 27A
P17	H	Polk Co., FL	US 27A, 5 km S of RT 640, along railroad track
P18*	H	Highlands Co., FL	RT C-17, 7 km N of US 27
P19	H	Highlands Co., FL	RT C-17, 1.6 km N of US 98
P20*	H	Highlands Co., FL	US 27A at Babson Park, corner of 5th Ave.
P21*	H	Polk Co., FL	Junction of US 27 and US 192
P22*	H	Lake Co., FL	RT 50, 4 km W of RT 91
P23*	H	Orange Co., FL	RT 545, 16 km N of US 192
P24*	H	Alachua Co., FL	RT 24, 2.1 km W of US 301
P25*	H	Alachua Co., FL	US 301, 7.2 km S of RT 24
P26*	H	Putnam Co., FL	In Ordway Preserve - RT 26, 3.7 km E of RT 21
P27*	H	Marion Co., FL	US 301, 2.1 km S of RT 318
P28*	H	Marion Co., FL	US 441, 8.8 km N of RT 25
P29*	H	Lake Co., FL	RT 44, 7.8 km W of RT 42
P30*	H	Clay Co., FL	Junction of RT 739 and RT 209
FL3*	C	Suwanee Co., FL	E of US 129, 16.5 km S of Live Oak
FL4	V	Dade Co., FL	US 1, 1.6 km S of RT 821, vacant lot at 220 St.
FL9	C	Wakulla Co., FL	Junction of US 319 and US 98
FL11	H	Leon Co., FL	US 20, 3.4 km E of William's Landing Rd.
FL19*	V	Collier Co., FL	RT 846 just E of RT 849
FL21*	V	DeSoto Co., FL	Junction of RT 31 and RT 763
FL22*	H	Citrus Co., FL	RT 491, 13.8 km S of RT 44
FL23*	H	Levy Co., FL	US 98, 1.9 km S of RT 347

* Soil collected from the population

For type, C = *P. caroliniana*, V = *P. viridis*, and H = hybrid.

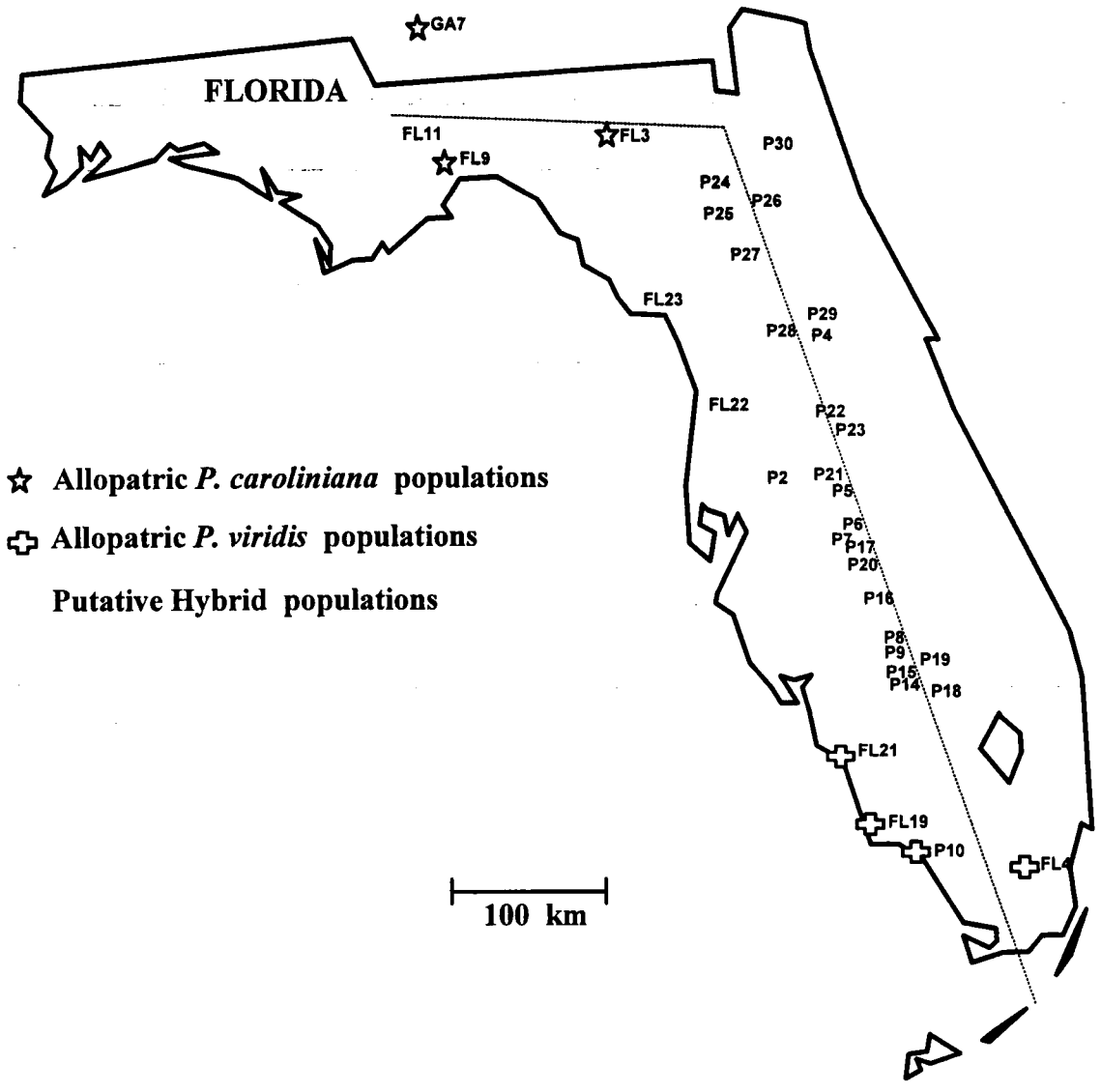

Figure 1. Map of *Piriqueta* populations sampled in this study. The transect begins in the south at Upper Matecumbe Key.

population (FL3), three allopatric *P. viridis* populations (P10, FL19, and FL21) and all twenty-seven hybrid populations. For several additional populations (GA1-GA5, FL9, FL11 and FL4), I sampled plants grown in the greenhouse from seed that had been previously collected in the field.

I sampled each population by collecting leaf material from up to forty individuals for genetic and morphological analyses. Leaf material for genetic analysis was placed in Eppendorf tubes and kept on ice until it was returned to the lab and snap frozen in liquid nitrogen. One leaf at the base of each individual was collected and dried in a plant press for morphological analysis. All sampling was done by walking along a transect through the population, and care was taken to collect from spatially-separated shoots to avoid resampling the same individual.

Morphological Marker Identification, Creation of Hybrid Index, and Morphological Measurements

A morphological hybrid index was calculated by scoring each plant sampled for a number of morphological characters. I used Optimas 5.2 image analysis program to measure leaf length, width, area, and perimeter and to calculate the width-to-length ratio. I counted the number of stellate hairs per mm^2 and number of hirsute hairs visible in the field of view of the dissecting scope (appr. 3 mm^2) on both the dorsal and ventral surfaces of all leaves. I also performed these morphological measurements on F_1 plants generated from greenhouse crosses.

The following morphological characters were used as markers because they showed clear differences between allopatric populations: 1) number of stellate hairs per

mm^2 on the dorsal leaf surface (SHD), 2) number of stellate hairs per mm^2 on the ventral leaf surface (SHV), 3) number of hirsute hairs visible in a 3 mm^2 area on the dorsal leaf surface (HHD), 4) number of hirsute hairs visible in a 3 mm^2 area on the ventral leaf surface (HHV), and 5) leaf width-to-length ratio (WLR). I created a morphological hybrid index for each marker by dividing the difference in means between the allopatric populations into six equally-spaced intervals. Six classes were used to coincide with the genetic hybrid index (see below). Like the genetic hybrid index, the morphological index extends from positive six (resembling *P. viridis*) to zero (resembling *P. caroliniana*). It then exceeds the genetic index in the negative direction to a score of negative eighteen because I had to create additional equivalently-sized intervals to account for morphological characters in hybrid populations whose means exceeded those of the allopatric *P. caroliniana* populations. Using this classification system, plants were assigned a score for each character and a mean morphological score (MSC) was calculated that included the scores from all characters. For each population, I also calculated the coefficient of variation in mean morphological scores (MCV).

Genetic Marker Identification, Creation of Hybrid Index, and Genetic Measurements

Nuclear DNA was isolated using the protocol described by Edwards et al. (1991). After precipitation with isopropanol, each sample was cleaned by binding the DNA to DEAE-cellulose as described by Maréchal-Drouard and Guillemont (1995). Samples were analyzed for RAPD markers (Williams et al. 1990) using arbitrary 10-base primers obtained from the University of British Columbia Biotechnology Laboratory.

Amplifications were carried out in 25 μL reaction mixtures, each containing 1 unit DNA polymerase (Sangon, Ltd.), 1.9 mM $MgCl_2$, 0.1 mM each of dATP, dCTP, dGTP, and dTTP (Boehringer Mannheim), 10X Sangon reaction buffer, and 50 ng of genomic DNA. I used a PTC-100 Programmable Thermal Controller set at 1 min at 92°C, 1 min at 35°C, and 2 min at 72°C for 45 cycles. Using two individuals of each species from allopatric populations (GA1-GA5, GA7, FL9, and FL3 for *P. caroliniana* and FL4, FL19, FL21, and P10 for *P. viridis*), I screened primers for diagnostic markers. Candidate markers identified in this initial screen were then scored for at least 33 individuals from allopatric populations for each species and their frequencies estimated. I identified two markers diagnostic for *P. caroliniana* (c28 and c59) and four diagnostic for *P. viridis* (v59, v61, v164, and v270 : Table 2). After determining that the genetic markers were diagnostic or nearly so, I screened all of the putative hybrid populations. The allopatric populations GA1-GA5 were only used in this study for screening marker c28.

I created a genetic hybrid index based on each individual's RAPD marker genotype (Table 3). A pure *P. caroliniana* genotype has both *P. caroliniana* markers and no *P. viridis* markers, and it received a score of zero. A pure *P. viridis* genotype has all four *P. viridis* markers and no *P. caroliniana* markers, and it received a score of six. Scores one through five are based upon relative gains and losses of markers (Table 3).

For each population, I calculated each marker's frequency, the mean genetic score (GSC) for the population, the coefficient of variation (GCV) in genetic scores, and the proportion of markers that were polymorphic in each population (P_P: Hamrick and Godt 1990). To calculate a genetic diversity index (H_{EP}), I used the formula described

Table 2. The primer sequence and frequency of occurrence (followed by the number scored) of each RAPD marker in allopatric populations. Markers designated "c" and "v" are diagnostic for *P. caroliniana* and *P. viridis*, respectively. Each marker number is the University of British Columbia primer number.

		Allopatric *Piriqueta* individuals	
Marker	Primer Sequence	*P. caroliniana*	*P. viridis*
c28	5'-CCGGCCTTAA-3'	0.98 (64)	0.04 (67)
c59	5'-TTCCGGGTGC-3'	0.97 (33)	0.18 (55)
v59	5'-TTCCGGGTGC-3'	0.00 (33)	0.98 (55)
v61	5'-TTCCCCGACC-3'	0.00 (33)	0.96 (55)
v164	5'-CCAAGATGCT-3'	0.00 (33)	0.95 (55)
v270	5'-TGCGCGCGGG-3'	0.03 (33)	0.95 (55)

Table 3. Hybrid scores based upon relative gains and losses of *P. caroliniana* and *P. viridis* RAPD markers. Marker ratios are *P. caroliniana* to *P. viridis*.

Score	Marker Ratio
0	2:0
1	2:1
	1:0
2	2:2
	1:1
	0:0
3	2:3
	1:2
	0:1
4	2:4
	1:3
	0:2
5	1:4
	0:3
6	0:4

by Hamrick and Godt (1990) and modified for use with dominant markers,

$$H_{EP} = 1 - (q^2 + (1-q)^2) = 2q - 2q^2,$$

where q is the frequency of the null banding phenotype and p = 1 - q, by assuming Hardy-Weinberg equilibrium. Because I had an unequal number of markers for each species, I made bootstrap calculations for the proportion of polymorphic loci (P'_p) and genetic diversity indices (H'_{EP}) using both *P. caroliniana* markers and all possible combinations of pairs of *P. viridis* markers for a total of 6 estimates. I calculated disequilibia statistics among pairs of RAPD markers using chi-square analyses (Cruzan and Arnold 1993).

Analysis of Soil Variables

I collected soil from the majority of populations (Table 1) by scraping away the surface layer and sampling to a depth of 10 cm, and had them analyzed for pH, phosphorous (ppm), potassium (ppm), calcium (ppm), magnesium (ppm), % organic matter, and cation exchange capacity (A&L Analytical Laboratories, Inc., Memphis, TN). I performed a principal components analysis (Proc PRINCOMP, SAS 1985) of all of the above variables to obtain composite variables of variation in soil characters.

Data Analysis

Although population FL11 is clearly allopatric in its distribution (Fig. 1), I detected several *P. viridis* markers that are present at relatively high frequencies, so I analyzed it as a hybrid population; it is not included in any allopatric *P. caroliniana* analyses. Due to small sample size, populations P14 and P15 were pooled into P1415, and P7 and P17 were pooled into P717. Populations in each pair of pooled sites are located within 1 km of each other.

I chose a point south of all the populations (Upper Matecumbe Key) as the origin of a transect that extended northward up the Florida peninsula and then westward along the panhandle following the distribution of *Piriqueta* populations that have been identified (Fig. 1). I measured the distance of each population from the origin along the transect in kilometers (KM).

I conducted ANOVAs both between and within allopatric populations for all morphological characters measured. I also performed an ANOVA between the variances in morphological scores in northern and southern hybrid populations. Southern populations were those up to and including population P20 at 332 km north of the transect origin.

For hybrid populations only, I performed correlation analyses for distance from the origin of the transect and the first four soil principal components axes with the morphological parameters (morphological character values, morphological scores, and coefficients of variation in morphological scores). I performed the same correlation analyses with the genetic parameters (RAPD marker frequencies, genetic scores, coefficients of variation in genetic scores, proportion of variable RAPD markers, and genetic diversity indices). All population correlations were weighted based on the appropriate morphological or genetic sample size. I also performed weighted correlations among the above morphological and genetic parameters. For all but one hybrid population (P5), the same individual plants were used for both the morphological and genetic measurements.

Chapter III

Results

Morphological Analysis

Significant differences were found between allopatric populations of the two species for the following morphological characters: leaf width, leaf area, leaf shape (WLR), the number of stellate hairs on the dorsal leaf surface (SHD), the number of stellate hairs on the ventral leaf surface (SHV), and the number of hirsute hairs on the dorsal leaf surface (HHD : Table 4, 5). Because leaf width and leaf area are size characters that are sensitive to developmental and environmental variation, I did not use them as diagnostic characters. Leaf shape (WLR), on the other hand, does not appear to be as subject to environmental influences so I chose it as a diagnostic morphological marker. The number of hirsute hairs on the ventral leaf surface (HHV) was not significantly different between allopatric populations; however, this was probably due to both a high variance for the trait in *P. caroliniana* populations and a small sample area on the leaf. Since their were no hairs on any of the *P. viridis* plants (Table 5), I used all of the indumentum characters as diagnostic markers (SHD, SHV, HHD, and HHV). There was a significant correlation between the number of stellate hairs on each leaf surface (SHD and SHV) as well as the number of hirsute hairs on each leaf surface (HHD and HHV: unpubl. data). However, I maintained the indumentum markers from both leaf surfaces since analyses were not performed with them individually, but rather each contributed in part to the composite morphological score (MSC).

Table 4. Results of the ANOVA for morphological characters diagnostic for allopatric *P. caroliniana* and *P. viridis* individuals.

	P. caroliniana	*P. viridis*	Between Allopatric Taxa			Within Allopatric Taxa		
Source	Mean	Mean	MS	F	P<F	MS	F	P<F
Length	42.58	47.42	232.64	0.85	0.391	309.43	1.69	0.161
Width	15.04	1.51	2094.46	168.93	**0.000**	15.70	3.58	0.099
Area	466.37	47.87	19129.46	39.14	**0.002**	64047.14	5.93	**0.000**
Perimeter	107.90	102.46	428.60	0.27	0.627	1910.70	2.14	0.084
SHD	16.09	0.00	3036.97	16.22	**0.014**	254.30	13.50	**0.000**
SHV	29.40	0.00	10832.97	48.73	**0.001**	291.18	5.90	**0.000**
HHD	5.73	0.00	389.90	19.88	**0.008**	25.24	4.57	**0.002**
HHV	2.67	0.00	64.86	3.17	0.143	27.31	8.11	**0.000**
WLR	0.36	0.03	1.25	2199.00	**0.000**	0.00	0.10	0.984

SHD and SHV = the number of stellate hairs per mm^2 on the dorsal and ventral leaf surfaces, respectively, HHD and HHV = the number of hirsute hairs per 3 mm^2 on the dorsal and ventral leaf surfaces, respectively, WLR = leaf width-to-length ratio.

Table 5. Morphological parameters for populations sampled.

POP	Type	KM	N	SHD	SHV	HHD	HHV	WLR	MSC	MCV
FL11	H	824	10	11.3 ± 2.19	21.3 ± 3.82	2.6 ± 0.69	0.4 ± 0.22	0.36 ± 0.022	2.68	42.10
GA7	C	811	4	14.3 ± 1.89	30.5 ± 6.24	5.3 ± 1.03	1.0 ± 0.58	0.35 ± 0.015	1.45	66.03
FL9	C	794	15	10.7 ± 2.01	23.1 ± 3.47	4.0 ± 0.62	1.1 ± 0.46	0.36 ± 0.019	2.24	60.67
FL3	C	662	14	22.4 ± 1.72	35.8 ± 2.04	7.7 ± 1.34	4.8 ± 1.07	0.36 ± 0.015	0.71	116.60
P30	H	593	8	22.9 ± 1.20	30.1 ± 1.34	2.8 ± 0.80	1.4 ± 0.42	0.37 ± 0.023	1.40	68.30
P24	H	558	13	29.9 ± 0.81	45.8 ± 1.93	2.1 ± 0.51	1.2 ± 0.55	0.32 ± 0.016	1.87	35.96
P26	H	551	4	19.5 ± 2.60	36.5 ± 2.72	14.0 ± 2.48	6.3 ± 2.39	0.28 ± 0.017	0.40	70.71
P25	H	548	15	17.5 ± 1.39	29.8 ± 1.90	2.8 ± 0.98	1.5 ± 1.33	0.29 ± 0.011	2.31	33.24
P27	H	512	6	23.7 ± 1.98	33.3 ± 3.09	2.7 ± 0.72	0.8 ± 0.48	0.23 ± 0.014	1.97	34.88
FL23	H	504	10	20.6 ± 1.31	36.2 ± 2.33	6.1 ± 0.90	1.1 ± 0.43	0.32± 0.021	1.26	56.52
P29	H	471	15	27.1 ± 1.54	39.9 ± 1.77	8.4 ± 1.39	5.2 ± 1.07	0.37 ± 0.011	0.52	163.70
P28	H	468	15	24.3 ± 1.80	40.9 ± 2.51	5.6 ± 1.80	3.9 ± 1.77	0.23 ± 0.028	1.89	55.61
P4	H	464	15	16.9 ± 1.74	35.9 ± 1.72	25.1 ± 2.54	14.9 ± 1.80	0.27 ± 0.012	0.51	68.15
FL22	H	436	15	15.7 ± 0.87	27.9 ± 1.86	3.1 ± 0.40	0.5 ± 0.19	0.20 ± 0.010	2.43	28.01
P22	H	418	16	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.11 ± 0.007	5.74	1.67
P23	H	411	12	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.05 ± 0.004	5.91	1.77
P21	H	393	15	19.5 ± 1.71	42.8 ± 3.20	22.0 ± 2.28	17.5 ± 2.22	0.24 ± 0.010	0.57	60.23
P2	H	391	15	22.4 ± 1.64	32.3 ± 1.48	3.5 ± 0.58	0.6 ± 0.27	0.37 ± 0.015	1.57	51.72
P5	H	379	15	16.5 ± 2.88	20.7 ± 2.66	10.1 ± 1.72	5.9 ± 1.31	0.22 ± 0.015	1.47	51.20
P6	H	347	30	24.3 ± 1.21	37.9 ± 1.83	0.8 ± 0.47	0.7 ± 0.32	0.12 ± 0.006	3.04	22.35
P717	H	337	15	16.1 ± 2.71	25.5 ± 4.16	0.1 ± 0.13	0.2 ± 0.15	0.09 ± 0.008	3.96	23.70
P20	H	332	20	19.0 ± 2.08	32.5 ± 3.03	0.0 ± 0.00	0.0 ± 0.00	0.06 ± 0.003	3.86	18.02
P16	H	320	17	9.9 ± 3.12	13.6 ± 4.26	0.0 ± 0.00	0.0 ± 0.00	0.09 ± 0.007	4.78	25.07
P8	H	299	11	18.9 ± 2.27	35.5 ± 4.73	0.0 ± 0.00	0.0 ± 0.00	0.10 ± 0.012	3.62	16.85
P9	H	291	11	20.5 ± 4.01	33.0 ± 5.92	0.0 ± 0.00	0.0 ± 0.00	0.17 ± 0.014	3.64	29.91
P19	H	289	30	25.2 ± 1.15	34.1 ± 1.71	0.1 ± 0.07	0.0 ± 0.00	0.19 ± 0.007	3.10	8.43
P1415	H	286	5	16.4 ± 1.78	23.8 ± 1.83	0.0 ± 0.00	0.0 ± 0.00	0.22 ± 0.007	3.28	3.34
P18	H	285	40	20.3 ± 2.37	27.9 ± 3.28	0.2 ± 0.10	0.0 ± 0.00	0.13 ± 0.007	3.99	27.92
FL21	V	246	4	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.03 ± 0.004	6.00	0.00
FL19	V	165	15	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.03 ± 0.003	6.00	0.00
P10	V	122	30	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.04 ± 0.003	5.99	0.61
F_1s	--	--	21	5.9 ± 1.38	9.3 ± 2.25	0.1 ± 0.10	0.0 ± 0.00	0.21 ± 0.022	4.56	17.63

POP = population, C = allopatric *P. caroliniana* population, H = hybrid population, V = allopatric *P. viridis* population, KM = latitude [the distance the populations are along the transect (Figure 1) that originates at Upper Matecumbe Key, SHD and SHV = the number of hairs per mm^2 on the dorsal and ventral leaf surfaces, respectively, HHD and HHV = the number of hirsute hairs per 3 mm^2 on the dorsal and ventral leaf surfaces, respectively, WLR = leaf width to length ratio, MSC = mean morphological score, MCV = coefficient of variation for morphological scores.

The five morphological markers displayed different levels of dominance in the F_1 generation (Fig. 2). Leaf shape (WLR) was intermediate to the parental genotypes, suggesting codominance for the alternative alleles at loci determining this character. The number of stellate hairs (SHD and SHV) were nearly intermediate as well, also suggesting codominance. However, there were no hirsute hairs (HHD and HHV) in the F_1 generation, therefore the absence of hirsute hairs appears to be dominant to their presence.

In the principal components analysis, the first four axes explained 94.5 % of the variation in the soil variables, with the first axis explaining 46.2 % and the second 27.3 %. The first principal component axis (PC1) was positively correlated with potassium (K), calcium (Ca), magnesium (Mg), and cation exchange capacity, while soil pH (negative correlation) and the percent organic matter (positive correlation) loaded heavily on the second principal component axis (PC2 : Table 6). Soil characteristics for the hybrid populations were not intermediate to those for allopatric populations; instead they clustered more closely with soils found in *P. viridis* populations (Fig. 3).

Several of the morphological markers were correlated with the soil principal component scores (Table 7) when only hybrid populations were considered. The number of dorsal hirsute hairs (HHD) and leaf shape (WLR) were positively correlated with PC1, and the number of ventral hirsute hairs (HHV) was also positively correlated with PC1, but not significantly. These results indicate that hybrid plants having a greater leaf width-to-length ratio and more hirsute hairs grow in soils with high levels of K, Ca, Mg, and a

Table 6. Results of the principal components analysis of soil variables. PC1 - PC4 are principle component axes 1 - 4, respectively.

Principal Component	PC1	PC2	PC3	PC4
Proportion Variation Explained	0.462	0.273	0.114	0.097
Cumulative Variation Explained	0.462	0.735	0.848	0.945
Soil pH	0.173	**-0.614**	0.201	0.307
Phosphorous (ppm)	0.191	0.369	**0.816**	0.360
Potassium (ppm)	**0.416**	0.150	**-0.479**	**0.480**
Calcium (ppm)	**0.463**	-0.365	0.094	-0.227
Magnesium (ppm)	**0.480**	0.185	-0.193	0.279
% Organic Matter	0.286	**0.511**	-0.021	**-0.462**
Cation Exchange Capacity	**0.484**	-0.191	0.133	**-0.450**

Table 7. Results of the correlation analysis between hybrid population morphological variables and both the soil principal component variables and distance in km the populations are along the transect (Fig. 1) that originates at Upper Matecumbe Key. Pearson correlation coefficients are given followed by p-values for each variable.

Variable	PC1	p	PC2	p	PC3	p	PC4	p	KM	p
SHD	0.259	0.315	0.167	0.523	0.020	0.941	0.314	0.219	-0.065	0.758
SHV	0.274	0.287	-0.064	0.808	0.166	0.525	0.190	0.465	0.058	0.784
HHD	**0.481**	**0.051**	**-0.503**	**0.039**	0.436	0.080	-0.315	0.218	0.270	0.192
HHV	0.444	0.074	**-0.506**	**0.038**	**0.484**	**0.049**	-0.200	0.441	0.181	0.387
WLR	**0.543**	**0.024**	0.146	0.577	0.108	0.680	-0.256	0.321	**0.674**	**0.000**
MSC	**-0.505**	**0.039**	0.422	0.091	-0.437	0.079	0.176	0.501	-0.246	0.236
MCV	**0.688**	**0.002**	0.236	0.362	-0.058	0.824	0.009	0.971	0.378	0.063

SHD and SHV = number of stellate hairs per mm^2 on the dorsal and ventral leaf surfaces, respectively. HHD and HHV = number of hirsute hairs per 3 mm^2 on the dorsal and ventral leaf surfaces, respectively. WLR = leaf width-to-length ratio, MSC = mean morphological score, MCV = coefficient of variation for the morphological scores, PC1 - PC4 = soil principal component variables for the first four axes, KM = distance along the transect, and p = p-value.

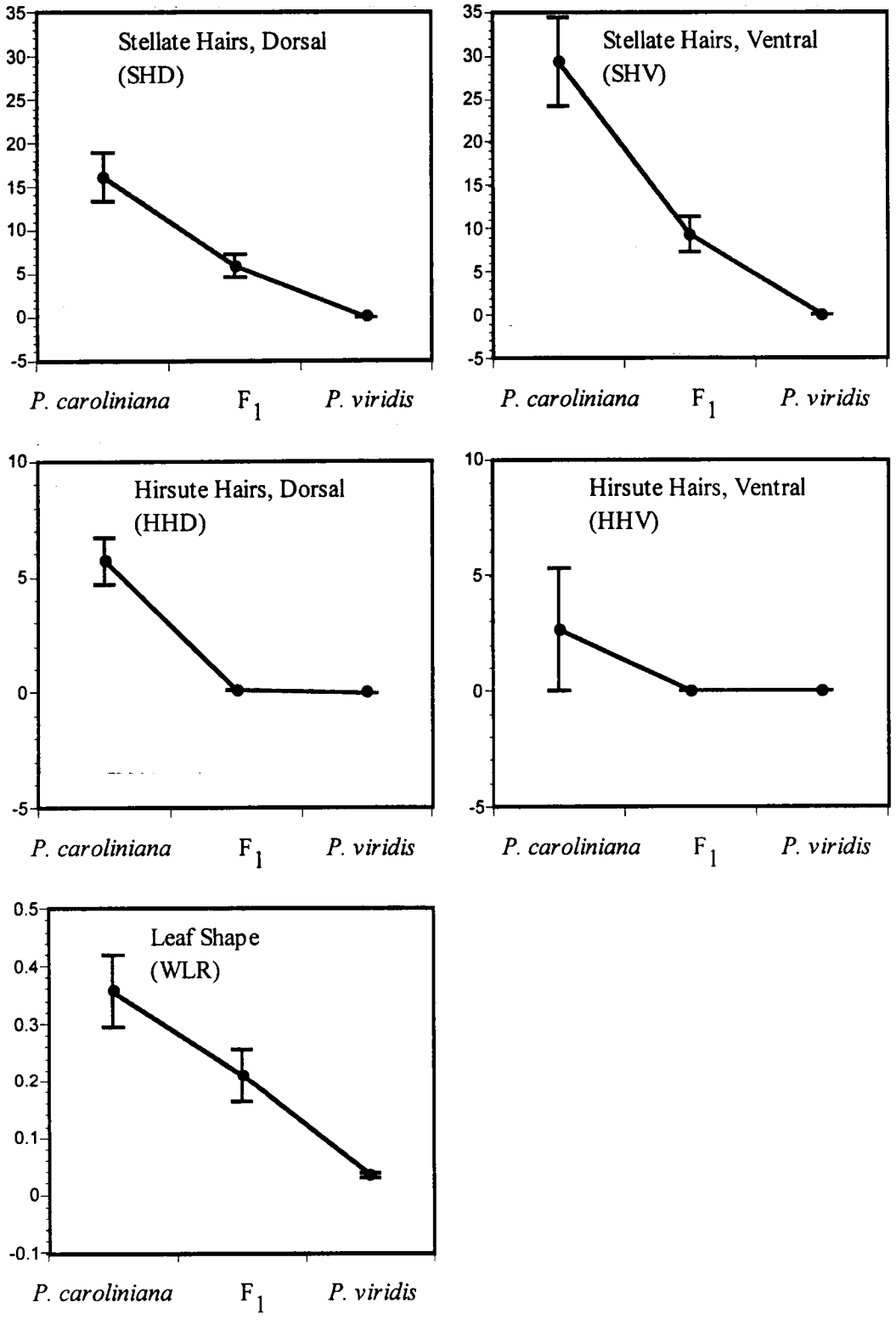

Figure 2. Mean values for morphological markers in plants of parental and F_1 genotypes. Hairs were counted on leaf surfaces in mm^2 (stellate) and 3 mm^2 (hirsute) areas .

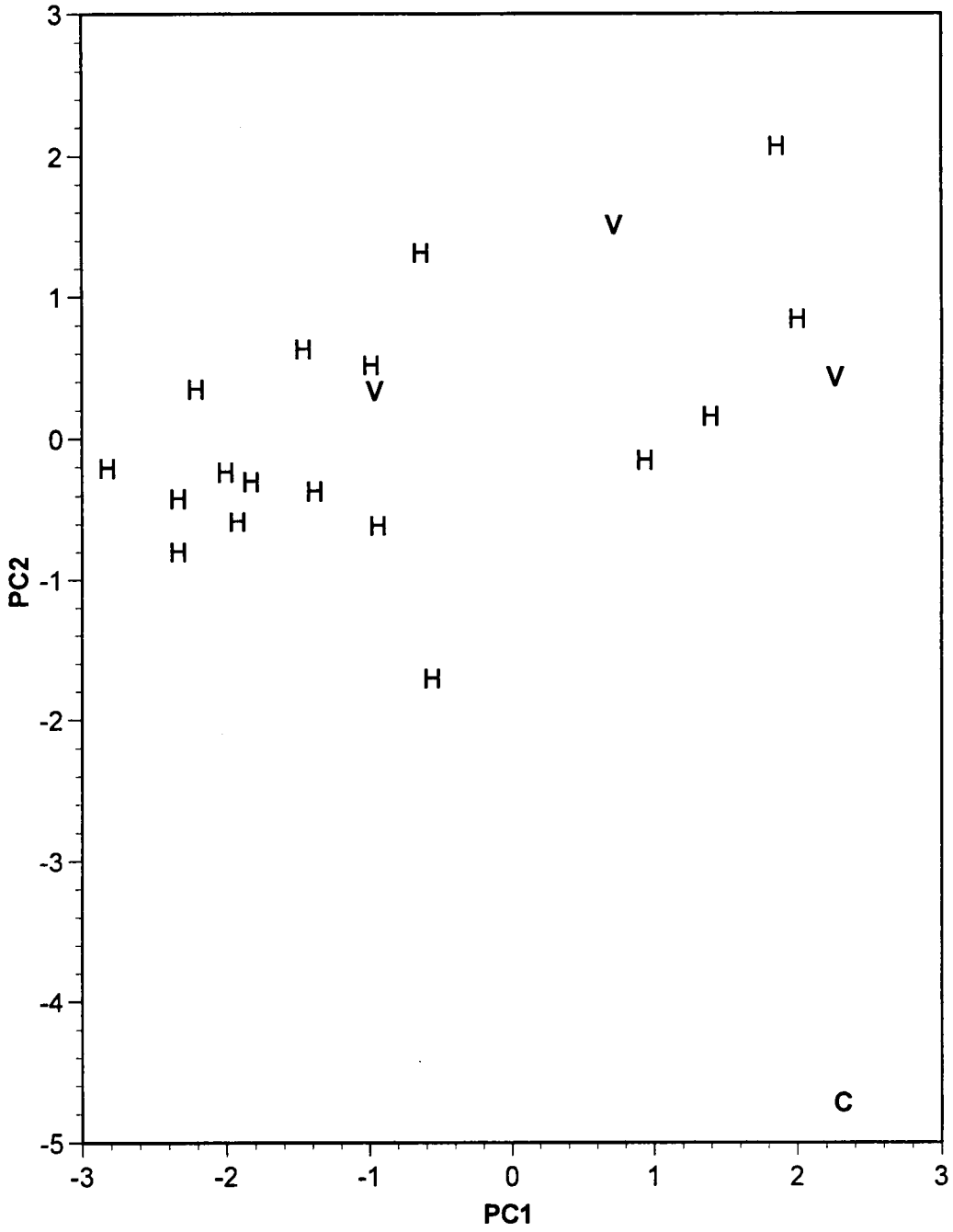

Figure 3. Distribution of populations along the first two principal component axes for soil characteristics. Values for allopatric *P. caroliniana* (C), allopatric *P. viridis* (V) and hybrid (H) populations are shown. The first two axes explain 73.5 % of the variation.

high cation exchange capacity relative to plants with a smaller leaf width-to-length ratio and few or no hirsute hairs. The negative correlation between the morphological scores (MSC) and PC1 and the positive correlation between the coefficients of variation in morphological scores (MCV) and PC1 indicate that hybrid individuals that are morphologically similar to *P. caroliniana* (score = 0) and those in populations with more morphological variation grow in soils with high levels of K, Ca, Mg, and a high cation exchange capacity. The negative correlation between the number of hirsute hairs (HHD and HHV) and PC2 indicates that plants with more hirsute hairs are found in soils with a higher pH and a lower percent organic matter than plants with few or no hirsute hairs.

Excluding allopatric populations, leaf shape (WLR) was positively correlated with population distance along the transect (KM : Table 7), indicating that plants in the northern end of the hybrid zone have a larger leaf width-to-length ratio than those in the southern end (Table 5, Fig. 4). Although there were no significant correlations with the indumentum markers (SSD, SSV, HHD, and HHV) and distance along the transect (KM : Table 7), they all showed an increase in frequency moving northward in the hybrid zone (Table 5, Fig. 4). For all of the morphological markers, there was a substantial amount of variation among populations in the area of hybridization (Fig. 4). In particular, in the northern portion of the hybrid zone higher levels of morphological variation and extreme morphological phenotypes were common in several populations (Fig. 5). The morphological scores (MSC) for hybrid populations were negatively correlated, but not significantly, with distance along the transect (KM : Table 7). This suggests that hybrid plants in the north are morphologically more similar to allopatric *P. caroliniana*

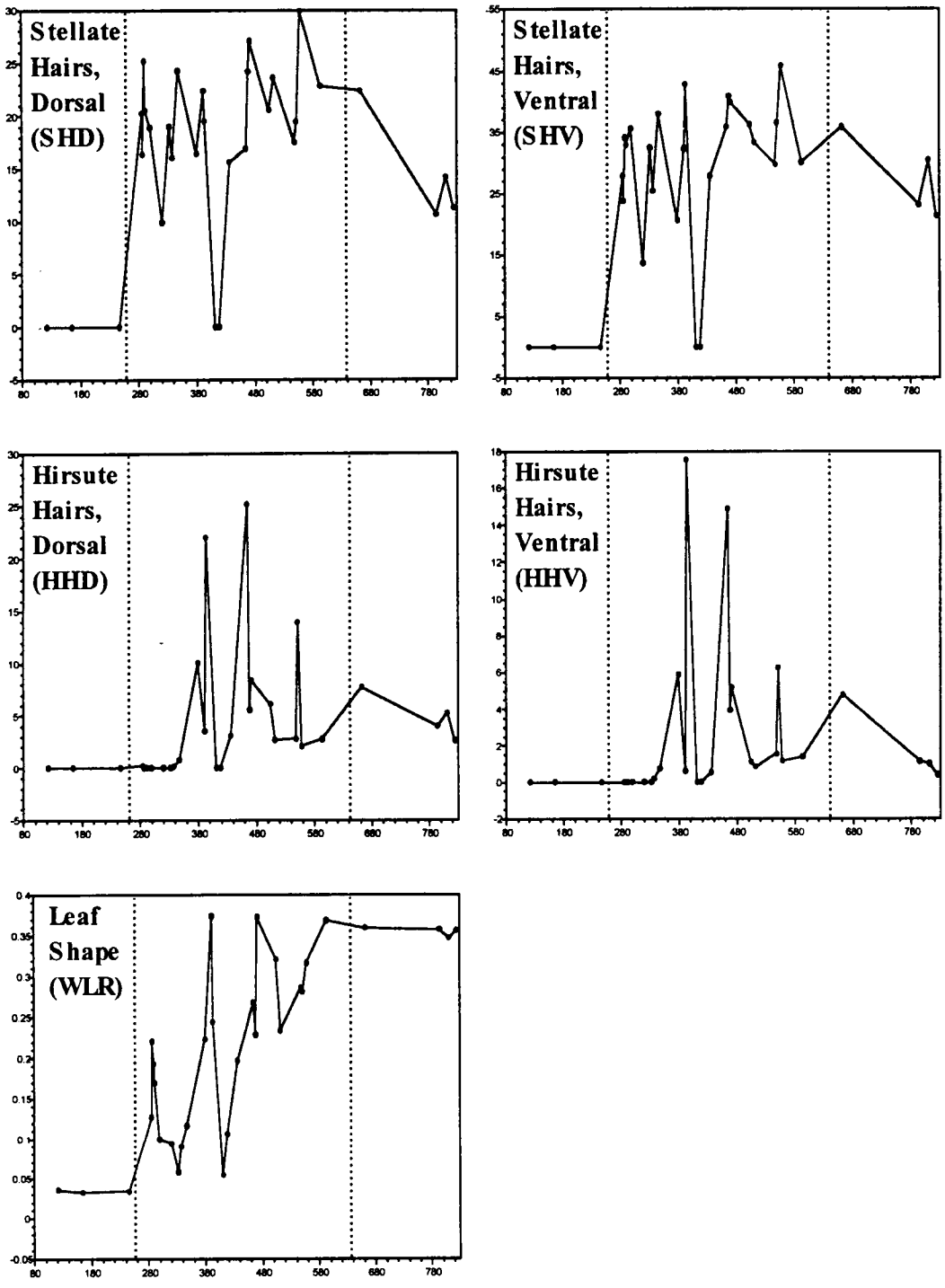

Figure 4. Mean morphological character values for all populations. The x-axes are population distances (km) along the transect from south to north Florida (Fig. 1). Dotted lines delineate the hybrid zone from allopatric parental populations. The most northern population (FL11) is also hybrid. Hairs were counted on leaf surfaces in mm^2 (stellate) and 3 mm^2 (hirsute) areas.

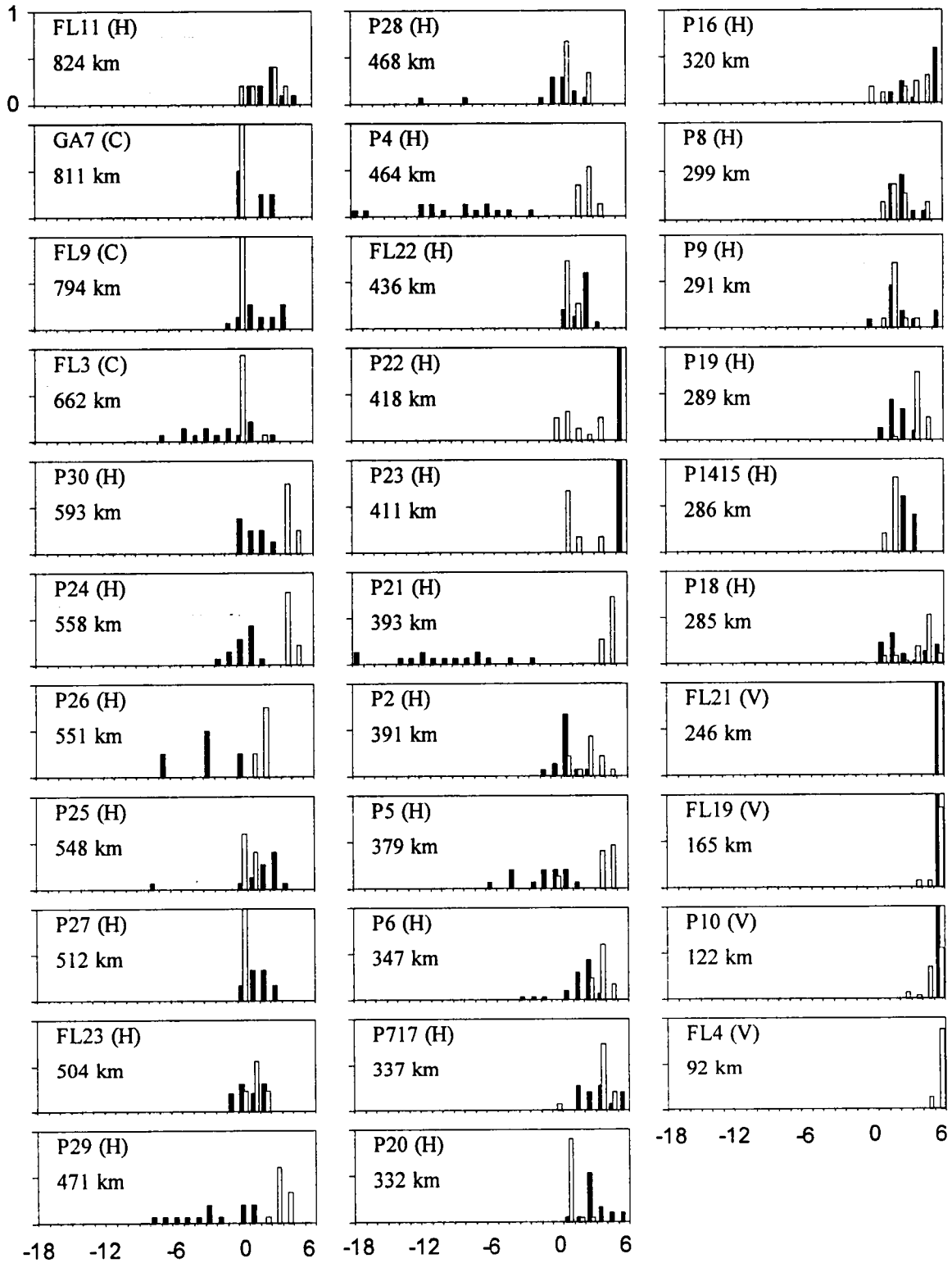

Figure 5. Distribution of morphological phenotypes and genetic marker genotypes among hybrid classes. The morphological index ranges from -18 to 6, and the genetic index from 0 to 6. Filled bars are for morphological characters, and open bars are for genetic markers. Populations are either allopatric *P. caroliniana* (C), allopatric *P. viridis* (V), or hybrid (H). The population distances along the transect from south to north Florida (Fig. 1) are given.

individuals, while those in the south are more similar to allopatric *P. viridis* individuals (Fig. 6A).

There was a positive, but not significant, correlation between distance along the transect (KM) and the amount of variation in morphological scores within hybrid populations (MCV : Table 7). Thus, in general, populations in the northern end of the hybrid zone are morphologically more variable than those in the southern end (Table 7, Fig. 7A).

Genetic Analysis

I screened a total of 345 ten-base primers. From the 215 primers that produced resolved bands, I identified two RAPD markers diagnostic for *P. caroliniana* (c28 and c59) and four diagnostic for *P. viridis* (v59, v61, v164, and v270: see Fig. 8 for an example of a diagnostic marker banding pattern). The number of markers identified here is similar to that found in other studies of hybrid zones (eg., four RAPD markers diagnostic for each of three species: Cruzan and Arnold 1993; three RAPD markers diagnostic for each of two species: Cruzan and Arnold 1994; three allozyme markers diagnostic for two species: Harrison 1986; five allozyme markers diagnostic for two species: Szymura and Barton 1986; and seven allozyme markers diagnostic for two species: Shoemaker et al. 1996) . All six of the markers identified here showed strong frequency differences for allopatric populations of the two taxa (Table 2), and plants in the putative hybrid populations displayed a mixture of the diagnostic markers (Table 8).

Both of the *P. caroliniana* markers had relatively high frequencies throughout the entire hybrid zone (Fig. 9). Marker c28 was almost fixed in populations south to

Table 8. Genetic parameters for populations sampled.

POP	Type	KM	N	c28	c59	v59	v61	v164	v270	GSC	GCV
GA1-GA5	C	----	10	0.90	----	----	----	----	----	----	----
FL11	H	824	5	1.00	0.60	0.60	0.60	0.00	0.60	2.20	74.69
GA7	C	811	5	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
FL9	C	794	14*	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
FL3	C	662	14	1.00	0.93	0.00	0.00	0.00	0.07	0.14	374.16
P30	H	593	8	1.00	0.75	1.00	1.00	1.00	1.00	4.25	10.89
P24	H	558	14	0.93	0.86	1.00	1.00	1.00	1.00	4.21	10.10
P26	H	551	4	1.00	1.00	0.00	1.00	0.75	0.00	1.75	28.57
P25	H	548	15	0.93	0.67	0.00	0.00	0.00	0.00	0.40	126.77
P27	H	512	6	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
FL23	H	504	9	1.00	0.78	0.00	0.00	0.78	0.00	1.00	70.71
P29	H	471	15	1.00	0.27	1.00	1.00	0.20	0.33	3.27	18.17
P28	H	468	15	1.00	1.00	0.33	1.00	0.00	0.33	1.67	58.55
P4	H	464	15	1.00	0.33	1.00	1.00	0.00	0.13	2.80	24.18
FL22	H	436	15	0.93	1.00	0.00	1.00	0.20	0.00	1.27	36.14
P22	H	418	16	1.00	0.69	0.31	0.63	0.19	0.31	1.75	89.77
P23	H	411	12	0.83	0.83	0.17	0.00	0.17	1.00	1.67	69.28
P21	H	393	15	1.00	0.27	1.00	1.00	1.00	1.00	4.73	9.67
P2	H	391	14	1.00	0.71	0.71	0.43	0.79	0.64	2.86	43.10
P5	H	379	30	1.00	0.53	0.87	0.87	0.87	0.87	3.93	41.66
P6	H	347	30	1.00	0.80	1.00	0.73	1.00	1.00	3.93	16.26
P717	H	337	15	1.00	0.80	0.93	0.93	0.93	0.93	3.93	29.57
P20	H	332	20	0.95	0.95	0.00	1.00	0.05	0.00	1.15	42.55
P16	H	320	17	0.65	0.59	0.71	0.71	0.18	0.71	3.06	62.75
P8	H	299	11	0.46	0.64	0.18	0.46	0.36	0.73	2.64	51.66
P9	H	291	10	0.10	1.00	0.10	1.00	0.20	0.00	2.20	35.86
P19	H	289	29	0.62	1.00	1.00	0.97	0.86	0.97	4.17	14.42
P1415	H	286	5	0.20	1.00	0.00	1.00	0.00	0.00	1.80	24.85
P18	H	285	38	0.47	0.61	0.82	0.95	0.82	0.82	4.32	32.37
FL21	V	246	4	0.00	0.00	1.00	1.00	1.00	1.00	6.00	0.00
FL19	V	165	14	0.00	0.07	1.00	0.93	1.00	0.93	5.79	10.01
P10	V	122	29	0.10	0.28	0.97	0.97	0.90	0.93	5.38	16.03
FL4	V	92	8**	0.00	0.13	1.00	1.00	1.00	1.00	5.88	6.02

POP = population, C = allopatric *P. caroliniana* population, H = hybrid population, V = allopatric *P. viridis* population, KM = the distance the populations are along the transect (Fig. 1) that originates at Upper Matecumbe Key, c28 - v270 = RAPD marker frequencies, GSC = mean genetic score, GCV = coefficient of variation for genetic scores.

* N = 35 for c28

** N = 20 for c28

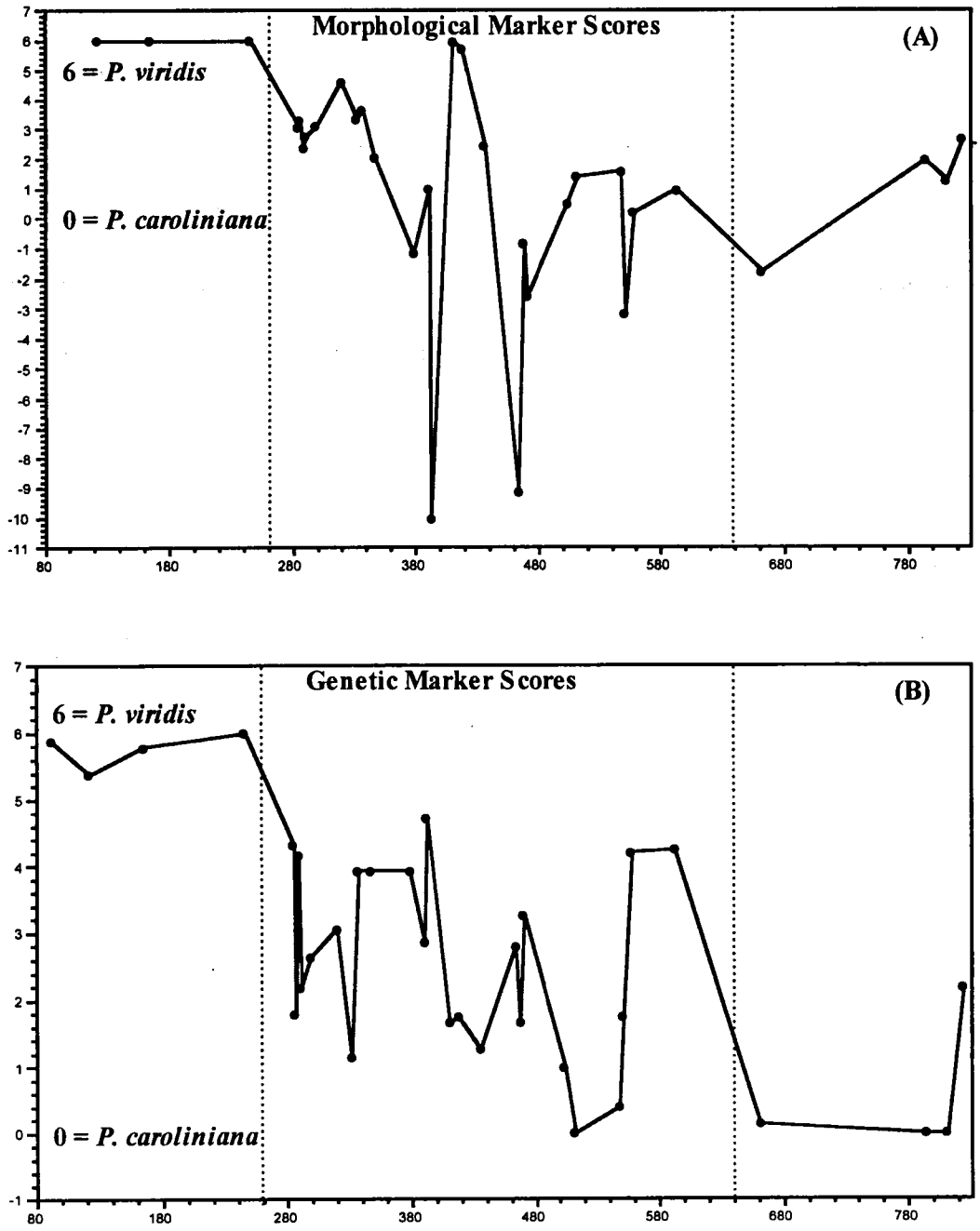

Figure 6. Mean morphological and genetic scores for all populations. The x-axes are population distances (km) along the transect from south to north Florida (Fig. 1). Dotted lines delineate the hybrid zone from allopatric parental populations. The most northern population (FL11) is also hybrid.

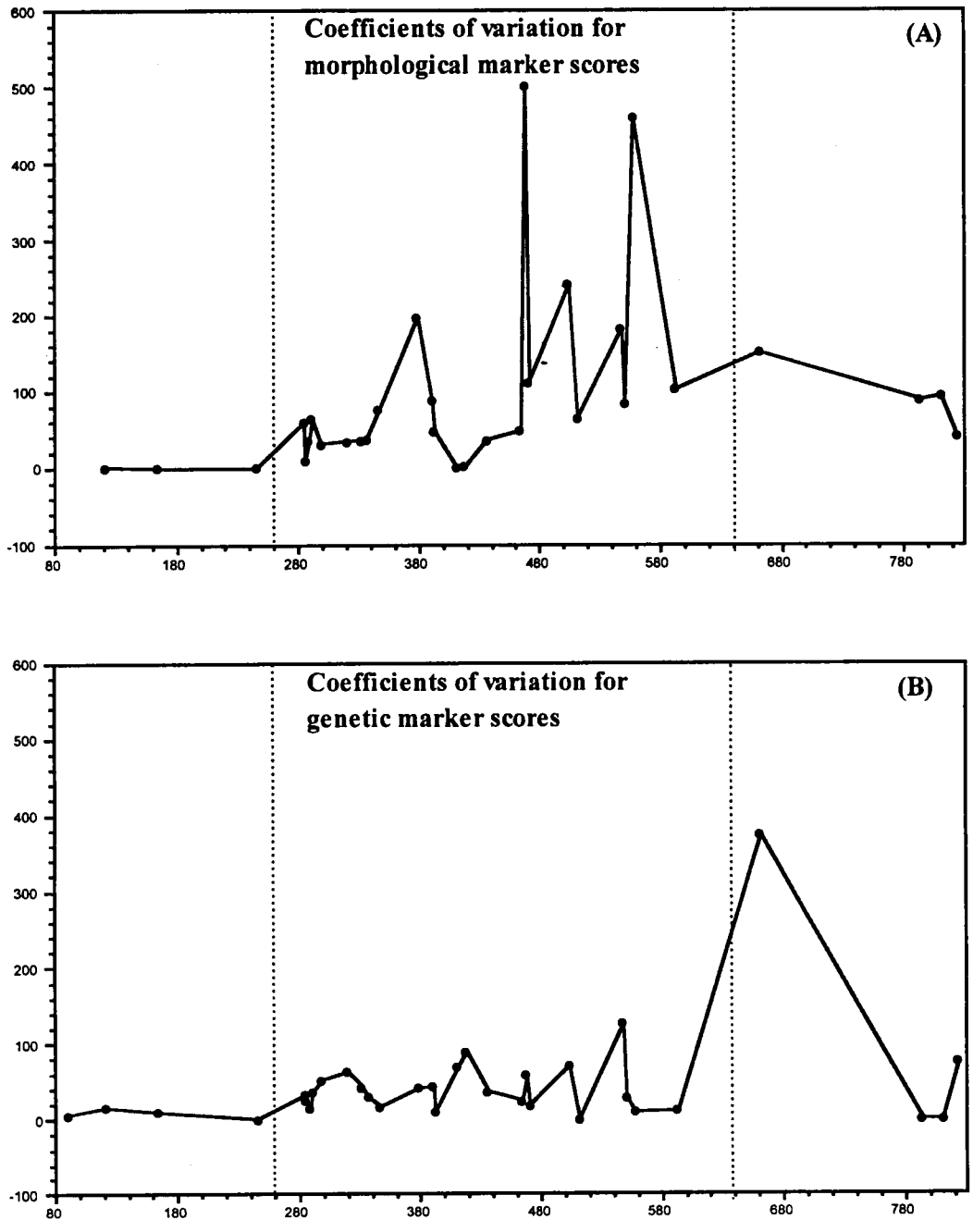

Figure 7. Coefficients of variation for mean morphological and genetic scores for all populations. The x-axes are population distances (km) along the transect from south to north Florida (Fig. 1). Dotted lines delineate the hybrid zone from allopatric parental populations. The most northern population (FL11) is also hybrid.

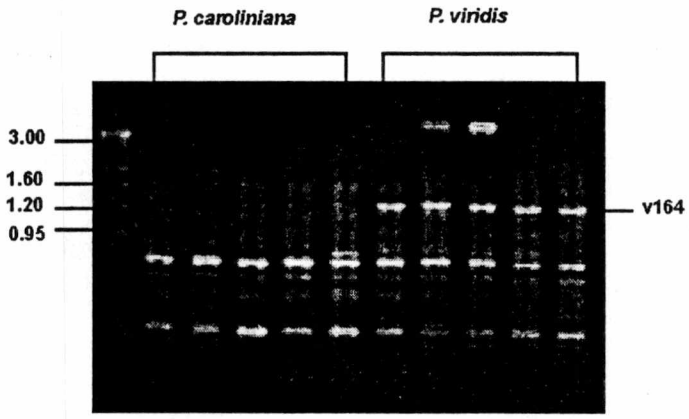

Figure 8. Amplification products for the RAPD marker v164 (diagnostic for *P. viridis*) in each of five allopatric *P. caroliniana* and *P. viridis* individuals. The first lane contains size marker fragments (kb).

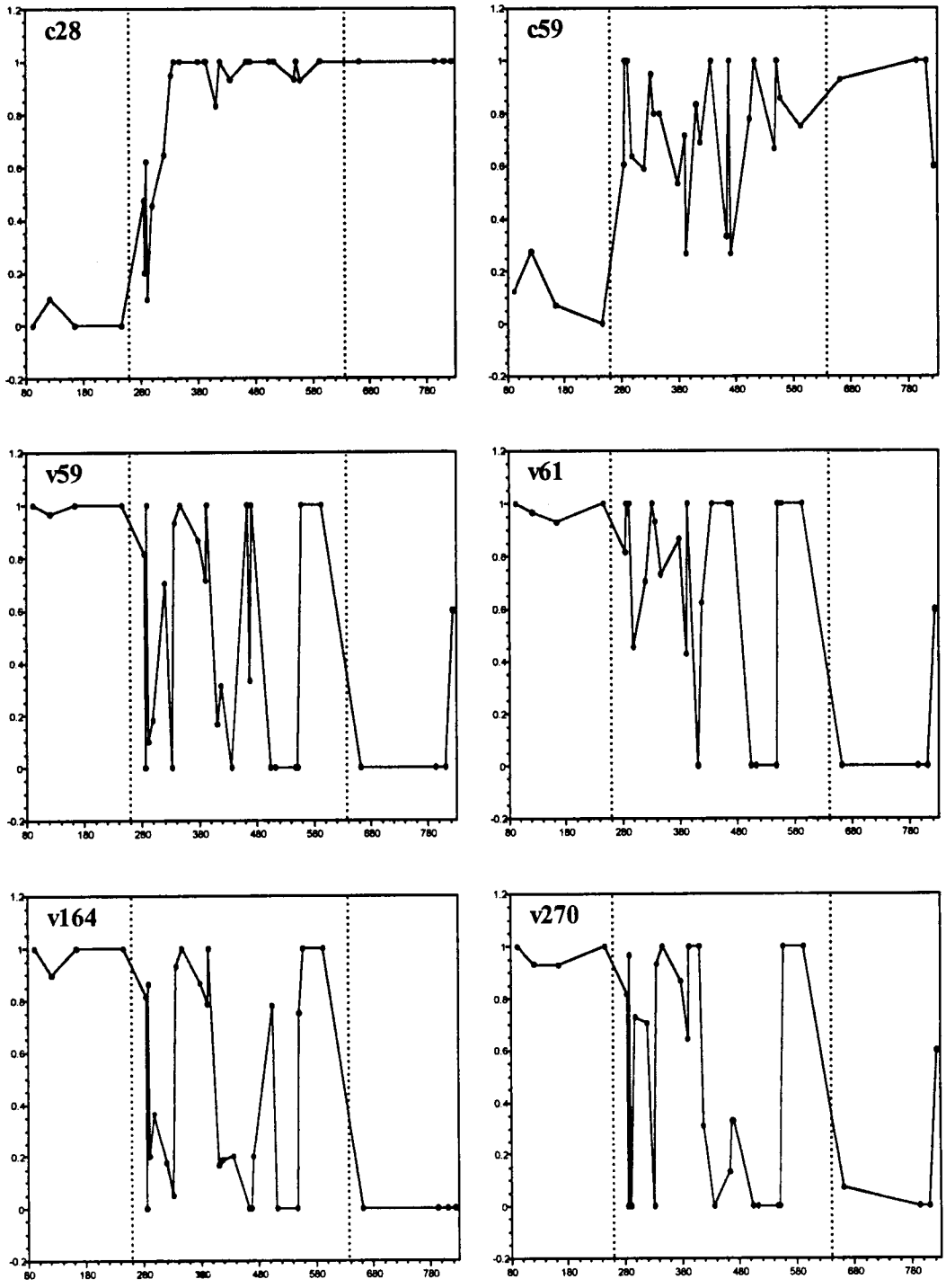

Figure 9. RAPD marker frequencies for all populations. The x-axes are population distances (km) along the transect from south to north Florida (Fig. 1). Dotted lines delineate the hybrid zone from allopatric parental populations. The most northern population (FL11) is also hybrid.

Highlands County (population P18 is the southern most in Highlands County: Table 1, Fig. 1), where it forms a sharp cline (Fig. 9). There was a significant positive correlation between marker c28 and distance along the transect (KM : Table 9). The other *P. caroliniana* marker (c59) was more variable in the northern end of the hybrid zone but remained at a higher frequency in populations where c28 exhibited a dramatic decrease in frequency (populations P9, P19, and P1415 : Table 8, Fig. 1, Fig. 9).

The *P. viridis* markers were highly variable in frequency across the entire hybrid zone (Fig. 9). Marker v59 was fixed in 7 of the 25 hybrid populations, v61 in 11, v164 in 4, and v270 in 5. Furthermore, the *P. viridis* markers were fixed for the null genotype in several hybrid populations, v59 in 7 populations, v61 in 4, v164 in 6, and v270 in 8. All of the *P. viridis* markers were fixed in populations P30, P24, and P21, all of which occur toward the northern end of the hybrid zone (Fig. 1). However, all of the *P. viridis* markers were fixed for the null genotype in populations P25 and P27, which are interspersed among the above populations in which the markers are fixed for the dominant state.

There was a negative, but not significant, correlation between genotypic scores (GSC) and distance along the transect (KM : Table 9, Fig. 6B). Thus, moving north in the hybrid zone, hybrid populations become genetically more similar to *P. caroliniana.* Although not significant, there was a positive correlation with the amount of variation in genotypic score within populations (GCV) and distance along the transect (KM : Table 9, Fig. 7B), indicating that there is more within-population variation in genotypic score moving north in the hybrid zone.

Table 9. Results of the correlation analysis between hybrid population genetic variables and both the soil principal component variables and the distance in km the populations are along the transect (Fig. 1) that originates at Upper Matecumbe Key. Pearson correlation coefficients are given followed by p-values for each variable.

Variable	PC1	p	PC2	p	PC3	p	PC4	p	KM	p
c28	0.420	0.106	-0.029	0.915	0.191	0.477	-0.378	0.149	**0.607**	**0.002**
c59	-0.124	0.647	0.325	0.220	-0.374	0.154	-0.018	0.947	-0.126	0.558
v59	0.425	0.101	-0.058	0.830	0.037	0.892	0.164	0.543	-0.154	0.473
v61	0.212	0.430	0.131	0.628	0.126	0.641	0.283	0.289	-0.275	0.193
v164	-0.002	0.994	-0.120	0.657	-0.478	0.061	0.302	0.255	-0.260	0.220
v270	0.134	0.621	-0.005	0.985	-0.246	0.359	0.245	0.361	-0.284	0.179
GSC	0.167	0.537	-0.067	0.804	-0.121	0.656	0.338	0.201	-0.371	0.074
GCV	-0.155	0.581	-0.043	0.879	-0.037	0.897	-0.110	0.695	0.298	0.167
P'_P	**-0.500**	**0.041**	-0.042	0.871	-0.255	0.323	0.382	0.130	**-0.512**	**0.009**
H'_{EP}	-0.270	0.311	0.080	0.770	-0.163	0.545	-0.224	0.405	-0.341	0.104

c28, c59, v59, v61, v164, and v270 = mean frequencies of these RAPD markers, GSC = mean genetic score, GCV = coefficient of variation for the genetic scores, P'_P = proportion of variable RAPD markers, H'_{EP} = genetic diversity index, PC1 - PC4 = soil principal component variables for the first four axes, KM = distance along the transect, and p = p-value.

There was a significant negative correlation between the bootstrap estimate of proportion of variable RAPD markers (P'_P) in hybrid populations and distance along the transect (KM : Table 9), indicating that populations at northern end of the hybrid zone have a higher proportion of fixed RAPD markers than those in the southern end (Fig. 10A). There was a negative, but not significant, correlation between the bootstrap estimate of genetic diversity indices of hybrid populations (H'_{EP}) and distance along the transect (KM : Table 9). Thus, there is slightly more genetic diversity in southern hybrid populations (Fig. 10B).

Several of the genetic markers displayed associations with morphological characters (Table 10). The frequency of marker c59 among hybrid populations was negatively correlated with the number of hirsute hairs on both the dorsal and ventral leaf surfaces (HHD and HHV : Table 10). Marker c59 was positively correlated with the mean morphological score, indicating that it is at a higher frequency in plants that are morphologically similar to *P. viridis*. Markers v59, v61, and v164 were positively correlated with the number of stellate hairs on both the dorsal or ventral leaf surface (SHD or SHV : Table 10).

Overall there was not a strong correlation between the morphological and genetic scores in the hybrid zone, but individual genetic markers displayed associations with hybrid morphological scores in some cases (Table 10; Fig. 5). Three of the *P. viridis* markers (v59, v61, and v164) were negatively correlated with the mean morphological scores (MSC), indicating that they are most frequent in plants morphologically similar to *P. caroliniana*. The coefficients of variation in the genetic scores were negatively

Table 10. Results of the correlation analysis between hybrid population genetic and morphological variables. Pearson correlation coefficients are given followed by p-values for each variable.

Variable	SHD	SHV	HHD	HHV	WLR	MSC	MCV
c28	-0.019 (0.932)	0.120 (0.575)	0.372 (0.073)	0.332 (0.113)	0.321 (0.126)	-0.338 (0.106)	-0.055 (0.797)
c59	0.102 (0.636)	-0.031 (0.887)	**-0.604 (0.002)****	**-0.630 (0.001)****	-0.258 (0.223)	**0.518 (0.010)****	0.029 (0.892)
v59	**0.403 (0.051)***	0.307 (0.145)	0.233 (0.274)	0.290 (0.169)	0.161 (0.451)	-0.349 (0.094)*	0.067 (0.756)
v61	0.374 (0.072)	0.364 (0.080)*	0.184 (0.388)	0.236 (0.267)	-0.046 (0.831)	-0.306 (0.146)*	-0.278 (0.189)
v164	**0.446 (0.029)***	0.338 (0.107)	-0.082 (0.703)	-0.030 (0.891)	0.030 (0.888)	-0.169 (0.431)	0.372 (0.073)**
v270	0.158 (0.460)	0.031 (0.885)	-0.165 (0.440)	-0.053 (0.804)	-0.182 (0.395)	0.066 (0.758)	0.154 (0.474)
GSC	0.386 (0.062)	0.280 (0.186)	0.076 (0.724)	0.168 (0.433)	-0.023 (0.915)	-0.250 (0.239)	0.111 (0.606)
GCV	**-0.604 (0.002)****	**-0.552 (0.006)****	-0.218 (0.318)	-0.253 (0.244)	-0.078 (0.723)	0.401 (0.058)**	0.002 (0.993)
P'_P	-0.335 (0.101)*	**-0.486 (0.014)****	**-0.477 (0.016)***	**-0.451 (0.024)***	**-0.475 (0.016)**[x]	**0.543 (0.005)***	-0.345 (0.092)
H'_{EP}	-0.337 (0.107)	**-0.450 (0.027)***	-0.277 (0.191)	-0.267 (0.207)	-0.246 (0.247)	0.351 (0.093)	-0.272 (0.198)

c28 - v270 = mean frequencies of these RAPD markers, GSC = mean genetic score, GCV = coefficient of variation for the genetic scores, P'_P = proportion of variable RAPD markers, H'_{EP} = genetic diversity index, SHD and SHV = number of stellate hairs per mm^2 on the dorsal and ventral leaf surfaces, respecyively, HHD and HHV = number of hirsute hairs per 3 mm^2 on the dorsal and ventral leaf surfaces, respectively, WLR = leaf width-to-length ratio, MSC = mean morphological score, MCV = coefficient of variation for the morphological scores.

[x] = the partial correlation with population distance along the transect held constant is not significant.
* = the partial correlation with population distance along the transect held constant is significant at $p < 0.05$.
** = the partial correlation with population distance along the transect held constant is significant at $p < 0.01$.

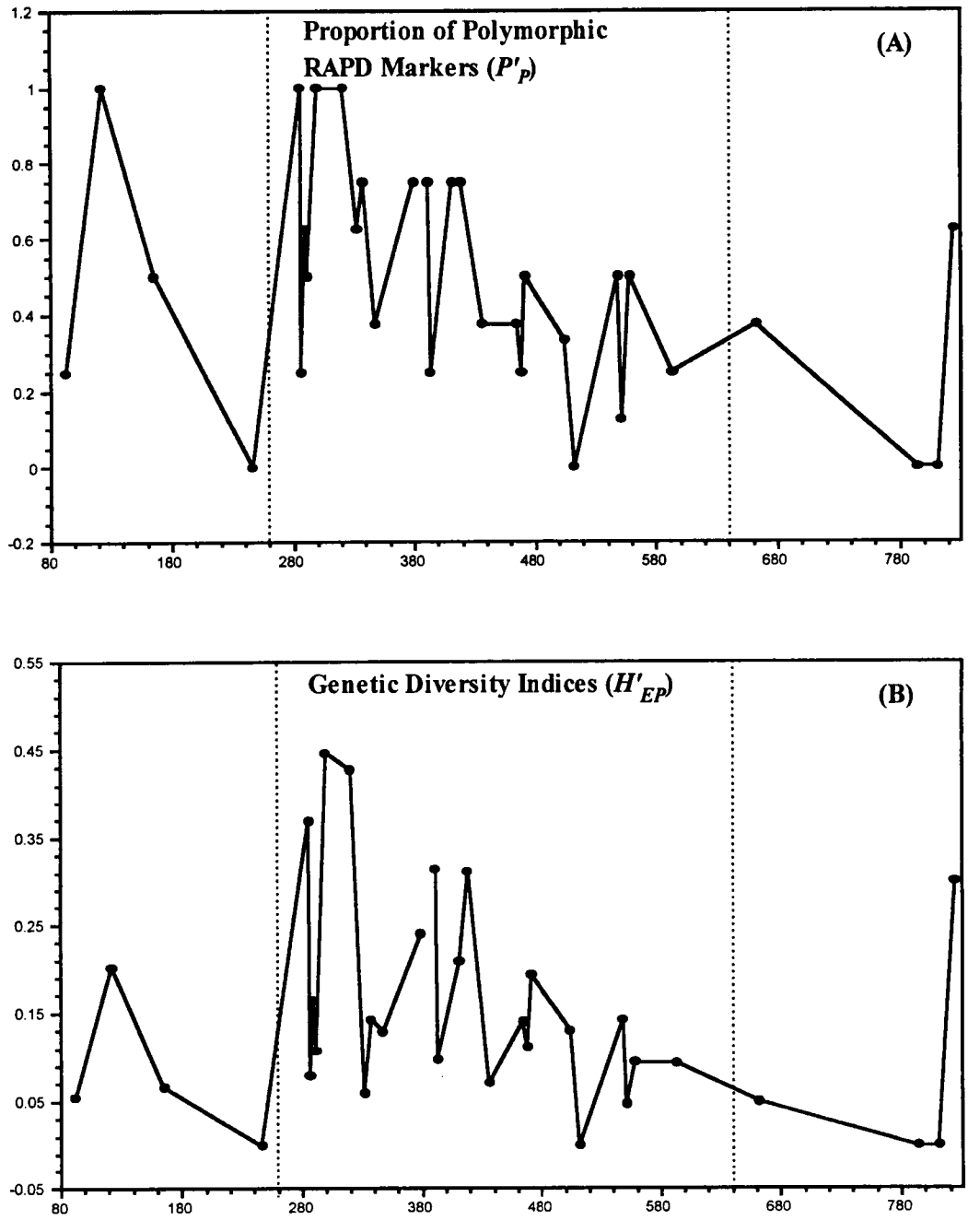

Figure 10. Proportions of variable RAPD markers and genetic diversity indices for all populations. The x-axes are population distances (km) along the transect from south to north Florida (Fig. 1). Dotted lines delineate the hybrid zone from allopatric parental populations. The most northern population (FL11) is also hybrid.

correlated with all of the morphological characters (significantly only with dorsal and ventral stellate hairs, SHD and SHV : Table 10) and positively correlated with the mean morphological scores (MSC), indicating that hybrid plants morphologically similar to *P. viridis* are found in populations with relatively high genetic variances.

The bootstrap estimates of the proportion of variable RAPD markers (P_P') were negatively correlated with the indumentum characters (SHD, SHV, HHD, and HHV : Table 10) and positively correlated with the mean morphological score (MSC :Table 10) indicating that plants in the hybrid zone with a greater proportion of variable RAPD markers are morphologically more similar to *P. viridis*. The same pattern resulted from the correlations between the bootstrap estimates of genetic diversities (H_{EP}') and morphological characters (although only the correlation between H_{EP}' and the number of stellate hairs on the ventral leaf surface, SHV, was significant : Table 10). This pattern suggests that plants with the greatest amount of genetic diversity are also morphologically similar to *P. viridis*.

The genetic markers displayed varying levels of association with each other (Table 11). Marker c28 was not associated with any of the other markers, probably because it occurred at a relatively high frequency over most of the hybrid zone. The other *P. caroliniana* marker (c59) was negatively associated with all of the *P. viridis* markers (but not significantly with v164). All of the *P. viridis* markers displayed positive associations with each other across populations in the hybrid zone (Table 11; Fig. 9).

Table 11. Disequilibria values among genetic (RAPD) markers for pooled hybrid populations. Phi coefficients are given in the upper right; chi-square likelihood probabilities in the lower left.

	c28	c59	v59	v61	v164	v270
c28		-0.082	0.064	-0.013	0.074	-0.047
c59	0.099		**-0.317**	**-0.106**	-0.085	**-0.180**
v59	0.216	**0.001**		**0.396**	**0.616**	**0.761**
v61	0.799	**0.033**	**0.001**		**0.220**	**0.184**
v164	0.150	0.095	**0.001**	**0.001**		**0.613**
v270	0.355	**0.001**	**0.001**	**0.001**	**0.001**	

Chapter IV

Discussion

These data indicate that there is a broad hybrid zone between *P. caroliniana* and *P. viridis* in central Florida. Plants with a mixture of genetic markers diagnostic for the parental taxa were found over a distance of approximately 300 km encompassing different climatic zones and environments. Moreover, the zone of hybridization appears to have advanced northward in recent history. Patterns of genetic diversity, proportions of polymorphic loci, the distribution of morphological and genetic markers among hybrid populations, and the variances in the frequencies of these markers within hybrid populations are consistent with the hypothesis that southern hybrid populations are older than hybrid populations in the north. In addition, it appears that dispersal and drift have influenced the distribution of *P. viridis* alleles, with abrupt frequency reversals occurring among adjacent populations in the northern portion of the hybrid zone. There is also evidence that environmental selection has influenced the distribution of hybrid genotypes, as suggested by the presence of correlations between morphological traits and soil characteristics.

An important assumption of this study is that secondary contact has occurred between the parental taxa resulting in the formation of the hybrid zone. Alternatively, it is possible that the two taxa have been continuously connected by a series of populations distributed along an environmental gradient (Endler 1977). Although it is difficult to distinguish secondary contact from primary intergradation (Harrison 1993), the patterns found here are more consistent with those expected from hybridization between

previously-isolated taxa. If this were a region of primary intergradation, I would expect the neutral marker clines to be concordant and display a smooth transition between the two parental taxa. With relatively high dispersal rates in a zone of primary integradation, I would also expect stronger associations between the morphological and genetic markers. This would be reflected by the concordance of morphological and genetic marker clines, which is not the case here. Although it is still possible that these taxa were never isolated, the distribution of genetic and morphological markers are more consistent with patterns expected from hybridization between previously-isolated taxa.

The mating system between *P. caroliniana* and *P. viridis* does not appear to play a significant role in the evolutionary dynamics of this hybrid zone. Experimental evidence indicates that their reproductive isolation barrier is weak (Ornduff 1970; Wang and Cruzan 1997). In reciprocal crosses, *P. viridis* has a higher F_1 seed set than *P. caroliniana*, suggesting that the latter taxon maintains the stronger reproductive barrier (Wang and Cruzan 1997). This mating system alone promotes stronger gene dispersal into hybrid populations from *P. caroliniana* parental populations, which, over time, would cause hybrid populations to resemble *P. caroliniana* more than *P. viridis*. In this analysis though, I did not find any morphological or genetic bias in hybrid populations being more similar to *P. caroliniana*. In fact, the two most northern hybrid populations (other than FL11) were genetically more similar to *P. viridis* than to *P. caroliniana*. This is particularly interesting because they are located an average of 557 km from the nearest parental *P. viridis* population but only 141 km from the nearest parental *P. caroliniana* population.

It appears that most of the hybrid populations are composed of advanced-generation hybrids rather than F_1 genotypes. F_1 progeny obtained in experimental crosses have stellate hairs but no hirsute hairs and should possess all of the diagnostic markers from both of the taxa. With the exception of southern populations up to 332 km north of the transect origin (including population P20 at 332 km), hybrid populations were composed of individuals with both stellate and hirsute hairs, and all of the hybrid populations consisted of individuals having mixtures of genetic markers from the two parental taxa. Although most individuals in the southern populations resembled experimental F_1 hybrids in having only stellate hairs, the majority of individual genotypes were not those expected for F_1 individuals. One exception to this pattern was population P19, in which most individuals did possess all of the diagnostic genetic markers. However, there are no known parental populations in close proximity to P19, so these are most likely advanced generation hybrids that have most of the diagnostic genetic markers by chance.

Several lines of evidence support the hypothesis that this hybrid zone has advanced northward. First, populations in the southern end of the hybrid zone appear older than those in the north because they have less variation in their morphological and genetic scores. When two differentiated populations first come into contact, it is expected that there will be an initial increase in the amount of genetic and morphological variation within hybrid populations due to a mixing of two distinct genomes. Over time though, this variation is expected to decrease as a result of drift and selection. Since there is an increase in both the genetic and morphological variation within populations

moving northward in this hybrid zone, it appears that the youngest hybrid populations are those in the north.

More evidence for hybrid zone movement emerges from analyses that provide information about the historical distribution of the parental taxa and the roles dispersal and drift may have played in this system. Looking at the genetic markers, the presence of both of the *P. caroliniana* markers at some frequency in all of the hybrid populations, coupled with the lack of some *P. viridis* markers in many populations, suggests that *P. caroliniana* has historically been present longer than *P. viridis* in the area of hybridization. In phylogeographic analyses, more cpDNA haplotypes have been found for *P. caroliniana* than *P. viridis*, which suggests that *P. caroliniana* has been present in the United States longer and had more time to diversify than *P. viridis* (S.D. Maskas pers. comm.). In addition, *P. caroliniana* haplotypes have been found in populations as far south as the southern end of the Lake Wales Ridge in Highlands County, indicating *P. caroliniana* was present in this region prior to formation of the hybrid zone (M. B. Cruzan pers. comm.) The precedence of *P. caroliniana* over *P. viridis* in the area of hybridization is also supported by information regarding Florida's biogeography. During the last glacial interval (Wisconsinan) of the Pleistocene, pollen records indicate that Florida was much more arid than it is presently, and most of the region was dominated by xeric vegetation including extensive scrub and sandhill communities (Webb 1990). This vegetation pattern is consistent with the hypothesis that *P. caroliniana* was more abundant further south than it is today. *P. viridis*, if present at all, was probably restricted to isolated wet areas. More mesic vegetation appeared in the pollen record

during the mid-Holocene approximately 5,000 years ago, at which time the Everglades formed and Florida experienced an influx of tropical vegetation (Webb 1990). During this period, more habitats suitable for *P. viridis* were probably established, allowing it to disperse northward.

The northward dispersal of *P. viridis* is also supported by the concordance among *P. viridis* genetic markers across all of the hybrid populations, as indicated by the correlation among allele frequencies (unpubl. data) and the strong positive associations among these markers in the hybrid zone. Furthermore, the high variation in frequency of all of the *P. viridis* genetic markers in hybrid populations, especially where they move from fixation for the dominant state to fixation for the null allele in adjacent populations, may be indicative of historical dispersal of *P. viridis* alleles northward, followed by the influence of drift in small populations. Similar patterns in both the concordance among markers and allele frequency reversals were found for genetic markers in a fire ant hybrid zone that has progressed northward within the last half century (Shoemaker et al. 1996). The loss of *P. viridis* alleles in the northern portion of the hybrid zone in Florida has resulted in a pattern of decreasing genetic diversity in northern hybrid populations.

Additional information about the historical distributions and dispersal patterns of *P. viridis* and *P. caroliniana* may be discerned from analyses of their habitat preferences. Greenhouse experiments show that *P. viridis* is tolerant to calcium-rich soils such as those in south Florida as well as more acidic, quartz sands similar to those in north Florida. In a pilot experiment I grew cuttings from parental and F_1 hybrid genotypes in two calcium/pH soil treatments (pure limestone sand pH vs. pure quartz sand) and found

evidence for higher calcium tolerance for *P. viridis* (31% survival in limestone sand, N = 38) and F_1 genotypes (18% survival, N = 38) than for *P. caroliniana* (7% survival, N = 27). Survival in quartz sand was higher for all three genotypes (56%, 53%, and 41% survival, respectively: unpublished data). Thus, it appears that while *P. caroliniana* is limited in the soil habitats it can occupy, *P. viridis* is capable colonizing a wider range of soils, which is consistent with the hypothesis that it is dispersing northward into central Florida.

The genetic composition of population FL11, which is the most northern of all the populations, is both interesting and enigmatic. It was treated as a hybrid population due to the presence of 75% of the *P. viridis* genetic markers, but its geographic position is well within the range of allopatric *P. caroliniana* populations. It is possible that the relatively high frequency of *P. viridis* markers is a consequence of a small sample size (N = 5) or may be the result of a chance long distance dispersal event.

While patterns of morphological and genetic variation suggest this hybrid zone has moved in recent history, it is less clear what processes are responsible for the northward introgression of *P. viridis* alleles into populations of *P. caroliniana*. One possibility is that this hybrid zone represents a tension zone (Key 1968; Barton and Hewitt 1985). Although this system appears to be driven by dispersal, which is an essential component of tension zones, there are other aspects of this hybrid zone that are not consistent with the tension zone model. According to this model, movement is driven by density gradients and will advance toward the less dense species (Barton and Hewitt 1985). In this study, *P. viridis* and hybrid populations in south Florida were not

obviously larger than *P. caroliniana* and hybrid populations in north Florida and south Georgia. More importantly, the tension zone model assumes reduced hybrid fitness and selection against hybrids. However, F_1 hybrids appear to be vigorous under greenhouse conditions (unpubl. data). Also, no particular hybrid class appears to be uniformly less represented in the distributions of genetic and morphological scores. Additional studies of hybrid fitness under greenhouse and field conditions will help determine whether conditions consistent with the tension zone model are present in this system.

Another force that may be driving introgression in this system is increased hybrid fitness in the environments found in central Florida. If hybrids were more fit in these areas, then environmental (extrinsic) selection would promote the displacement of parental genotypes by hybrids. Increased reproductive output by hybrids would then facilitate the dispersal of *P. viridis* alleles and introgression into *P. caroliniana* populations. A better understanding of parental and hybrid fitnesses in central Florida would be necessary to assess the relative roles of selection and dispersal in the dynamics of this hybrid zone.

Environmental selection does seem to have played a role in the distribution of hybrid populations and is likely responsible for the patterns of distribution of morphological characters among hybrid populations. In particular, I found associations between morphological characters and the soil principal component axes. This was in contrast to the lack of any significant correlations between the genetic markers and the soil properties. Furthermore, the two northern hybrid populations (P22 and P23) with individuals morphologically similar to *P. viridis* were associated with vegetation

characteristic of allopatric *P. viridis* populations in south Florida (pers. obs.). This pattern of morphological character reversals among hybrid populations is similar to that found by Shoemaker et al. (1996) and is expected for mosaic hybrid zones (Rand and Harrison 1989). These data suggest that environmental selection has played a significant role in determining the distribution of hybrid genotypes and has produced some characteristics expected under the mosaic hybrid zone model.

Although most of the genetic markers appear neutral to selection, c28 exhibits a sharp cline suggesting that it is influenced by environmental selection (Endler 1977). The frequency of this allele is consistently high across the northern portion of the hybrid zone and is not correlated with the frequency of the other *P. caroliniana* marker, c59 (unpubl. data). It is interesting that this cline is concordant with one described for two subspecies of slash pine occurring in the same vegetation communities as both *Piriqueta* taxa (Squillace 1966) and is also coincidental with the southern extent of longleaf pine (Myers 1990). The cline in c28 is occurs in the same region where there is a transition in vegetation type, as indicated by the replacement of longleaf and typical slash pine by south Florida slash pine along the southern end of the Lake Wales Ridge in Highlands County. These patterns of variation in vegetation types and the unique clinal pattern exhibited by c28 suggest that this marker may be associated with quantitative trait loci that are responding to environmental selection.

The level of morphological uniformity and the unique combination of parental characteristics at the southern end of the hybrid zone are similar to features indicative of homoploid hybrid species (Arnold 1993; Rieseberg et al. 1995), suggesting that these

populations may represent an intermediate stage in hybrid speciation. In particular, plants in these populations possess only stellate hairs, making them distinct from both parental taxa and the other hybrid populations. Furthermore, the variance in the morphological hybrid score is significantly lower for the southern compared to the northern hybrid populations ($F = 8.10$, $P < 0.01$, $N = 305$ and 92 for the northern and southern hybrid populations, respectively), indicating that individuals within these populations are morphologically uniform compared to those in the north. While these populations appear to be morphologically distinct, the establishment of geographic or reproductive isolation barriers would be required for hybrid speciation to occur.

Introgression has already occurred across the entire width of the hybrid zone as is evident from the presence and fixation of *P. viridis* alleles in northern hybrid populations, and moreover, from the fact that the most northern population, FL11 had enough *P. viridis* alleles to be treated as a hybrid population. The extent of introgression by *P. viridis* alleles into *P. caroliniana* populations is impressive given the limited seed dispersal and the small population sizes exhibited by these taxa. From the present data it is not clear whether the northern extent of introgression is limited by environmental selection or whether there has not been sufficient time for *P. viridis* alleles to disperse into northern populations. Examination of the distribution of maternally inherited markers and patterns of selection in this hybrid zone will provide insights into expected future patterns of introgression. It is possible that the two parental taxa may continue to fuse in the future, with environmental selection influencing the distribution of specific

morphological characters resulting in a mosaic of phenotypes associated with different habitats.

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Vita

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