Selbyana 27(2): 201–207. 2006.

GEOGRAPHICAL VARIATION IN THE WIDESPREAD TEMPERATE EPIPHYTE, *EPIDENDRUM MAGNOLIAE* MUHLENBERG (ORCHIDACEAE)

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ABSTRACT. *Epidendrum magnoliae* is a widespread epiphytic orchid that occurs in Mexico and the United States. We investigated patterns of genetic divergence among populations of *E. magnoliae* distributed from North Carolina to south-central Florida. We found that populations of *E. magnoliae* maintain a high level of genetic diversity, 89.5% of the total variation within the species, while genetic diversity among populations was only 10.5%. Extensive gene flow has occurred within and among the northern and southern sampled regions of *E. magnoliae*. However there was also a significant relationship between genetic and physical distance, indicating that gene flow is reduced between more distantly spaced populations. The three populations sampled at the extreme ends of the species distribution in the United States were less diverse than more centrally located populations, suggesting a reduced rate of immigration and an increase in genetic drift. It has recently been proposed that speciation in orchids is likely due to the rapid and combined effects of genetic drift and sporadic selection. Such a scenario is more likely to occur in peripheral populations like those of *E. magnoliae*.

Key words: epiphyte orchid genetics, peripheral populations

INTRODUCTION

Although genetic diversity has been examined in numerous orchid species (see reviews in Hamrick & Godt 1996, Forrest et al. 2004, Tremblay et al. 2005), a clear pattern of diversity within the family has yet to emerge. An initial survey suggested that while orchid polymorphism and genic diversity were similar to other herbaceous taxa, the distribution of this variation within and among populations was atypical (Hamrick & Godt 1996). Population differentiation was found to be exceptionally low in orchids (8.7%), perhaps due to the specialized pollination systems and wind-dispersed seeds that characterize the family (Hamrick & Godt 1996). This trend is consistent with that found across the flowering plants, in which outcrossing species and taxa with seeds dispersed by wind maintain significantly lower levels of population divergence than species with contrasting traits (Hamrick & Godt 1996). However, it has also been argued that gene flow, while high in some orchids, is typically much more restricted among wild orchid populations than in other plants (Sun & Wong 2001). Perhaps reduced gene flow among small, geographically isolated populations leads to reduced population variation in orchids, despite mechanisms that favor outcrossing (Sun & Wong 2001). More recent reviews have suggested that the amount of population differentiation varies greatly among orchids (Forrest et al. 2004, Tremblay et al. 2005), reflecting a great diversity of reproductive ecologies, geographic ranges, and population densities in this species-rich family (Forrest et al. 2004). Estimates of population genetic divergence range from a virtual absence of differentiation in some species to near complete population isolation in others (Forrest et al. 2004). Based upon a significantly greater number of species, the mean estimate of genetic diversity among orchid populations (18.7%) also appears slightly higher than initial estimates (Hamrick & Godt 1996, Forrest et al. 2004).

Approximately two-thirds of all orchids are epiphytic, however investigations of orchid population genetics have been highly biased towards terrestrial species. Of the nearly 80 species included in the two recent orchid reviews (Forrest et al. 2004, Tremblay et al. 2005), only two were epiphytic. The terrestrial and canopy habitats differ in important features that may influence genetic diversity, and the patterns of variation found in terrestrial orchids may not be representative of epiphytic taxa. For example, tiny wind-

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dispersed orchid seeds may travel farther when released from an elevated position within the canopy, possibly resulting in higher gene flow in epiphytes (Bush et al. 1999, Murren 2003). Additionally, a presumed benefit of the epiphytic habit is an enhanced capacity to attract pollinators (Dressler 1981, Trapnell et al. 2004), thereby increasing gene flow among canopy plants. Conversely, aspects of the epiphytic habit could also lead to reduced genetic diversity. Reproductive success is generally skewed in orchids due to resource limitation, thereby reducing effective population size and possibly increasing genetic drift (Tremblay et al. 2005). These effects may be amplified in canopy-dwellers, where water and nutrients may be more limiting than in the terrestrial habitat. Genetic diversity in epiphytic orchids may be fundamentally different than in their terrestrial relatives. Numerous studies of a range of epiphytic taxa are needed in order to generate an understanding of diversity in orchids. To that end, we have examined genetic diversity in the temperate epiphytic orchid *Epidendrum magnoliae.*

Epidendrum magnoliae is a widespread epiphyte that ranges from North Carolina to south-central Florida, westward along the gulf coast to Louisiana, and south into Mexico (Nuevo León, San Luis Potosi, Tamaulipas) (Correll 1950, Luer 1972, Flora of North America 1993). *E. magnoliae* has an unusually broad ecological amplitude relative to most epiphytic orchids and is the only epiphytic orchid found outside of Florida in the United States. Populations in the United States occur in a variety of wetlands, including those by lakes, rivers, and in swamp forests and in sub-tropical hammocks. Plants can occasionally be found in slightly drier habitats as well. *E. magnoliae* has therefore been described as a common species in Florida (Luer 1972) and in South Carolina (Porcher & Rayner 2001), with contiguous populations distributed along the coastal plain of Georgia as well. In a previous study (Bush et al. 1999) we examined local genetic diversity in *E. magnoliae* by sampling three closely spaced populations in North Carolina and South Carolina (all separated by less than 85 km). A moderate level of population divergence was detected (14.9%), within the range typically found in terrestrial orchids.

Here we report an investigation of genetic diversity in more widely spaced populations of *Epidendrum magnoliae,* examining patterns of genetic divergence over a broad geographical and ecological range. Additionally, we asked: 1) Is there a relationship between genetic distance and physical distance in *E. magnoliae*? 2) Is migration restricted among different geographical regions? 3) Is there a reduction in diversity at the extreme ends of the species distribution within the United States?

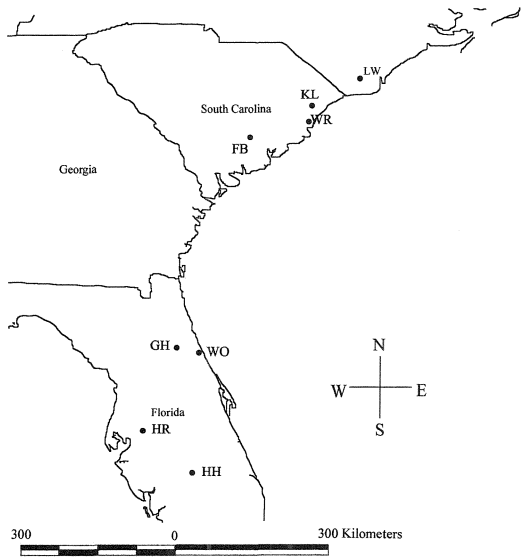

FIGURE 1. Locations of the eight *Epidendrum magnoliae* populations, sampled at Washington Oaks State Gardens (WO), Mike Roess Gold Head Branch State Park (GH), Highlands Hammock State Park (HH), and Hillsborough River State Park (HR) in Florida; Francis Beidler Forest (FB), Kingston Lake (KL), and on the Waccamaw River (WR), in South Carolina; and at Lake Waccamaw, North Carolina (LW).

We selected RAPD (random amplified polymorphic DNA) markers to examine diversity in *Epidendrum magnoliae.* RAPDs are dominant, selectively neutral, nuclear DNA markers. The majority of orchid population genetic studies have surveyed allozyme diversity. Recently, RAPD markers have also proven effective in the study of genetic variation in orchids (Bush et al. 1999, Wallace 2002). Generally, RAPDs detect higher levels of variation among populations and greater levels of polymorphism within orchid populations than do allozymes (Wong & Sun 1999, Sun & Wong 2001).

METHODS

Populations of *Epidendrum magnoliae* were sampled from a span of over 800 km, ranging from the northern to the southern limits of the species distribution, excluding Mexico. Four populations were sampled in Florida, located at Washington Oaks State Gardens (WO), Mike Roess Gold Head Branch State Park (GH), Highlands Hammock State Park (HH), and Hillsborough River State Park (HR) (FIGURE 1). One additional population was sampled in South Carolina at the Francis Beidler Forest (FB). The

data gathered from our previous study (Bush et al. 1999) of three northerly populations (located at Lake Waccamaw, North Carolina (LW); at Kingston Lake (KL) and on the Waccamaw River (WR) in South Carolina) was also included in this study. Twelve to sixteen plants were sampled from each population. No more than one plant was sampled per tree. The plants were accessed using a series of stackable pole saws that allowed us to reach plants located up to 15 m in the canopy. Approximately 1.0 g of leaf tissue was collected from each plant. Leaf tissue was placed in liquid nitrogen upon collection in the field, and was later stored at −80°C until DNA extraction.

The sampled populations were found in a variety of wetland habitats. Two populations (HH and HR) were located at the southern perimeter of the species distribution within the United States (Luer 1972). The HH population was situated within a sub tropical hammock, while the HR and GH populations were located on the banks of two moderately sized rivers. The WO population was positioned in a live oak grove found adjacent to a salt marsh. All of the populations in the northern region were closely associated with swampland. The FB population was located in a seasonally inundated swamp forest, and the KL and WR populations were positioned in the backwaters of the Waccamaw River. Finally, the LW sample was collected from the shores of Lake Waccamaw in North Carolina, which is the most northerly extant population of *Epidendrum magnoliae.* Lake Waccamaw is also at the headwaters of the Waccamaw River, which forms a corridor of *E. magnoliae* habitat from the LW population to the nearby KL and WR populations.

Laboratory Analysis

Complete details of DNA extraction and polymerase chain protocols are given elsewhere (Bush et al. 1999). Briefly, each plant was subjected to PCR analysis using five different random primers (OP-GO8, OP-T12, OP-P03, OP-X03, and OP-X17) (Operon, Alameda, CA). Two primers used in our previous study were not used in the present study due to concerns regarding the reproducibility of their amplification products. All PCR reactions were performed twice to ensure reproducibility. The PCR products were separated on 1.5% gels and photographed using a polaroid camera. The amplicons (DNA bands visible on the gel) were scored as either present or absent.

Statistical Analysis

The percentage of polymorphic loci and the band frequencies were calculated for each population. The band frequencies among all 120 individuals were also determined. An analysis of molecular variance (AMOVA version 1.55, Excoffier et al. 1992) was employed to generate Phi statistics, which are estimates of the distribution of genetic variation within and among populations. AMOVA was applied to a distance matrix of band sharing, calculated on a pairwise basis between all sampled individuals. In this study, a Euclidean distance matrix was used, with the Euclidean metric given by:

$$E = \{\varepsilon_{xy}^2\} = n\left[1 - \frac{2n_{xy}}{2n}\right]$$

where n is the number of bands, and n_{xy} is the number of shared bands. This metric is a direct total of the number of band differences between two individuals. Using AMOVA, 1000 permutations were then performed upon the distance matrix to partition genetic variation and to generate the significance of all of the variance components. AMOVA was utilized to assess population divergence in *Epidendrum magnoliae* in several ways. Diversity was first partitioned into within population and among population components, yielding an overall estimate of population divergence in the species, Phi_{ST}. Secondly, differentiation was also estimated between all population pairs. Finally, to estimate divergence among the northern (KL, LW, WR, and FB) and southern (WO, GH, HH, and HR) sampled regions of *E. magnoliae,* AMOVA was used to partition variation among regions, among populations within regions, and among individuals within populations. The relationship between physical and genetic distance was examined via a Mantel test. Nei's genetic distance (Nei 1973), an estimate of population genetic differentiation, was calculated for all population pairs using POPGENE (Yeh et al. 1999). The Mantel test was applied to the matrices of physical distance and Nei's genetic distance via TFPGA software (Tools for Population Genetic Analysis, Miller 1997), with the significance of the correlation assessed by 1000 permutations.

Results

The five random primers yielded nine polymorphic loci. The average proportion of loci fixed within a population (13.8%) was low. However, 33.3% of the loci were fixed in HH and in HR, while the fixation was much lower (7.4%) in the remaining populations. Overall,

TABLE 1. The band frequencies for nine loci within populations of *Epidendrum magnoliae*. The number of individuals sampled per population (*N*), the percent loci polymorphic within each population, and the band frequencies among all 120 individuals sampled are also reported.

	KL	WR	LW	FB	WO	GH	HH	HR	
N	18	18	16	12	16	12	13	15	
Locus									Mean
OP-T12 840	0.333	0.167	0.188	0.667	0.125	0.413	0.077	0.000	0.233
OP-G8 1600	0.278	0.389	0.625	0.250	0.313	0.250	0.000	0.000	0.275
OP-G8 1500	0.500	0.667	0.688	0.583	0.438	0.583	0.230	0.260	0.500
OP-G8 1300	0.889	0.778	0.750	0.083	0.062	0.083	0.077	0.930	0.908
OP-G8 620	0.556	0.500	0.875	0.830	0.875	0.500	0.384	0.600	0.641
OP-G8 350	0.056	0.000	0.188	0.250	0.000	0.083	0.000	0.200	0.092
OP-P3 720	0.167	0.111	0.125	0.417	0.250	0.333	0.077	0.060	0.183
OP-X3 560	0.778	0.944	0.938	0.667	0.625	0.750	0.230	0.730	0.725
OP-X17 580	0.278	0.167	0.063	0.000	0.025	0.000	0.000	0.000	0.117
% polymorphism	100.0	88.9	100	88.9	88.9	88.9	66.7	66.7	

the average percentage of polymorphic loci in the eight sampled populations of *Epidendrum magnoliae* was 86.3%, ranging from 100% in LW and KL to 67% polymorphic in HH and HR (TABLE 1). Therefore, populations differed primarily in band frequency.

Genetic diversity among the eight populations of *Epidendrum magnoliae* was low (Phi_{ST} = 0.105). Of the total variation detected, a highly significant AMOVA ($P < 0.001$) partitioned 89.5% within populations and only 10.5% among populations (TABLE 2). In the nested AMOVA analysis, the proportion of the variation due to differences among populations within the same region was 7.0%, while the among region component was 5.6%.

Populations were generally genetically distinct from one another, as 20 of the 28 pairwise Phi values were significant at the 5% level (TABLE 3). However, most of the significant differences involved the three populations located at the periphery of the species range in the United States. The HH and HR populations differed significantly from all of other populations (including each other), while Lake Waccamaw differed significantly from all but the Waccamaw River population. Among the remaining populations, only three of 10 comparisons yielded significant differences. There was however, a significant association between genetic distance and physical distance ($r = 0.4623$, $P < 0.002$).

DISCUSSION

RAPDs proved effective in uncovering variation in *Epidendrum magnoliae*. We extracted highly significant AMOVAs in partitioning variation within and among populations, as well as in estimating the differentiation between the northern and southern sampled regions. The ma-

TABLE 2. Analysis of molecular variance based upon eight populations of *Epidendrum magnoliae*. a) The estimated distribution of variation within and among the eight populations sampled. b) The estimated distribution of variation among groups, among populations within groups, and within populations.

a)

Source of variation	df	SSD	MSD	Variance component	% total	*P* value
Among populations	7	364.49	52.07	2.21	10.45	0.001
Within populations	112	2124.22	18.96	18.96	89.55	

b)

Source of variation	df	SSD	MSD	Variance component	% total	*P* value
Among groups	1	114.7	114.70	1.22	5.60	0.001
Among populations within groups	6	249.78	41.63	1.52	7.01	
Within populations	112	2124.22	18.96	18.86	87.39	

Note: Values reported are mean squared deviations (MSDs), sums of squared deviations (SSD), estimates of variance components, percent of total variance contributed by each component (% total), and the probability that the estimated component differs from zero due to chance.

TABLE 3. Pairwise Phi values between eight populations of *Epidendrum magnoliae*. The Phi values are indicated below the diagonal and significance of each value is located above the diagonal.

	KL	WR	LW	FB	WO	GH	HH	HR
KL	X	0.3417	0.0460	0.2138	0.2967	0.8042	0.0001	0.0001
WR	0.0024	X	0.1758	0.0001	0.0100	0.0829	0.0001	0.0500
LW	0.0520	0.0260	X	0.0001	0.0210	0.0240	0.0001	0.0001
FB	0.0260	0.1364	0.1120	X	0.0470	0.6513	0.0001	0.0001
WO	0.0145	0.1110	0.0927	0.0739	X	0.1169	0.0001	0.0460
GH	−0.0298	0.0382	0.0973	−0.0214	0.0411	X	0.0320	0.0001
HH	0.1451	0.2690	0.3457	0.2309	0.1395	0.1395	X	0.0310
HR	0.0656	0.1213	0.1908	0.1753	0.0795	0.0752	0.1071	X

jority of the pairwise estimates of population divergence were also significant (20 of 28). The eight non-significant estimates suggest a complete lack of genetic difference among the population pairs. However if we had used a greater number of markers, slight differences between populations may have been detected.

Populations of *Epidendrum magnoliae* maintain a high level of diversity, 89.5% of the total variation. There are also small, significant genetic differences among *E. magnoliae* populations. These differences were primarily due to the sampling of populations at the extreme ends of the species range within the United States, and populations separated by large distances. The degree of population divergence in *E. magnoliae* (0.105) is lower than the most recent mean estimate for orchids (0.187) (Forrest et al. 2004). In *Tolumnia variegata,* the only other epiphytic orchid studied on a geographical basis, population differentiation (G_{ST} = 0.11) was similar to that found here (Ackerman & Ward 1999). Two other epiphytic orchids, both studied by intensively sampling within closely associated patches, documented even lower levels of diversity among populations (Trapnell et al. 2004, Murren 2003). Clearly additional studies are needed before broad generalizations regarding the level of diversity in epiphytic orchids can be made. However, among the species studied to date, the initial trend is one of high gene flow and high within population diversity.

The low level of among-population genetic divergence suggests extensive gene flow within and among the sampled regions of *Epidendrum magnoliae.* Direct measures of pollen and seed movement are needed to precisely assess their relative contributions to gene flow. For example, a recent investigation tracked pollen mobility via molecular markers in a tropical epiphytic orchid. Pollen movement averaged hundreds of meters, including instances that exceeded 1 km (Trapnell & Hamrick 2005). The pollinators of *E. magnoliae* have not been identified, however our results are consistent with those of an outcrossing species. A ballistic seed dispersal model developed for epiphytic orchids predicted dispersal distances of 100 m to over 1 km, varying according to wind speed and the height of initial seed release from the canopy (Murren & Ellison 1998, Murren 2003). Canopy density likely influences dispersal as well, with thicker canopies reducing dispersal distances. In *E. magnolia,* dispersal distances are likely heterogeneous. Populations are frequently punctuated by canopy breaks, occurring at river and swamp edges and at sites of anthropogenic disturbances, from which dispersal distances could be considerable. Seed dispersal as well as pollen movement are likely important in migration within and among the sampled regions of *E. magnoliae.*

There is a strong relationship between genetic and physical distance in *Epidendrum magnoliae* (r = 0.4623), indicating that gene flow is restricted over greater, geographical distances. Populations separated by less than 100 km were often genetically indistinguishable, while more distantly spaced populations were usually significantly different. However, factors other than physical distance also influence the level of divergence among *E. magnoliae* populations. The pattern of diversity among the northernmost (LW) and the two southernmost United States populations (HH and HR) was quite different from that found in more centrally located populations. For the genetic distances calculated on a population by population basis, 17 of 18 comparisons involving the peripheral populations yielded significant differences. Of those comparisons involving the remaining populations, only three of 10 were significantly different. The estimates of genetic divergence also indicated that the northernmost and southernmost United States populations had diverged to a greater degree.

The reduction in diversity in the populations located at the extremes of the species range in the southeastern United States is not unexpected. While more centrally located populations can potentially receive immigrants from any direc-

tion, the populations at the edge of the range lack neighbors in one or more directions. The resulting reduction in gene flow should lead to a greater degree of divergence. The two southernmost studied populations of *Epidendrum magnoliae,* HH and HR, also show a reduction in diversity due to genetic drift. Within each population, 33.3% of the loci were fixed, while fixation was not common otherwise (7.4%). Furthermore, marginal populations are presumably subjected to more intense selection (Carson 1959, Eckert & Barrett 1993). Although most individual RAPD loci are selectively neutral, no technique is insensitive to the forces of selection. For example, immigrant propagules may be selected against when arriving in the northernmost and southernmost *E. magnoliae* populations. The resulting population differentiation is detectable via RAPD markers (Wallace 2002).

The ecological processes possibly associated with the great taxonomic diversification of the orchids have recently received a great deal of attention. It has been proposed that, in addition to natural selection, genetic drift has been a driving force in orchid speciation (Tremblay et al. 2005). Reproductive success is characteristically low in orchids, reducing effective population size and thus likely increasing genetic drift. Therefore, it has been proposed that orchid speciation is more likely due to the rapid and combined effects of genetic drift and sporadic selection, rather than to the gradual effects of selection alone (Tremblay et al. 2005). Such a scenario is more likely in peripheral populations similar to the *Epidendrum magnoliae* populations located at the extreme ends of the species range within the United States, where genetic diversity and gene flow are reduced.

Acknowledgments

We thank the Francis Beidler Forest, the North Carolina Division of Parks and Recreation, and the Florida Division of Recreation and Parks for allowing sampling within their boundaries. We also thank Zach Gurick, Karen Strawser, Jamie Davis, and Natali Amaradasa for assistance with the lab work, and Jim Luken, Eric Hágsater, and one anonymous reviewer for suggestions regarding this manuscript. This study was supported in part by a Coastal Carolina University Academic Enhancement Grant.

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