Selbyana 20(1): 120–124. 1999.

RAPD Variation in Temperate Populations of the Epiphytic Orchid *Epidendrum conopseum* and the Epiphytic Fern *Pleopeltis polypodioides*

Stephen P. Bush,* Wendy E. Kutz and Julie M. Anderton

Department of Biology, Coastal Carolina University, Conway, SC 29528 USA
E-mail: bush@coastal.edu

Abstract. Although 10% of all vascular plant species are epiphytic, few studies of genetic diversity in epiphytes have been completed. We used random amplified polymorphic DNA (RAPD) markers to investigate genetic diversity in temperate populations of the epiphytic orchid *Epidendrum conopseum* and in the epiphytic fern *Pleopeltis polypodioides* (resurrection fern). Three populations of each species were sampled in North Carolina and South Carolina, totaling 52 plants of *E. conopseum* and 20 specimens of *P. polypodioides.* Seven random primers produced 11 polymorphic RAPD markers in *E. conopseum,* while three primers yielded 14 variable markers in *P. polypodioides.* An analysis of molecular variation partitioned 85.1% of the variation within populations of *E. conopseum* and only 14.9% among populations. *Pleopeltis polypodioides* was found to maintain slightly greater population subdivision, with 76.4% of the total variation within populations and 23.6% among populations. The results suggest extensive gene flow among populations of both species, particularly in *E. conopseum.* The lack of population differentiation in these epiphytes may be associated with the effective wind dispersal of spores and seeds from the canopy habitat.

Key words: RAPDs, genetics, diversity, epiphytes, orchids

Introduction

Although the epiphytic habit encompasses a diversity of taxa, key epiphytic groups share reproductive characters associated significantly with genetic diversity. Self-compatibility is prevalent among epiphytes, particularly in tropical forests, where other life forms tend to be self-incompatible (Bush & Beach 1995). Pollinator limitation and potential resource limitation (Benzing 1990) may select for self-compatibility, since one of the benefits of self-compatibility is an increased probability of self-fertilization (Bawa 1974). The high frequency of self-compatibility suggests that inbreeding is possible among epiphytic taxa (Benzing 1990, Bush & Beach 1995, Hooper & Haufler 1997); however, self-compatibility may also be selectively neutral.

Epiphytes tend to have highly specialized plant-pollinator relationships, and geitonogamy may be avoided through pollinator foraging patterns and low daily flower production (Ackerman 1986). A great number of epiphytes also produce numerous, small, wind-dispersed seeds. Wind dispersal is significantly correlated with high levels of genetic diversity in terrestrial plants (Hamrick & Godt 1990), and propagules dispersed by canopy herbs may travel greater distances than the propagules of their terrestrial relatives (Akiyama 1994). The complex nature of epiphytic reproductive systems does not suggest a general pattern of genetic diversity among epiphytes. Interactions between mating system and migration, as well as other genetic determinants of epiphyte population structure, may produce diverse patterns of population genetics across a range of species.

Despite the species richness of the epiphytic habit, which comprises an estimated 10% of all vascular plant species (Madison 1977, Kress 1986), genetic diversity among epiphytes has received little attention. More than 2200 studies of allozyme variation in plant populations have been completed (Hamrick & Godt 1996), but relatively few have examined epiphytes. Epiphytic ferns and mosses have been found to maintain generally high levels of genetic variation (Ranker 1992, Akiyama 1994, Hooper & Haufler 1997), while levels of diversity among several species of Bromeliads range from moderate to complete monomorphism (Soltis et al. 1987). Although genetic variation has been examined in numerous terrestrial orchids, no population genetic data have been reported for epiphytic orchids.

This study examined molecular diversity in temperate populations of *Epidendrum conopseum* R. Br. (green-fly orchid) and *Pleopeltis polypodioides* (L.) E.G. Andrews & Windham (resurrection fern). *Epidendrum conopseum,* the most widespread epiphytic orchid in the southeastern United States, is the only epiphytic orchid occurring outside of Florida. *Epidendrum conopseum* ranges along the eastern coastal plain, reaching its northern limits in North Car-

* Corresponding author.

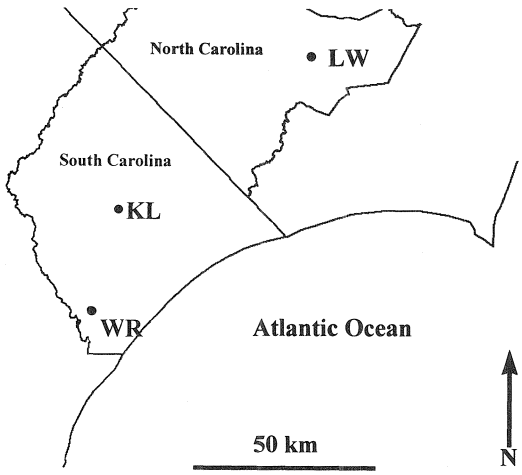

FIGURE 1. Locations of populations of *Epidendrum conopseum* and *Pleopeltis polypodioides* sampled at Kingston Lake (KL) and Waccamaw River (WR) in South Carolina and Lake Waccamaw (LW) in North Carolina.

olina; populations are found along the Gulf Coast and in Mexico as well (Correll 1950, Luer 1972). *Pleopeltis polypodioides* is distributed widely in the southeast and extends northward to Maryland and Ohio (Hamilton & Pryor 1994). The frost-tolerance of *E. conopseum* and *P. polypodioides* is atypical of epiphytes, which are primarily limited in distribution by frost (Benzing 1990). Given the potential advantages of self-fertilization to epiphytes, particularly in unfavorable conditions (Benzing 1990), we were interested in whether or not diversity in these temperate populations reflected a history of inbreeding. Diversity was assessed using random amplified polymorphic DNA (RAPD) markers. RAPDs are a useful technique for population genetic analyses, as a high number of variable DNA markers can be generated by a small number of primers.

MATERIALS AND METHODS

Three populations of *Epidendrum conopseum* and three of *Pleopeltis polypodioides* were sampled in the fall of 1998. For each species, populations were sampled in North Carolina on Lake Waccamaw and in Horry County, South Carolina, on the Waccamaw River and Kingston Lake (FIGURE 1). The number of individuals sampled per population is provided in TABLE 1. Only one plant per tree was sampled to avoid sampling more than one ramet of a genet. DNA was extracted by following the extractions protocol of Bernatzky and Tanksley (1986). For *P.*

TABLE 1. Sample sizes for populations of *Epidendrum conopseum* and *Pleopeltis polypodioides.*

Species Locality	No. of individuals
Pleopeltis polypodioides	
Kingston Lake (KL)	6
Waccamaw River (WR)	7
Lake Waccamaw (LW)	7
Epidendrum conopseum	
Kingston Lake (KL)	18
Waccamaw River (WR)	18
Lake Waccamaw (LW)	15

polypodioides, samples were purified further, using the NucleiClean kit (Sigma). Several plants of *E. conopseum* were screened with 180 random primers (all of the primers from Operon kits A, B, G, H, K, P, S, and X); and seven primers that demonstrated bright, reproducible polymorphisms were selected for the study. Similarly DNA samples of *P. polypodioides* were screened with 60 primers (Operon kits A,B, and P); and three that yielded reproducible polymorphisms were used for population analysis.

Prior to the polymerase chain reaction (PCR), the concentrations of all DNA samples were determined using a Hoefer DynaQuant fluorometer, and then each sample was diluted with sterile deionized water to 5.0 ng/μl. DNA amplification was performed in a Perkin Elmer 480 thermocycler. For *Pleopeltis polypodioides,* each 12.5 μl PCR reaction mixture contained 12.5 ng DNA, 6.3 μl H_20, 1.25 μl 10× buffer, 1.0 μl of 2.5 mM each dNTP, 1.0 μl of 20 mM $MgCl_2$, 0.5 μl of 10 μM primer, and 0.1 ul of 5 U/ul Taq polymerase (Promega). Reaction mixtures were overlaid with one drop of mineral oil. Cycling parameters were three cycles: 94°C, 1 min.; 35°C, 1 min.; 72°C, 2 min., followed by 42 cycles: 94°C, 5 sec.; 35°C, 1 min.; 72°C, 2 min. For *Epidendrum conopseum,* each 12.5 μl PCR reaction mixture contained 12.5 ng DNA, 5.93 μl H_2O, 1.25 μl 10× buffer, 1.0 μl of 2.5 mM each dNTP, 1.1 μl of 20 mM $MgCl_2$, 0.75 μl of 10 μM primer, and 0.12 ul of 5U/ul Taq polymerase (Promega). Reaction mixtures then were overlaid with one drop of mineral oil, and cycling parameters were as indicated above. Reaction products were separated on 1.5% gels and then stained with ethidium bromide.

Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was used to provide estimates of variance components from RAPD phenotypes. AMOVA was performed on a distance matrix generated by pairwise comparisons of all individuals to determine the number of marker differences. In the proposed study, a Eu-

TABLE 2. RAPD band frequencies in three populations of *Epidendrum conopseum.*

Amplicon	Population KL	WR	LW	Mean
OP-T12				
840 bp	0.333	0.167	0.188	0.229
OP-H1				
1700 bp	0.167	0.222	0.188	0.192
OP-P7				
780 bp	0.722	0.888	0.938	0.849
OP-P3				
720 bp	0.167	0.111	0.125	0.403
OP-G8				
1600 bp	0.278	0.388	0.625	0.430
1500 bp	0.500	0.666	0.688	0.618
1300 bp	0.889	0.778	0.750	0.806
620 bp	0.555	0.500	0.875	0.643
350 bp	0.056	0.000	0.188	0.081
OP-X3				
560 bp	0.778	0.944	0.938	0.887
OP-X17				
580 bp	0.278	0.167	0.063	0.169

clidean distance matrix was used, with the Euclidean metric given by:

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

where n is the number of markers, and n_{xy} is the number of shared markers. This metric is a direct total of the number of marker differences between two individuals. In all, 9000 permutations were performed, partitioning genetic variation at the levels of among individuals/within populations and among populations/within the species.

RESULTS

For *Epidendrum conopseum*, seven primers produced 11 markers polymorphic in at least one population (TABLE 2). With the exception of OP-G8 350, which was absent from the Waccamaw River population, all markers were polymorphic in all populations. AMOVA indicated highly significant genetic differences between populations ($P < 0.001$). The majority of the variation, however, was found within populations, as 85.1% of the total variation was due to differences between individuals within populations, and 14.9% was due to differences among populations (TABLE 3).

TABLE 4. RAPD band frequencies in three populations of *Pleopeltis polypodioides.*

Amplicon	Population KL	WR	LW	Mean
OP-P11				
970 bp	0.167	0.000	0.000	0.056
1050 bp	1.000	0.714	1.000	0.904
1190 bp	0.330	0.429	0.289	0.349
OP-A7				
710 bp	0.330	0.714	0.857	0.633
730 bp	0.330	0.000	0.000	0.110
890 bp	0.000	0.000	0.142	0.047
990 bp	0.000	0.142	0.000	0.147
1150 bp	0.167	0.142	0.000	0.100
OP-P4				
690 bp	0.000	0.229	0.142	0.123
780 bp	0.167	0.142	0.289	0.199
800 bp	0.000	0.142	0.142	0.094
1050 bp	0.167	0.429	0.000	0.198
1300 bp	0.830	0.429	0.714	0.657

For *Pleopeltis polypodioides,* 14 variable markers were produced by the three primers applied (TABLE 4). Nine of the markers were at fixation in at least one population, and four of these markers were found in only one population. Highly significant differences ($P < 0.002$) were detected among the three sampled populations. Of the total genetic variation present, 76.4% was due to differences between individuals within populations, while 23.6% was due to differences between individuals among populations (TABLE 5).

DISCUSSION

This is the first study of DNA variation in natural populations of epiphytes, and the first

TABLE 3. Analysis of molecular variance (AMOVA) based on three populations of *Epidendrum conopseum.*

Source of variation	df	SSD	MSD	Variance component	% total	*P*-value
Among populations	2	19.57	9.79	0.43	14.9	<0.0001
Within populations	42	119.02	2.43	2.43	85.1	<0.0001

Note: Values reported are mean squared deviations (MSDs), sums of squared deviation (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 5. Analysis of molecular variance (AMOVA) based on three populations of *Pleopeltis polypodioides.*

Source of variation	df	SSD	MSD	Variance component	% total	*P*-value
Among populations	2	15.42	7.71	0.78	23.6	<0.002
Within populations	17	42.88	2.52	2.52	76.4	<0.002

Note: see TABLE 3.

study of any kind to document variation in an epiphytic orchid. Although we sampled few populations and our sample sizes were small in *Pleopeltis polypodioides,* we extracted a highly significant AMOVA for both *P. polypodioides* and *Epidendrum conopseum.* The AMOVA results indicated moderate population divergence within each species; nevertheless, considerable diversity was present within populations, 85.1% of the total diversity within *E. conopseum* and 76.4% for *P. polypodioides.* These values are comparable to outcrossing species of buffalograss (*Buchloe dactyloides*), which ranged from 72.9% to 80.5% by region (Huff et al. 1993), and the arctic herb *Saxifraga oppositifolia* (86% to 64.8% by region) (Gabrielsen et al. 1997).

Epidendrum conopseum is not likely autogamous, as many plants of this species produce flowers that never bear fruit. The capacity for vectored self-fertilization in *E. conopseum* is unknown; and the pollinators, which are likely nocturnal, have not been observed. The results suggest, however, that geitonogamy, if possible in *E. conopseum,* is infrequent. The diversity maintained in the species suggests outcrossing with considerable gene flow between populations. The terrestrial Orchidaceae exhibit levels of genetic diversity similar to that found in other herbaceous families; however, orchids maintain unusually low levels of population differentiation. Tiny wind-dispersed seeds and highly specialized pollination systems may maintain gene flow among terrestrial orchid populations (Hamrick & Godt 1996). Similar mechanisms may operate in *E. conopseum* as well, and the elevated habit of the canopy may enhance seed dispersal distance.

Populations of temperate epiphytes may be transient in response to severe frosts; and within population diversity may be reduced because of the founder effects and genetic drift associated with frequent recolonization. *Epidendrum conopseum,* however, maintains diversity within temperate populations, and no decrease in diversity was detected at the northern limits of the species at Lake Waccamaw in North Carolina. Although *E. conopseum is* considered rare and listed as threatened in several states, Porcher (1995) maintains that the species is common in South Carolina, often concealed in the canopy. Our findings suggest that *E. conopseum* is neither rare nor colonizing in temperate regions; instead, sustained gene flow between established populations appears likely.

In six neotropical species of *Pleopeltis,* allozyme variation was high, with little differentiation among populations and no evidence of inbreeding (Hooper & Haufler 1997). *Pleopeltis polypodioides* also maintains within population variation; however, AMOVA suggests greater population divergence than among neotropical *Pleopeltis.* Estimates of population divergence based upon allozyme data (G_{st}) may not reflect inbreeding in ferns (Soltis & Soltis 1990), and the same may hold true for RAPD data. Laboratory and field tests, coupled with molecular data, are needed to determine the breeding system of *P. polypodioides.*

This paper reports genetic structure on a local scale, as have most of the investigations of epiphyte genetic structure. We currently are collecting and analyzing more distant populations of *Epidendrum conopseum* and *Pleopeltis polypodioides* to examine the geographical distribution of genetic variation. Future analysis also will include larger sample sizes for *P. polypodioides* to refine further the trends in population structure revealed by this analysis.

LITERATURE CITED

Ackerman, J.D. 1986. Coping with the epiphytic existence: pollination strategies. Selbyana 9: 52–60.

Akiyama, H. 1994. Allozyme variability within and among populations of the epiphytic moss *Leucodon* (Leucodontaceae: Musci). Amer. J. Bot. 81: 1280–1287.

Bawa, K.S. 1974. Breeding systems of a lowland tropical community. Evol. 28: 85–92.

Benzing, D.H. 1990. Vascular Epiphytes. Cambridge Univ. Press, Cambridge.

Bernatzky, R. and S.D. Tanksley. 1986. Genetics of actin-related sequences in tomato. Theor. Appl. Gen. 72: 314–321.

Bush, S.P. and J.H. Beach. 1995. Breeding systems of epiphytes in a tropical montane wet forest. Selbyana 16: 155–158.

Correll, D.S. 1950. Native Orchids of North American North of Mexico. Chronica Botanica, Waltham.

Excoffier, L., P.E. Smouse and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application

to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Gabrielsen, T.M., K. Bachmann, K., K.S. Jakobsen and C. Brochmann. 1997. Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia.* Mol. Ecol. 6: 831–842.

Hamilton, R.G. and J.S. Pryor. 1994. Size hierarchies in natural populations of *Polypodium polypodioides.* Amer. Fern Journal 84: 41–47.

Hamrick, J.L. and M.J.W. Godt. 1990. Allozyme diversity in plant species. Pp. 43–63 *in* A.H.D. Brown, M.T. Clegg, A.L. Kahler and B.S. Weir, eds. Plant Population Genetics, Breeding and Genetic Resources. Sinauer, Sunderland.

———. 1996. Effects of life history traits on genetic diversity in plant species. Phil. Trans. R. Soc. Lond. B 351: 1291–1298.

Hooper, E.A. and C.H. Haufler. 1997. Genetic diversity and breeding system in a group of neotropical epiphytic ferns (*Pleopeltis*; Polypodiaceae). Amer. J. Bot. 84: 1664–1674.

Huff, D.R., R. Peakall and P.E. Smouse. 1993. RAPD variation within and among populations of outcrossing buffalograss [*Buchloe dactyloides* (Nott.) Engelm.]. Theor. Appl. Genet. 86: 927–934.

Kress, W.J. 1986. The systematic distribution of vascular epiphytes: an update. Selbyana 9: 2–22.

Luer, C.C. 1972. The Native Orchids of Florida. New York Botanical Garden, New York.

Madison, M. 1977. Vascular epiphytes: their systematic occurrence and salient features. Selbyana 2: 1–13.

Porcher, R.D. 1995. Wildflowers of the Carolina Lowcountry. University of South Carolina Press, Columbia.

Ranker, T.A. 1992. Genetic diversity of endemic Hawaiian epiphytic ferns: implications for conservation. Selbyana 13: 131–137.

Soltis, D.E., A.J. Gilmartin, L. Rieseberg and S. Gardener. 1987. Genetic variation in the epiphytes *Tillandsia ionatha* and *T. recurvata* (Bromeliaceae). Amer. J. Bot. 74: 531–537.

Soltis, P.S. and D.E. Soltis. 1990. Genetic variation within and among populations of ferns. Amer. Fern Journal 80: 161–172.