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## Genetic structure in a population of a tropical tree *Ocotea tenera* (Lauraceae): influence of avian seed dispersal

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**Abstract** We studied the influence of avian seed dispersal on the structuring of genetic diversity in a population of a tropical tree, *Ocotea tenera* (Lauraceae). The seeds of *O. tenera* are principally dispersed by four, relatively specialized, fruit-eating bird species (emerald toucanets, keel-billed toucans, resplendent quetzals, and three-wattled bellbirds). We found high genetic diversity within the overall population and significant, nonrandom structuring of that diversity among subpopulations. Subpopulations contained members of several sibling groups, and most saplings within subpopulations were shown not to be the progeny of adult trees within the same subpopulation. Our data indicate that *O. tenera* subpopulations are founded with several seeds from few maternal families, and that this mode of establishment is an important determinant of population genetic architecture.

**Key words** Colonization · Frugivory · Genetic differentiation · *Ocotea* · Seed dispersal

### Introduction

Many tropical trees produce fruits which are consumed by birds (Howe and Smallwood 1981). Depending on their behavior, different bird species tend to remain in the fruiting tree or fly away to another tree to digest the fruits. Birds regurgitate or defecate the seeds undamaged, thereby serving as vectors for seed dispersal. Various ecological aspects of relationships between plants and avian frugivores have been investigated, such as frugivore diet composition (Howe and Estabrook 1977; Martin 1985), frugivore foraging behavior (Wheelwright 1991), and patterns of seed deposition (Tomback and

Linhart 1990; Horvitz and Le Corff 1993; Willson 1993). However, little detailed information exists concerning the population genetic consequences of avian seed dispersal in tropical forest tree populations. Seed dispersal syndromes in tropical forests vary in terms of characteristic patterns of seed collection and deposition, number of seeds moved, and distance of gene flow (Howe and Smallwood 1981). These characteristics of seed dispersal influence demographic phenomena and, thus, shape genetic attributes of plant populations (Hamrick and Lovell 1988, Howe 1989).

We studied the population genetic consequences of avian seed dispersal in *Ocotea tenera* (Lauraceae) to determine patterns of successful seed dispersal. *O. tenera* seeds are dispersed by emerald toucanets (*Aulacorhynchus prasinus*), keel-billed toucans (*Ramphastos sulfuratus*), resplendent quetzals (*Pharomachrus mocinno*), and three-wattled bellbirds (*Procnias tricarunculata*). Birds swallow one to three fruits from a tree and fly to another site to digest the fruit and regurgitate the seeds (Wheelwright 1991). This provides the potential for extensive gene flow throughout the forest. Yet, because *O. tenera* is a forest-gap colonist, successful seed dispersal may be patchy. If gaps are colonized with seeds of many maternal trees and there is high gene flow through migration, genetic diversity should be high in the total population and subpopulations, and genetic differentiation among subpopulations should be low. If gaps are colonized with seeds from few maternal trees and there is little migration, then total population and subpopulation genetic diversity should be low, but genetic differentiation should be high. We primarily focused on gene flow by seed dispersal since there is little gene flow via pollen (Gibson 1995).

### Materials and methods

The study population of *O. tenera* grows throughout the lower montane forest surrounding Monteverde, Costa Rica (10°18'N, 84°48'W, 1300–1500 m above sea level). The "natural" population of *O. tenera* described in this paper consists of all reproductively

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mature trees known in 1980 to occur within the 16-km<sup>2</sup> survey area of Wheelwright (1986), and saplings found in the immediate vicinity of mature individuals. Subpopulations exist as distinct clusters of trees in forest gaps and along forest edges next to cattle pastures (Fig. 1).

Two experimental plots, established in 1981 and 1984 by N.T.W. for studies of growth and reproduction of *O. tenera* were included in this study (Fig. 1) and are referred to as the "experimental" population. The methods used to establish experimental plots were devised before this genetic analysis was conceived; thus they provide a unique opportunity to compare genetic traits of natural subpopulations, whose establishment history is unknown, with artificial subpopulations, whose establishment history is known. The 1981 plot was established with seeds collected from ten maternal trees and grown in an abandoned banana patch. The 1984 plot was established with seeds collected from seven maternal trees and grown in a site similar to the 1981 plot. Five maternal lineages are common to both groups. Genetic parameters measured from experimental plots were compared with values from naturally occurring subpopulations to (1) estimate the number of parents that contributed to the establishment of a subpopulation, and (2) evaluate the degree of relatedness of individuals within natural subpopulations. Further details of experimental plots are presented in Wheelwright and Bruneau (1992).

Leaf samples were collected from 170 trees and saplings throughout the Monteverde site in February 1991 and transported on ice to the University of Colorado. Leaf tissue for each sample was crushed in liquid nitrogen with a mortar and pestle, and proteins extracted using a phosphate polyvinyl pyrrolidone buffer (Mitton et al. 1979). Protein extracts were absorbed onto filter paper wicks, placed in 96-well microtitre plates, and stored in a -60°C ultracold freezer.

Samples were electrophoresed on 11% starch gels using four gel/electrode buffer systems (Soltis et al. 1983). A total of 18 putative loci in ten isozyme systems were resolved from field samples (Soltis et al. 1983; Gibson 1995): acid phosphatase (*AcpH*),

diaphorase (*Dia1*, *Dia2*, *Dia3*), fluorescent esterase (*Fe1*, *Fe2*), glutamate dehydrogenase (*Gdh*), glutamate oxaloacetate transaminase (*Got*), menadione nitrate reductase (*Mnr*), malate dehydrogenase (*Mdh1*, *Mdh2*), peroxidase (*Per1*, *Per2*, *Per3*), uridine diphosphoglucose pyrophosphorylase (*Udp*) and 6-phosphoglucose dehydrogenase (*6Pgdh1*, *6Pgdh2*, *6Pgdh3*). Different loci for the same enzyme were numbered sequentially, with the most anodally migrating locus being given the lowest number. The same procedure was used to designate alleles at individual loci. For example, allele 2 at the second Fe locus was designated by *Fe2-2*.

We calculated statistics of genetic diversity and structure for the natural population and six subpopulations. The same values were also calculated for the two experimental plots. Five standard measures of genetic diversity were calculated for the entire population and each subpopulation: percentage of polymorphic loci (%P), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), average number of alleles per locus ( $A$ ), and effective number of alleles ( $A_e$ ). Wright's fixation index ( $F$ ) was calculated for each locus in each subpopulation to measure deviations in genotype frequencies from Hardy-Weinberg expectations.

Total genetic diversity ( $H_T$ ) for the natural population was calculated and decomposed into diversity within ( $H_S$ ) and among ( $D_{ST}$ ) subpopulations (Nei 1977). Mean values were obtained for each locus and averaged across all loci. Distribution of genetic diversity within and among subpopulations was evaluated through Wright's  $F$  statistics,  $F_{IT}$  and  $F_{IS}$  (Wright 1965), and Nei's  $G_{ST}$  (Nei 1977).  $F_{IT}$  and  $F_{IS}$  represent deviations of genotype frequencies from Hardy-Weinberg expectations over all subpopulations and within subpopulations, respectively. Positive  $F_s$  indicate excess homozygosity.  $G_{ST}$  measures the proportion of the total genetic diversity partitioned among subpopulations, and, therefore, indicates genetic differentiation among subpopulations.  $F_{IT}$ ,  $F_{IS}$ , and  $G_{ST}$  were calculated for each polymorphic locus and averaged across all polymorphic loci.

Statistical significance of deviations of  $F_{IT}$  and  $F_{IS}$  from zero was tested with a  $\chi^2$ -statistic:

$$\chi^2 = F^2 N(a-1), df = a(a-1)/2$$

where  $N$  is the total sample size and  $a$  is the number of alleles (Li and Horvitz 1953). Likewise, a  $\chi^2$  was calculated for the  $G_{ST}$  values to detect significant differences in allele frequencies among subpopulations for each locus:

$$\chi^2 = 2NG_{ST}(a-1), df = (a-1)(n-1)$$

where  $N$  is the total sample size,  $a$  is the number of alleles at a locus, and  $n$  is the number of subpopulations (Workman and Niswander 1970).

Gene flow among subpopulations,  $Nm$ , was indirectly estimated using  $G_{ST}$  (Slatkin and Barton 1989) where:

$$Nm = (1 - G_{ST}) / 4G_{ST}$$

Genetic identity ( $I$ ) (Nei 1972) was calculated for all pairs of subpopulations. The relatedness coefficient of Queller and Goodnight (1989) ( $R$ ) was calculated for each subpopulation to test whether related seeds were dispersed together.  $R$  can range from 0.0 in a population of unrelated individuals to 0.5 in a population of full siblings.  $R$  was also calculated for different cohorts (individuals of the same size/age class) within subpopulations and compared among cohorts.

## Results

After transport, 165 of the 170 leaf samples still contained viable enzymes. Of these, 112 samples were from naturally occurring trees, and 53 were from trees in the experimental plots (Table 1).

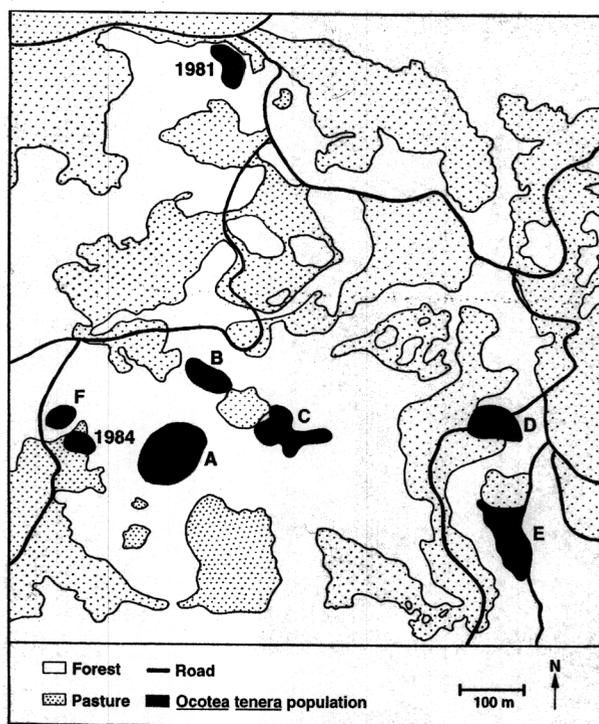


Fig. 1 Locations of six "natural" subpopulations (A-F) and two experimental plots (1981 and 1984) of *Ocotia tenera* at Monteverde, Costa Rica

Genetic diversity of natural subpopulations

Of the 18 resolved loci 8 (44%) were polymorphic (Table 1). Three loci (*Fe2*, *Mdh2*, and *Mnr*) expressed two alleles, three loci (*Fe1*, *Per2*, and *Per3*) expressed three alleles, and two loci (*Gdh* and *Per1*) expressed four alleles. This yielded a mean of 1.83 alleles per locus and 2.88 alleles per polymorphic locus. The eight loci were polymorphic in all subpopulations, except for *Mdh2* in subpopulation F which was fixed for *Mdh2-2*. Mean effective number of alleles per locus (the number of alleles in equal frequency that would give the observed level of heterozygosity at Hardy-Weinberg equilibrium) was 1.47. No allele was consistently in the highest frequency in all subpopulations. Expected heterozygosity averaged across subpopulations was 0.205 (Table 1). The highest expected heterozygosity was estimated in subpopulation B (0.226), and the lowest in F (0.182).

Mean observed heterozygosity (0.174) and expected heterozygosity (0.205) were not significantly different from one another in the natural population (Table 1). Observed heterozygosity was lower than but not significantly different from expected in all subpopulations.  $\chi^2$  analyses of Wright's fixation index (*F*) for each polymorphic locus in each subpopulation showed significant deviations above 0.0 in 10 of 48 instances. Significant positive *F* values were found for *Per2* in subpopulations D and E; *Per3* in subpopulations A, B, C, and D; *Mdh2* in B and C; and *Gdh* in A and C.

Genetic structure of natural subpopulations

The mean  $F_{IT}$  (0.275) was significantly different from 0, suggesting an overall heterozygote deficiency if the natural population is considered a single, panmictic unit. Significant positive  $F_{IT}$  values were estimated for *Gdh*, *Mdh2*, *Mnr*, *Per2*, and *Per3* (Table 2). The mean  $F_{IS}$  value (0.167) was not significantly different from 0, which suggests that most loci within subpopulations are in Har-

**Table 1** Levels of allozyme diversity calculated across 18 loci for six NATURAL subpopulations and two experimental subpopulations of *Ocotea tenera*

SUBPOP	N	H <sub>o</sub>	(sd)	H <sub>e</sub>	(sd)	%P	A <sub>e</sub>
A	26	0.160	0.065	0.185	0.063	44.44	1.44
B	22	0.169	0.067	0.226	0.067	44.44	1.56
C	27	0.172	0.058	0.216	0.066	44.44	1.50
D	14	0.196	0.080	0.203	0.062	44.44	1.47
E	12	0.209	0.091	0.218	0.063	44.44	1.47
F	11	0.141	0.085	0.182	0.063	35.29	1.40
mean	12	0.174	0.031	0.205	0.026	42.92	1.47
1981	28	0.186	0.059	0.228	0.070	44.44	1.59
1984	25	0.175	0.065	0.221	0.065	44.44	1.49
mean	53	0.180	0.044	0.225	0.048	44.44	1.54

N, number of individuals; H<sub>o</sub>, observed heterozygosity; H<sub>e</sub>, expected heterozygosity; %P, percentage polymorphic loci; A<sub>e</sub>, effective number of alleles

**Table 2** Estimates of genetic structure calculated across eight polymorphic loci for NATURAL subpopulations

LOCUS	H <sub>T</sub>	H <sub>S</sub>	F <sub>IT</sub>	F <sub>IS</sub>	G <sub>ST</sub>
Fe1	0.655	0.610	0.057	-0.014	0.082**
Fe2	0.467	0.387	-0.048	-0.185	0.135**
Gdh	0.621	0.551	0.458**	0.390**	0.134**
Mdh2	0.479	0.405	0.668**	0.607**	0.180**
Mnr	0.480	0.357	0.242**	-0.018	0.292**
Per1	0.737	0.629	0.193	0.058	0.167**
Per2	0.457	0.418	0.204*	0.131	0.100**
Per3	0.662	0.604	0.422**	0.367**	0.104**
mean	0.565	0.495	0.275**	0.167	0.128**

H<sub>T</sub>, total genetic diversity; H<sub>S</sub>, genetic diversity within subpopulations; F<sub>IT</sub> total population fixation index; F<sub>IS</sub>, subpopulation fixation index; G<sub>ST</sub>, proportion of total diversity among subpopulations

( $\chi^2$  test: \* p<0.05, \*\* p<0.001)

**Table 3** Relatedness coefficient (R) estimates for NATURAL and experimental subpopulations

SUBPOPULATION	R
A	0.225
B	0.091
	0.014
D	0.172
E	0.185
F	0.265
mean	0.179
1981	0.007
1984	0.129

dy-Weinberg equilibrium (Table 2).  $\chi^2$  analysis of  $F_{IS}$  values for individual loci, however, showed significant deviation from zero at *Gdh*, *Mdh2* and *Per3*.

Large differences in allele frequencies were detected among subpopulations for all polymorphic loci. Genetic differentiation among subpopulations was estimated at 0.031, which yielded a mean  $G_{ST}$ -value of 0.128 (Table 2).  $\chi^2$  tests of  $G_{ST}$  for the population and individual loci indicated significant genetic differences among subpopulations for all polymorphic loci, but approximately 87% of the population's genetic variation is common to all subpopulations. Gene flow was estimated at  $Nm=1.73$  migrants per generation.

Genetic identity and relatedness

Mean genetic identity of subpopulations was  $I=0.925$ . The two most similar subpopulations, B and C ( $I=0.960$ ), are in close proximity to one another. Subpopulations separated by similar distances, however, did not show correspondingly high genetic identity (e.g., D and E,  $I=0.902$ ) The most dissimilar subpopulations, A and E ( $I=0.871$ ), are isolated from one another by extensive forest and are composed of trees which from their sizes appear to be of different ages.

Over the natural population, *R* was estimated at 0.179 (Table 3). Individual subpopulation relatedness values ranged from  $R=0.091$  in subpopulation B to  $R=0.265$  in

**Table 4** Relatedness coefficients (R) and number of individuals (N) for adult and sapling cohorts in three NATURAL subpopulations

SUBPOPULATION	COHORT	N	R
B	sapling	16	0.060
	adult	6	-0.090
C	sapling	21	0.147
	adult	6	0.145
	sapling	7	0.213
	adult	4	-0.157

subpopulation F. It is interesting to note that subpopulations B and F also showed the highest and lowest genetic diversity values, respectively (Table 1).

In subpopulations B, C, and F, there were reproductively mature

trees and saplings underneath them. In B there were 3 females and 16 saplings, in C, there were 2 potential female trees and 21 saplings, and in F there were 3 females and 7 saplings (directly beneath one female). Fruit production of trees at these sites has been documented for the past 14 years, which would encompass the lifespan of the saplings. Thus, we knew which individuals in these subpopulations could potentially have produced the saplings. We compared the multi-locus genotypes of the female trees to the saplings to investigate potential maternity. In subpopulation C it was possible to show that 15 of the 21 saplings had genotypes which could not have been produced by the trees in that subpopulation. Likewise, in subpopulation B, 12 of the 16 saplings were found to not be the progeny of the adult trees, and in subpopulation F, five saplings directly beneath one of the females were not her progeny.

Because there was a strong indication that groups of seeds from another locality were deposited in these three subpopulations, we divided each subpopulation into two cohort groups (adults and saplings) and calculated relatedness among individuals in the same cohort at each site (Table 4). In subpopulation C, saplings and adults showed similar levels of relatedness. In B, there was little relatedness among saplings and even less among adults. In F, there was high relatedness among saplings, but little among adults.

### Genetic analyses of experimental subpopulations

The same eight loci were polymorphic in experimental plots, and no new alleles were found at these loci in experimental plots. Significant differences in allele frequencies between the two experimental plots were found for *Fe1*, *Gdh*, and *Per1*. Differences in allele frequencies between natural and experimental trees were found for *Gdh*, *Fe1*, and *Fe2*. Mean observed and expected heterozygosity did not significantly differ in either plot (Table 1). Significant positive fixation index values were calculated for two loci (*Gdh* and *Per3*) in the 1981 plot

**Table 5** Estimates of genetic diversity and structure from reviews and studies of woody, tropical species

SPECIES	%P	H <sub>e</sub>	G <sub>ST</sub> (F <sub>ST</sub> )
Tropical trees <sup>a</sup>	49.0		
Animal dispersed tropical trees <sup>a</sup>	49.4		
Tropical woody spp. <sup>b</sup>	39.8		
Tropical woody spp. with animal ingested seeds <sup>b</sup>	60.3	0.208	0.051
<i>Ocotea tenera</i>	44.4	0.205	0.128
<i>Ocotea skutchii</i> <sup>c</sup>	11.1	0.039	ND
<i>Schwartzia simplex</i> <sup>c d</sup>	48.4	0.209	0.037
<i>Brosimum alicastrum</i> <sup>c d</sup>	28.6	0.125	0.051

%P, percentage of polymorphic loci; H<sub>e</sub>, expected heterozygosity; G<sub>ST</sub> (F<sub>ST</sub>), genetic differentiation among populations.

(<sup>a</sup> Loveless, 1992; <sup>b</sup> Hamrick et al. 1992; <sup>c</sup> Hamrick and Loveless 1986; <sup>d</sup> Hamrick et al. 1993; ND data not available)

and one locus in the 1984 plot (*Per2*). Relatedness was estimated at R=0.007 and R=0.129 in the 1981 and 1984 plots, respectively (Table 3).

### Discussion

There is high genetic diversity in the *O. tenera* population at Monteverde, which is consistent with trends observed in woody taxa (Hamrick and Godt 1990). *Ocotea skutchii* was the subject of a limited isozyme analysis (Hamrick and Loveless 1986), and showed much less genetic diversity than we found in *O. tenera* (Table 5). Comparison of *O. tenera* values to those presented in reviews of allozyme diversity in woody taxa in general (Hamrick et al. 1992) and tropical woody taxa in particular (Hamrick and Loveless 1986; Loveless 1992) shows that *O. tenera* possesses typical population genetic diversity for a tropical tree (Table 5).

There is also significant spatial genetic structure in this *O. tenera* population. Although our G<sub>ST</sub> estimate was higher than previous estimates in tropical trees with animal seed dispersal, it is similar to mean values for tropical trees (Table 5). Allele frequencies were different for all polymorphic loci among subpopulations, but genotype frequencies tended to be near Hardy-Weinberg proportions or show a slight heterozygote deficiency within subpopulations. We suspect the population level heterozygote deficiency detected at some loci is due to a Wahlund effect, whereby significant differences in allele frequencies among subpopulations give the "statistical appearance" of a heterozygote deficiency in the entire population (Hartl and Clark 1989). A Wahlund effect is essentially a sampling error caused when subpopulations are established with groups of colonists whose allele frequencies differ from other subpopulations or the overall population. However, three loci did not show expected Hardy-Weinberg genotypic frequencies within subpopulations, which may be due to strong selection against heterozygous embryo genotypes (Gibson 1995).

Family structure was also found in the study population. In two subpopulations, R was estimated at approxi-

mately 0.25 which is expected in populations composed exclusively of half-siblings (Queller and Goodnight 1989). In two other subpopulations,  $R$  was approximately 0.18. Depression of  $R$  values below 0.25 could be caused by the presence of progeny from several different maternal families (overlapping seed shadows). Subpopulation B had a very low  $R$  (0.093) and high genetic diversity (0.226). It is possible that B was established by few members of numerous maternal families. We can also propose another plausible explanation. Because subpopulation B is smaller than other subpopulations, its trees are larger, and the canopy has grown over these trees, B may be successional older than other subpopulations. As the subpopulation aged, previously high relatedness among trees may have decreased, due to random mortality of individuals in the same sibling groups that colonized the site, or intense sibling competition (Hamrick et al. 1993). Our analyses are not able to differentiate between multiple colonizations and decay of family structure.

The high  $G_{ST}$  and low  $Nm$  estimated for *O. tenera* were counter to our expectations. If birds are vectors for extensive gene flow and if seeds from all subpopulations have an equal chance of contributing seeds to the colonization of a site, we would not expect the observed high genetic differentiation among subpopulations. Our data do not disprove the notion that birds move seeds long distances between localities. Since *O. tenera* is a gap colonist, subpopulations must be established with colonists from distant subpopulations. Therefore, our data suggest that nonrandom collection and clumped dispersal of groups of seeds that are not genetically representative of the entire population are causes of the elevated  $G_{ST}$  (Table 3), which results in an estimate of highly restricted gene flow based upon our indirect estimate of  $Nm$ . Thousands of fruits are produced by *O. tenera* trees and dispersed by avian frugivores within a year, yet extensive establishment of seedlings in the forest does not occur (Gibson 1995). This is most likely because many seeds are not deposited in favorable sites in the forest, and most dispersed seeds are predated by rodents (Wheelwright 1988). Therefore, the low estimate of gene flow in our study reflects limitations on seedling establishment more than it indicates restrictions on distance of seed dispersal.

The most interesting observation was the lack of relatedness between *O. tenera* saplings and adult trees at several sites. This confirms the occurrence of post-colonization gene flow among subpopulations. In *O. tenera*, birds collect fruit from a maternal tree and then regurgitate those seeds under a distant plant. If birds forage in several closely spaced trees which are themselves related, dispersal of those seeds together would elevate the average relatedness among the total group of seed being dispersed (cf. Linhart and Tomback 1985). The number of seeds dispersed together at any one time and site of deposition is dependent on the frugivores' species-specific behavior. Resplendent quetzals feed alone or in pairs. Emerald toucanets travel together in small flocks (<20). Thus, resplendent quetzals which eat one or two

fruits would move very few seeds and may have less of an effect on population genetic structure than toucanets. This pattern is markedly different from many temperate-zone trees which show the greatest dispersal and establishment in the immediate vicinity of a maternal tree.

Hamrick et al. (1993) investigated the effect of different seed dispersal mechanisms on genetic characteristics in tree species of a low-elevation tropical forest. Two species in their study, *Brosimum alicastrum* (Moraceae) and *Swartzia simplex* var. *ochracea* (Caesalpineaceae) have similar dispersal ecology to *O. tenera*. *B. alicastrum* is a dioecious, canopy species whose seeds are dispersed by various arboreal mammals. *S. simplex* is a hermaphroditic, understory tree whose seeds are bird-dispersed. In these two species, high genetic relatedness was estimated among individuals of the same diameter classes within subpopulations.  $F_{ST}$ -values for these two species (Table 5) were significant but lower than the  $G_{ST}$  (which is similar in interpretation to  $F_{ST}$ ) of *O. tenera*. The genetic architecture of *B. alicastrum* and *S. simplex* was proposed to be due to overlapping seed shadows from different maternal trees and clumped dispersal of seeds from the same maternal line. Because the  $G_{ST}$  is greater in *O. tenera*, it appears that there is minimal seed shadow overlap among maternal individuals.

We cannot determine the exact number of individuals or families establishing *O. tenera* subpopulations. However, comparison of natural subpopulations and experimental plots allows us to make rough estimates of colonization and gene flow parameters. Experimental plots had slightly higher genetic diversity values than most natural subpopulations (Table 1). However, relatedness among trees within experimental plots was much lower than in natural subpopulations (Table 3). The lower genetic diversity and higher relatedness in natural subpopulations as compared to experimental plots indicates that natural subpopulations contain progeny from few maternal lineages (<5), and that several siblings (>7) from the same family group are present in each subpopulation.

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## References

- Gibson JP (1995) Evolutionary ecology of reproduction in *Ocotea tenera* (Lauraceae), a gynodioecious tropical tree. Ph.D. Dissertation. University of Colorado, Boulder
- Hamrick JL, Godt MJ (1990) Allozyme diversity in plant species. In: Brown ADH, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland, pp 43–63
- Hamrick JL, Loveless MD (1986) Isozyme variation in tropical trees: procedures and preliminary results. *Biotropica* 18: 201–207

- Hamrick JL, Loveless MD (1988) The genetic structure of tropical tree populations: associations with reproductive biology. In: Bock JH, Linhart YB (eds) The evolutionary ecology of plants. Westview, Boulder, pp 129–146
- Hamrick JL, Godt MJ, Sherman-Broyles SL (1992) Factors influencing levels of genetic diversity in woody plant species. *New For* 6:95–124
- Hamrick JL, Murawski DA, Nason JD (1993) The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetatio* 107/108:281–297
- Hartl DL, Clark AG (1989) Principles of population genetics, 2nd edn. Sinauer, Sunderland
- Horvitz CC, Le Corff J (1993) Spatial scale and dispersion patterns of ant- and bird-dispersed herbs in two tropical lowland forests. *Vegetatio* 107/108:351–362
- Howe HF (1989) Scatter- and clump-dispersal and seedling demography: hypotheses and implications. *Oecologia* 79:417–426
- Howe HF, Estabrook GF (1977) On intraspecific competition for dispersal agents in tropical trees. *Am Nat* 111:817–791
- Howe HF, Smallwood J (1981) Ecology of seed dispersal. *Annu Rev Ecol Syst* 13:201–228
- Li CC, Horvitz DG (1953) Some methods of estimating the inbreeding coefficient. *Am J Hum Genet* 5:107–117
- Linhart YB, Tomback DF (1985) Seed dispersal by nutcrackers causes multi-trunk growth form in pines. *Oecologia* 67:107–110
- Loveless MD (1992) Isozyme variation in tropical trees: patterns of genetic organization. *New For* 6:67–94
- Martin TE (1985) Resource selection by tropical frugivorous birds: integrating multiple interactions. *Oecologia* 66:563–573
- Mitton JB, Linhart YB, Sturgeon KB, Hamrick JL (1979) Allozyme polymorphism detected in mature needle tissue of ponderosa pine. *J Hered* 70:86–89
- Nei M (1972) Genetic distance between populations. *Am Nat* 106:283–292
- Nei M (1977) F-statistics and analysis of gene diversity in subdivided populations. *Ann Hum Genet* 41:225–233
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* 43:258–275
- Slatkin M, Barton NH (1989) A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* 43:1349–1368
- Soltis DE, Haufler CH, Darrow DC, Gastony GJ (1983) Starch gel electrophoresis of ferns: a compilation of crushing buffers, gel and electrode buffers, and staining schedules. *Am Fern J* 73:373–380
- Tomback DF, Linhart YB (1990) The evolution of bird-dispersed pines. *Evol Ecol* 4:185–219
- Wheelwright NT (1986) A seven-year study of individual variation in fruit production in tropical bird-dispersed tree species in the family Lauraceae. In: Estrada A, Fleming TH (eds) Frugivores and seed dispersal. Junk, Dordrecht, pp 19–35
- Wheelwright NT (1988) Four constraints on coevolution between fruit-eating birds and fruiting plants a tropical case history. Proceedings of the 19th International Ornithology Congress, Ottawa, pp 827–845
- Wheelwright NT (1991) How long do fruit-eating birds stay in the plants where they feed? *Biotropica* 23:29–40
- Wheelwright NT, Bruneau A (1992) Population sex ratios and spatial distribution of *Ocotea tenera* (Lauraceae) trees in a tropical forest. *J Ecol* 80:425–432
- Willson MF (1993) Dispersal mode, seed shadows, and colonization patterns. *Vegetatio* 107/108:261–280
- Workman PL, Niswander JD (1970) Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *Am J Hum Genet* 22:24–49
- Wright S (1965) The interpretation of population genetic structure by F-statistics with special regard to systems of mating. *Evolution* 19:395–420