Lundiana 3(2):99-109, **2002**

ISSN 1676-6180

Genetic differentiation and diversity of natural populations of *Cryptocarya* spp. (Lauraceae) from the Brazilian Atlantic rain forest

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Abstract

The genetic variability and structure of ten populations of *Cryptocarya* spp. (Lauraceae) were investigated by means of isozymes. Leaf samples of adult individuals of *C. aschersoniana*, *C. moschata*, and *Cryptocarya* sp., from two regions of São Paulo state, Brazil, were collected. Seeds from 35 families of *C. moschata* and juveniles of three populations from diaspores dispersed by "muriquis", *Brachyteles arachnoides* (Primates - Cebidae), were also collected. From twenty loci analysed, the expected average heterozygosity of progenies, juveniles, and adults of *C. moschata* was respectively 0.313, 0.227, and 0.351, whereas it was 0.258 and 0.393 for *C. aschersoniana* and *Cryptocarya* sp., respectively. Results indicated that a greater variability occurred within populations for all categories analysed. For adult populations of *C. moschata*, the divergence obtained through G_{ST} estimates suggests the existence of significant genetic drift and/or natural selection effects between regions. The level of gene differentiation ($\hat{G}_{ST}$ = 0.107) was relatively high, and comparable to what would be expected for groups of plants having a half-sib family structure in a single generation. For juvenile populations, the genetic differentiation was also high ($\hat{G}_{ST}$ = 0.084). This could be explained by differential feeding behaviour of "muriquis" within their home range, allied to the distance among sites of seed dispersion. Nei's average genetic distance (*D*) between pairs of *C. moschata* adult populations was 0.07, indicating again a pronounced genetic differentiation. Present values obtained from isozymes support morphological and wood anatomy evidences that *C. moschata* and *C. aschersoniana* are closely related species.

Keywords: *Cryptocarya aschersoniana*, *C. moschata*, Genetic distance, Genetic structure, Genetic variability, Population differentiation, Lauraceae

Introduction

The use of isozymes in plant biology has expanded rapidly in recent years. Current accumulation of knowledge from isozyme studies on the genetic structure of tropical woody species shows that they possess high levels of genetic variation and among-population differentiation (Loveless, 1992; Liengsiri et al., 1995).

Knowledge about the genetic structure of tropical tree populations can help to explain the evolution of high diversity in the tropics, and to design adequate forest conservation and management strategies (Eguiarte et al., 1992; Moraes et al., 1999). However, genetic diversity in tropical rain forest trees is at risk due to deforestation, logging operations, and habitat fragmentation. The consequences of tropical deforestation on the loss of biodiversity at the species level are well known and have received considerable attention (Chase et al., 1995). However, less is known about the effects of deforestation on the loss of genetic diversity within species. Deforestation may reduce population size and gene flow or, altogether, eliminate local populations (Nason et al., 1997). Moreover, forest fragmentation may lead to genetic isolation of once continuous populations; this in turn may cause further losses in genetic diversity as a result of inbreeding and genetic drift (Rocha & Lobo, 1998). Furthermore, in developing countries, which are largely in the tropics, habitat loss and fragmentation, over-exploitation, and the introduction of exotic species are decisive factors in the rapid destruction of tropical forests which have been estimated to be home to at least two-thirds of the world's living organisms. Therefore, the persistence of evolutionarily viable populations of tropical forests is crucial to the

Received 19.09.2001
Accepted 17.04.2002

preservation of tropical ecosystems and global biological diversity (Liengsiri et al., 1995).

The Brazilian Atlantic rain forest is an area experiencing one of the highest rates of deforestation in the world. It is a typical Neotropical rainforest, one of the seven moist forest areas recognized in this realm. It is characterized by a complex and diversified vegetation, which is adapted to rainfall and wetness conditions, with the predominance of species of Leguminosae, Bignoniaceae, Lauraceae, Sapotaceae, and Myrtaceae. It has only been preserved in steep mountain slopes areas, which are inadequate for large-scale farming (Por, 1992). A series of São Paulo state deforestation maps (Victor, 1975) shows the destruction at the level of 90%. In spite of that, and according to SOS Mata Atlântica (1991), São Paulo state had, in 1991, with 34,448 square kilometers, the largest nucleus of Atlantic rain forest remnants.

The genus *Cryptocarya* is one of the principal genera of trees of the Brazilian Atlantic rain forest, being regularly cited in species lists from both floristic composition and phytosociological structure studies (P.L.R. Moraes, unpublished data). Among the Brazilian species of this genus, *Cryptocarya moschata* is the commonest and the most widespread one, being accounted to its important medicinal properties since Spix & Martius (1828, p. 553) and Martius (1843, p. 110). Although the fruit of *Cryptocarya moschata* possesses the mammal dispersal syndrome (van der Pijl, 1969), and it is known to be dispersed by "muriqui" primates, most of the seeds fall from the trees, under which seedlings form dense "carpets" (Moraes & Paoli, 1995). However, despite the fact that a seedling bank develops, the species still needs the formation of gaps for the recruitment of new individuals, since there is a virtually complete absence of juveniles in understory (Moraes & Paoli, 1999). Therefore, the diaspores dispersed by "muriquis" present a higher probability of survival, and consequently of new individuals recruitment, because of less incidence of predation, lower density, and greater distance from the mother-trees (Moraes & Paoli, 1995).

This paper provides a description of the genetic differentiation and diversity of natural populations of canopy tree species of *Cryptocarya* spp. occurring in natural reserves of the Atlantic rain forest in São Paulo state, Brazil. In *C. moschata*, progenies, juveniles, and adults were investigated with the objective of detecting possible genetic differentiation among them, considering this species occurs mainly in clusters of two to five individuals at the same place, far 10 to 20 m from each other, and presents a mixed mating system (P.L.R. Moraes, unpublished data). Additionally, because juvenile populations are provenient from primate dispersed diaspores, the influence of seed dispersion by primates was also investigated.

Materials and methods

Cryptocarya

According to the classification by Rohwer (1993), the genus *Cryptocarya* is placed in the tribe Perseeae due to the exinvolucrate inflorescences, basically of the thyrso-paniculate type, with the pollen sacs of the third staminal whorl being usually extrorse. According to van der Werff & Richter's classification (1996), based on inflorescence structure and wood and bark anatomy, the genus is placed in tribe Cryptocaryeae, with a paniculate-±cymose inflorescence. However, the newest phylogenetic classification of Lauraceae, proposed by Rohwer (2000), pointed that this separation between taxa with involucrate and non-involucrate inflorescences, which had been one of the basic subdivisions in all systems of the family so far, was not supported by *matK* gene sequencing data. Although a *Beilschmiedia-Cryptocarya* clade, that had been recognized by wood anatomy, but not in most of the recent morphological systems, was well supported.

The genus was established by R. Brown in 1810 with only three species (Kostermans, 1952), comprehending today about 350 spp.; it is pantropical, centred in Malaysia, absent from Central Africa, with ten or fewer neotropical species, mostly in Southern Brazil and Chile, but also known from French Guyana and adjacent Brazil, Andean Venezuela, Ecuador, and Peru (Hyland, 1989). *Cryptocarya* (tribe Cryptocaryeae, sub-tribe Cryptocaryneae) is considered isolated among the neotropical genera of Lauraceae (Raj & van der Werff, 1988). Based just on the pollen morphology (Raj & van der Werff, 1988; van der Merwe et al., 1990), Rohwer (1993) suggested that the genus may not be a natural group. Christophel et al. (1996), using leaf architecture and cuticular features of all species of leafy Lauraceae found in Australia, also pointed the possibility that the genus, as now defined, is not natural and perhaps even polyphyletic.

Cryptocarya moschata Nees is a Brazilian tree species with a widespread distribution, mainly in the Atlantic rain forest, which yields fruit with nutmeg smell and taste ("Brazilian nutmeg"). Individuals of *C. moschata* are trees 15 to 30 m tall x 20 to 104 cm dbh (diameter at breast height) (P.L.R. Moraes, unpublished data). The flowers are bisexual, 3-merous, tepals 3 + 3, stamens 6 introrses + 3 extrorses, 2-locular, staminodes 3, ovary ± sessile, stigma inconspicuous. The nucule-type fruit is 1.45-3.06 cm long (= 2.26 ± 0.28 cm; N = 1,892), 1.29-2.55 cm wide (= 1.90 ± 0.24 cm; N = 1,892), one-seeded. The seeds are 1.34-3.00 cm long (= 2.17 ± 0.27 cm; N = 1,764), 1.16-1.92 cm wide (= 1.52 ± 0.11 cm; N = 1,764; Moraes & Alves, 1997). The chromosome number is 2n = 24 (Moraes & Gardingo, 1996). This species is rich in alkaloids, styryllpyrones, and flavonoids (Cavalheiro & Yoshida, 2000). Its bark, bitter and scented, is considered to be stomachical and helpful in fighting colics and diarrhea, in folk medicine (Vattimo-Gil, 1957). The tea from its seeds is used against stomachache, and its crushed leaves against aches and colics; the fruit is carminative, and widely consumed by primate populations [brown howler monkeys (*Alouatta fusca*), brown capuchins (*Cebus apella*), and "muriquis" (*Brachyteles arachnoides*)], and cracids (*Pipile jacutinga*, and *Penelope obscura*); its wood is used in canoes manufacturing.

The other two species of *Cryptocarya* investigated in this current paper, *C. aschersoniana* Mez and *Cryptocarya* sp. present a more restricted distribution in the Atlantic rain forest of São Paulo state than *C. moschata*. *Cryptocarya aschersoniana* is mainly distributed in Planalto Forests of this state, and *Cryptocarya* sp. (an still unidentified species) is only known, up to now, from the sampled area in this present study. Those species are morphologically very similar to *C. moschata*,

what promotes great taxonomical misinterpretation for identification of herbarium specimens.

Plant collections

Study was carried out on open-pollinated progenies of single trees of *Cryptocarya moschata* collected from Parque Estadual Carlos Botelho (PECB-P; 24°44' to 24°15'S, 47°46' to 48°10'W; Moraes et al., 1999). Additionally, leaves of adult trees from four populations of *C. moschata* were sampled, as follows: 141 randomly distributed individuals within an area of 647 ha at Parque Estadual Carlos Botelho (PECB-A); 36 individuals collected from three trails: Quilombo, Rio Saibadela, and Azul at Parque Estadual Intervales, Núcleo Saibadela (PEI-NS; 24°13' to 24°14'S, 48°04' to 48°06'W); 10 individuals sampled along the trail of Corisco at Parque Estadual da Serra do Mar, Núcleo Picinguaba (PESM-NP; 23°20' to 23°22'S, 44°48'45" to 44°52'30"W); and 27 individuals collected from three distinct sites: Poço do Pito, Itamambuca, and Salto Grande at Parque Estadual da Serra do Mar, Núcleo Santa Virgínia (PESM-NSV; 23°17' to 23°24'S, 45°11' to 45°30'W). From the same area at PECB, three samples of 20 individuals each were collected from different sites of seed defecation of "muriqui" monkeys, comprehending sub-populations of 6 year-old juveniles (PECB-D1, PECB-D2, and PECB-D3). Population PECB-D1 was circa one kilometer far from the others, while PECB-D2, and PECB-D3 were distant about 100 m from each other. In addition to that, 20 adult trees of *C. aschersoniana* Mez were collected at Mata de Santa Genebra (MSG; 22°49'45"S, 47°06'33"W), municipality of Campinas, and seven adult trees of *Cryptocarya* sp. were collected from the same area of *C. moschata* at PECB. All these samples were collected in pristine forests or patches with little anthropogenic disturbance, except *C. aschersoniana* population which was sampled from an expressively man-disturbed forest.

A total of 35 open-pollinated families, the great majority of which consisting of 20 seedlings, were analysed. One eophyll of the first pair emitted from each seedling in each family was collected for electrophoresis run. The collected eophylls were immediately submitted to extraction. For adult trees in the field, it was collected at least a foliar stem with mature leaves, which was packed in plastic bags, and stored in stiff foam-boxes with ice for the transportation to the laboratory. In the laboratory, they were kept at ≅ 5°C, and the third mature leaf from the stem apex was selected, and extracted after two days on average.

Electrophoresis

Starch gel electrophoresis was carried out according to procedures described by Alfenas et al. (1991). Sixteen enzyme systems were assayed. Among them, a total of 20 putative loci in six isozyme systems (Table 1) were resolved from field samples: acid phosphatase (Acph-1, Acph-2, Acph-3), alkaline phosphatase (Alph-1, Alph-3, Alph-4, Alph-5), cathalase (Cat-1, Cat-2, Cat-3, Cat-4), glutamate-oxaloacetate transaminase (Got-1, Got-2), peroxidase (Per-1, Per-2, Per-3, Per-4, Per-5), and polyphenoloxidase (Ppo-4, Ppo-5). Different loci for the same enzyme were sequentially numbered, with the most anodally migrating locus being given the lowest number, and so on. Likewise, within each locus the most frequent migrating band was designated allele 1, and each successively slower band was numbered 2, 3, etc.

Data Analysis

The BIOSYS-2 computer program (Swofford & Selander, 1989; current release modified from BIOSYS-1 by William C. Black IV) was used to analyse the isozyme data for allelic frequencies, percentage of polymorphic loci, mean number of alleles, and estimates of observed and expected heterozygosities. Genetic variability measures were determined using 20 isozyme loci which had scorings for all populations. The effective number of alleles, taking into account both the number of alleles and their frequencies, was calculated according to Crow & Kimura (1970). Exact tests for deviation from Hardy-Weinberg expectations (HWE) was performed using conventional Monte Carlo method, according to Guo & Thompson (1992) through TFPGA program (Miller, 1997). Allelic frequencies were compared among populations and among seedlings, juveniles and adults of *C. moschata* for PECB through contingency table approach (Fisher's RxC test), to determine if significant differences in allele frequencies exist among these groups of individuals (Sokal & Rohlf, 1995). This analysis was performed using TFPGA program, and its description can be found in Raymond & Rousset (1995).

Table 1. Details of enzymes stained for, their abbreviations, enzyme commission (EC) numbers, the gel buffer systems on which they were scored, and references for staining.

Enzyme name and abbreviation		EC number	Gel buffer[a]	Reference
Acid phosphatase	Acph	EC3.1.3.2	TC	Alfenas et al. (1991)
Alkaline phosphatase	Alph	EC3.1.3.1	TC	Boyer (1961)
Cathalase	Cat	EC1.11.1.6	LB	Scandalios (1969)
Glutamate-oxaloacetate transaminase	Got	EC2.6.1.1	LB	Brewbaker et al. (1968)
Peroxidase	Per	EC1.11.1.7	LB	Brewbaker et al. (1968)
Polyphenoloxidase	Ppo	EC1.10.3.1	TC	Alfenas et al. (1991)

[a] Gel buffer systems used were: LB: gel, 0.19 M boric acid, adjusted to pH 8.3 with lithium hydroxide 0.05 M (buffer A, Scandalios, 1969); 0.008 M citric acid, adjusted to pH 8.3 with tris 0.051 M (buffer B, Scandalios, 1969); mixing of buffers A and B in 1:9 proportion; tray, buffer A. TC, gel, 3.5% dilution of tray buffer; tray, 0.223 M tris + 0.086 M citric acid, pH 7.5 (Alfenas et al., 1991).

Genetic differentiation between populations was analysed by the G_{ST}-statistic of Nei (1987). The G_{ST}-statistic is derived from the formula $H_T = H_S + D_{ST}$, where H_S is the average gene diversity within populations, and D_{ST} is the average gene diversity among populations. The relative genetic differentiation between populations is obtained as $G_{ST}=D_{ST}/H_T$. The genetic differentiation between populations within and between the two geografic regions, i.e. the southern (PECB-A and PEI-NS) and northern (PESM-NP and PESM-NSV) coastal regions of São Paulo state, was evaluated by pooling all populations within the same geographic region. The diversity statistics and standard error of the mean were calculated using Ritland's (1990) GDD program. The statistical significance of G_{ST} values at each locus was calculated using a chi-square test (Workman & Niswander, 1970).

The hierarchical genetic structure between populations of species of *Cryptocarya* was estimated by Nei's (1972) genetic distance (*D*), which is the most widely used measure of genetic distance, and is appropriate for long-term evolution when populations diverge due to drift and mutation. Nei's genetic distance is proportional to the time since divergence in the special case of the infinite alleles mutation model and equilibrium in the ancestral population (Weir, 1996). Through another approach, if populations are a series of interconnected islands wherein gene flow occurs only between adjacent islands (stepping-stone model), then the genetic identity should decrease exponentially with distance (Ritland, 1989). Since Nei's *D* is the logarithm of gene identity (*I*), a linear increase in *D* with geographical distance is predicted. The dendrogram was plotted using the unweighted pair-group method with arithmetic averaging (UPGMA) as described by Sneath & Sokal (1973). TFPGA program was used to generate the dendrogram, and to check its confidence and consistency for each node generated by the original data set, through bootstrapping over loci. NTSYS-Pc program version 1.70 (Rohlf, 1992) was employed to generate the matrix of cophenetic values, and to test the goodness of fit of the cluster analysis to the original data.

Results and discussion

Genetic variability

Allelic frequencies of ten natural populations of *Cryptocarya* spp. are presented in Table 2. The values obtained from progenies of *C. moschata* ranged from complete fixation, as allele 1 of Alph-4, to fairly low frequencies, as allele 1 of Acph-3. Likewise, for adult populations of *C. moschata*, the values also varied from complete fixation (e.g. allele 2 of Alph-5 in PEI-NS, PESM-NP, and PESM-NSV populations) to reduced frequencies (e.g. allele 1 of Cat-2 in PEI-NS population). The existence of exclusive alleles was detected in some adult populations, as allele 1 of Acph-3 and Alph-5. For juveniles of *C. moschata* there were no exclusive alleles. Alleles 2 of Acph-1 and Acph-2 were detected in progenies at extremely low frequencies and were not found in adult and juvenile individuals, but occurred in *Cryptocarya* sp. at much higher frequencies. Besides, several alleles showed very distinct frequencies among populations (e.g., alleles 1 and 2 of Cat-1 and Cat-2 from adults).

These results showed divergence among populations of *C. moschata*, particularly between the PECB-A population and the others. Moreover, the occurrence of alleles in PECB-P which were not found in PECB-A suggests an insufficient sampling of the latter, or the existence of gene flow from non-sampled individuals. On the other hand, the complete fixation of alleles in nine loci of juvenile individuals, and in five and four loci of PESM-NP and PESM-NSV, respectively, also suggests an insufficiency of sampling, since these were the populations with the smallest sample sizes. However, populations of *C. aschersoniana* and *Cryptocarya* sp., which also presented small sample sizes, showed relatively less proportion of fixed alleles. This may indicate that these species have different life history features from *C. moschata*, such as the mating system, that might avoid fixation.

Allelic frequencies differed significantly ($p < 0.05$) from HWE at 16 of the 18 loci tested for PECB-P population (Table 3). All but one of these deviations (Ppo-4) were due to heterozygote deficits. In juveniles, population PECB-D3 rejected the null hypothesis of HWE in 40.0% of polimorphic loci while populations PECB-D1 and PECB-D2 rejected it in 27.3%. In adults, population PECB-A and PEI-NS rejected the null hypothesis in 44.44% and 41.17% of polimorphic loci, respectively, while populations PESM-NP and PESM-NSV rejected the null hypothesis respectively in 13.33% and 18.75% of polimorphic loci. In *C. aschersoniana*, five loci rejected the HWE null hypothesis (33.33%), and in *Cryptocarya* sp. two loci (11.76%) rejected the null hypothesis.

Levels of isozyme diversity averaged over 20 loci for ten natural populations of *Cryptocarya* spp. are given in Table 4. The mean number of alleles per locus (A) ranged from 1.6 to 2.0, whereas the effective number of alleles (A_e) ranged from 1.37 to 1.67. The difference between A and A_e reveals the presence of alleles of low frequency. Relatively smaller differences were found in juveniles, when compared to those found in adults and progenies, since eveness was greater in juveniles. It is worth mentioning that these values are similar to those found for other tropical tree species (Loveless & Hamrick, 1987; Chase et al., 1995; Gibson & Wheelwright, 1995). The percentage of polymorphic loci (0.95 criterion) ranged from 50.0% to 85.0%. These values reinforce the fact that a greater fixation of alleles was found in juvenile populations than in the others, reflecting a lower polymorphism. The mean expected ($\hat{H}_e$) heterozygosity for adult populations of *C. moschata* was 0.303, whereas it was 0.313 for progenies, 0.211 for juvenile populations, 0.258 for *C. aschersoniana* and 0.393 for *Cryptocarya* sp. These values of heterozygosity for adults are among the highest found for other tropical tree species (Moran et al., 1989; Alvarez-Buylla & Garay, 1994; Murawski & Bawa, 1994). Despite the differences among populations for diverse age categories, their fixation indices ($\hat{F}_{(IS)}$) were not significant except for progenies, which presented an excess of homozygotes. An explanation for this general heterozygous deficit in progenies is not well understood, since information about brazilian *Cryptocarya* spp. flower biology is not available. However, as discussed by Moraes et al. (1999), *C. moschata*, with its minute, scented and whitish flowers, with low alimentary reward to pollinators (small flower glands functioning as nectar-secreting emergences), among other features, would be adapted to pollination by small insects, such as flies and small bees, which

Table 2. Allelic frequencies at 20 isozyme loci for seedlings (PECB-P), juveniles, and adults of *Cryptocarya moschata* Nees, and adults of *C. aschersoniana* Mez and *Cryptocarya* sp.

Locus/allele	Population									
	PECB-P[a]	PECB-A[c]	PECB-D1[b]	PECB-D2[b]	PECB-D3[b]	PEI, NS[c]	PESM, NP[c]	PESM, NSV[c]	*C. aschersoniana*[d]	*Cryptocarya* sp.[e]
Acph-1										
1	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.571
2	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.429
Acph-2										
1	0.983	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.786
2	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.214
Acph-3										
1	0.006	0.053	0.000	0.000	0.000	0.194	0.000	0.000	0.194	0.000
2	0.994	0.947	1.000	1.000	1.000	0.806	1.000	1.000	0.806	1.000
Alph-1										
1	0.728	0.791	0.925	0.750	0.775	0.542	0.722	0.690	0.350	0.833
2	0.272	0.209	0.075	0.250	0.225	0.458	0.278	0.310	0.650	0.167
Alph-3										
1	0.807	0.734	1.000	1.000	1.000	0.444	0.450	0.340	1.000	1.000
2	0.193	0.266	0.000	0.000	0.000	0.556	0.550	0.660	0.000	0.000
Alph-4										
1	1.000	0.911	1.000	1.000	1.000	0.708	1.000	0.780	0.400	0.786
2	0.000	0.089	0.000	0.000	0.000	0.292	0.000	0.220	0.600	0.214
Alph-5										
1	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	1.000	0.982	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Cat-1										
1	0.631	0.493	0.875	0.700	0.650	0.861	0.350	0.611	0.731	0.429
2	0.369	0.507	0.125	0.300	0.350	0.139	0.650	0.389	0.269	0.571
Cat-2										
1	0.635	0.645	0.075	0.100	0.100	0.014	0.050	0.481	0.111	0.286
2	0.365	0.355	0.925	0.900	0.900	0.986	0.950	0.519	0.889	0.714
Cat-3										
1	0.586	0.674	0.850	0.725	0.900	0.694	0.850	0.926	0.150	0.571
2	0.414	0.326	0.150	0.275	0.100	0.306	0.150	0.074	0.850	0.429
Cat-4										
1	0.872	0.840	0.250	0.900	1.000	0.944	0.800	0.778	0.650	0.500
2	0.128	0.160	0.750	0.100	0.000	0.056	0.200	0.222	0.350	0.500
Got-1										
1	0.791	0.713	0.475	0.675	0.750	0.111	0.250	0.037	0.974	0.286
2	0.040	0.110	0.050	0.075	0.025	0.750	0.700	0.944	0.026	0.429
3	0.169	0.177	0.475	0.250	0.225	0.139	0.050	0.019	0.000	0.286
Got-2										
1	0.438	0.401	0.350	0.325	0.325	0.431	0.600	0.426	0.125	0.143
2	0.121	0.230	0.450	0.100	0.225	0.125	0.050	0.204	0.750	0.143
3	0.441	0.369	0.200	0.575	0.450	0.444	0.350	0.370	0.125	0.714
Ppo-4										
1	0.613	0.635	0.550	0.575	0.575	0.319	0.550	0.667	0.225	0.571
2	0.387	0.365	0.450	0.425	0.425	0.681	0.450	0.333	0.775	0.429
Ppo-5										
1	0.892	0.848	1.000	1.000	1.000	0.875	0.800	0.778	1.000	0.375
2	0.108	0.152	0.000	0.000	0.000	0.125	0.200	0.222	0.000	0.625
Per-1										
1	0.559	0.748	0.000	0.000	0.000	0.639	0.950	0.907	0.667	0.857
2	0.441	0.252	1.000	1.000	1.000	0.361	0.050	0.093	0.333	0.143
Per-2										
1	0.464	0.741	0.000	0.000	0.000	0.917	0.950	0.815	0.425	0.143
2	0.536	0.259	1.000	1.000	1.000	0.083	0.050	0.185	0.575	0.857
Per-3										
1	0.733	0.617	0.900	0.775	0.600	0.639	0.950	0.704	0.079	0.429
2	0.267	0.383	0.100	0.225	0.400	0.361	0.050	0.296	0.921	0.571
Per-4										
1	0.735	0.684	0.725	0.675	0.725	0.750	0.600	0.685	0.225	0.214
2	0.186	0.202	0.100	0.200	0.225	0.250	0.100	0.130	0.025	0.071
3	0.079	0.113	0.175	0.125	0.050	0.000	0.300	0.185	0.750	0.714
Per-5										
1	0.686	0.571	0.375	0.500	0.525	0.444	0.600	0.852	0.900	0.786
2	0.314	0.429	0.625	0.500	0.475	0.556	0.400	0.148	0.100	0.214

[a] seedlings of *C. moschata*; N = 692
[b] juveniles of *C. moschata*; N = 20
[c] adults of *C. moschata*: PECB-A, N = 141; PEI-NS, N = 36; PESM-NP, N = 10; PESM-NSV, N = 27; [d] N = 20; [e] N = 7

Table 3. Goodness of fit test for Hardy-Weinberg proportions through conventional Monte Carlo method[a], using allelic frequencies at 20 isozyme loci for seedlings (PECB-P), juveniles, and adults of *Cryptocarya moschata* Nees, and adults of *C. aschersoniana* Mez and *Cryptocarya* sp.

Locus	Population									
	PECB-P[b]	PECB-A	PECB-D1[c]	PECB-D2[c]	PECB-D3[c]	PEI, NS	PESM, NP	PESM, NSV	*C. aschersoniana*	*Cryptocarya* sp.
Acph-1	1.000	–	–	–	–	–	–	–	–	0.165
Acph-2	1.000	–	–	–	–	–	–	–	0.231	
Acph-3	0.000[d]	1.000	–	–	–	1.000	–	–	0.0034[d]	–
Alph-1	0.000[d]	0.796	0.078	0.049[d]	0.527	0.0002[d]	0.523	0.603	0.631	0.088
Alph-3	0.000[d]	0.046[e]	–	–	–	0.0019[e]	0.040[e]	0.020[e]	–	–
Alph-4	–	0.594	–	–	–	0.691	–	0.304	0.638	1.000
Alph-5	–	1.000	–	–	–	–	–	–	–	–
Cat-1	0.000[d]	0.046[e]	0.247	0.115	1.000	1.000	0.477	0.224	0.009[d]	1.000
Cat-2	0.000[d]	0.139	0.074	1.000	1.000	1.000	1.000	1.000	0.166	0.439
Cat-3	0.000[d]	0.440	1.000	0.258	1.000	0.014[e]	1.000	1.000	1.000	0.151
Cat-4	0.000[d]	0.002[d]	0.000[d]	0.154	–	1.000	0.307	0.007[d]	0.628	1.000
Got-1	0.000[d]	0.028[d]	0.034[d]	0.806	0.022[d]	0.001[d]	0.653	1.000	1.000	0.053
Got-2	0.000[d]	0.000[d]	0.068	0.046[d]	0.200	0.025[d]	0.679	0.0002[d]	1.000	0.021[d]
Ppo-4	0.000[e]	0.000[e]	0.020[e]	0.002[e]	0.002[e]	0.006[e]	0.044[e]	1.000	0.539	0.157
Ppo-5	0.0001[d]	1.000	–	–	–	0.444	0.312	0.570	–	0.427
Per-1	0.000[d]	0.0001[d]	–	–	–	0.292	1.000	1.000	0.004[d]	0.075
Per-2	0.000[d]	0.665	–	–	–	1.000	1.000	0.549	0.002[e]	1.000
Per-3	0.000[d]	0.157	1.000	0.523	0.006[e]	0.001[e]	1.000	0.065	1.000	1.000
Per-4	0.000[d]	0.000[d]	1.000	0.449	1.000	0.653	0.061	0.253	1.000	0.019[d]
Per-5	0.000[d]	0.495	0.054	1.000	0.029[d]	0.195	0.079	0.443	0.001[d]	0.241

[a] 10 batches per analysis, 1,000 permutations per batch, 10,000 total permutations
[b] seedlings of *C. moschata*
[c] juveniles of *C. moschata*
[d] heterozygote deficit
[e] heterozygote excess
– Locus fixed for one allele

might promote pollen flow mostly at short distances. Another important contributing factor is that *C. moschata* has self-compatible hermaphroditic flowers and a mixed-mating system, with significant biparental inbreeding and nonrandom mating situations (P.L.R. Moraes, unpublished data). The homozygous excess in progenies may also indicate a possible selection favoring heterozygotes through this species life cycle until its adulthood phase.

When the diversity indices were analysed through different forms of pooled populations, a higher gene diversity was found for populations of southern coastal region (PECB and PEI-NS) in comparison to northern coastal region (PESM-NP and PESM-NSV). There was also an indication that the diversity of all populations of adults of *C. moschata*, analysed as a pooled population, might be determined mainly by individuals of the southern region.

Contingency table analyses of heterogeneity among different classes of age for *C. moschata* from PECB showed heterogeneity among all analysed categories. This could be associated with the effects of the reproductive biology of the species such as non-random mating or internal structuring in populations. Additionally, the existence of overlapping generations in adult populations, and the survival and/or recruitment of juveniles (under selection and/or drift effects) could enhance, at least partially, the differences found especially in allelic frequencies.

Moraes et al. (1999) emphasized the important role of "muriquis" as seed dispersors of *C. moschata* at PECB, based on their feeding behaviour which comprehends: i) eating fruit from a single tree, subsequently settling several diaspores (at least half-sibs) in areas which may be safe sites for a new recruitment; and ii) eating fruit from various trees, with diaspore settlement along their home range. The former behaviour can establish a high genetic heterogeneity among these recruitment sites, which, however, may be diminished if several independent events occur. It is expected that the latter behaviour, compared to the former, would promote a lower genetic heterogeneity among recruitment sites (Hamrick et al., 1993). Considering that *C. moschata* presents seedling banks in the understory, and that their mortality rate is lower at primate's dispersion sites when compared to the ones observed under mother trees (Moraes & Paoli, 1995), it is expected that the probability of new individuals recruitment for the reproductive phase be higher to the first ones, implying an effective gene flow, which, even occurring at low levels, might be sufficient to avoid local populational structure. However, the coefficient of gene differentiation ($\hat{G}_{ST}$ found for juvenile populations, coming from sites of "muriqui" seed dispersion, was 0.084 and highly significant (Table 5). Moreover, contingency table analysis of heterogeneity among the three distinct juvenile

Table 4. Measures of genetic variability averaged over 20 isozyme loci for 10 natural populations of *Cryptocarya* spp.

Population	Mean ± SE sample size/locus	Mean ± SE alleles/locus	Effective number of alleles ± SE	% Loci polymorphic[a]	Mean ± SE heterozygosity		Fixation Index
					Observed	H-W exp.[b]	
C. moschata							
PECB-P[c]	676.2 ± 13.9	2.0 ± 0.1	1.57 ± 0.14	75.0	0.211 ± 0.046	0.313 ± 0.045	0.326**
PECB-A[e]	141.0 ± 0.0	2.0 ± 0.1	1.62 ± 0.15	85.0	0.330 ± 0.045	0.336 ± 0.042	0.018
PECB-D1[d]	20.0 ± 0.0	1.7 ± 0.2	1.37 ± 0.16	55.0	0.182 ± 0.062	0.199 ± 0.051	0.085
PECB-D2[d]	20.0 ± 0.0	1.7 ± 0.2	1.40 ± 0.15	55.0	0.260 ± 0.065	0.226 ± 0.051	-0.150
PECB-D3[d]	20.0 ± 0.0	1.6 ± 0.2	1.39 ± 0.16	50.0	0.223 ± 0.063	0.209 ± 0.053	-0.067
PEI, NS[e]	36.0 ± 0.0	2.0 ± 0.1	1.55 ± 0.14	80.0	0.325 ± 0.055	0.310 ± 0.045	-0.048
PESM, NP[e]	9.9 ± 0.0	1.9 ± 0.1	1.46 ± 0.14	75.0	0.268 ± 0.063	0.269 ± 0.050	0.004
PESM, NSV[e]	26.5 ± 0.3	2.0 ± 0.1	1.52 ± 0.15	80.0	0.310 ± 0.051	0.297 ± 0.045	-0.044
Southern Region[f]	177.0 ± 0.0	2.0 ± 0.1	1.66 ± 0.15	85.0	0.329 ± 0.043	0.352 ± 0.043	0.065
Northern Region[g]	36.5 ± 0.4	2.0 ± 0.1	1.52 ± 0.14	80.0	0.298 ± 0.048	0.297 ± 0.043	-0.003
Total adults	213.4 ± 0.4	2.0 ± 0.1	1.66 ± 0.15	85.0	0.323 ± 0.042	0.351 ± 0.043	0.079
Total juveniles	60.0 ± 0.0	1.7 ± 0.2	1.43 ± 0.16	55.0	0.222 ± 0.059	0.227 ± 0.052	0.022
C. aschersoniana (MSG)	18.6 ± 0.6	1.9 ± 0.1	1.42 ± 0.14	70.0	0.208 ± 0.054	0.258 ± 0.044	0.193
Cryptocarya sp. (PECB)	6.6 ± 0.3	2.0 ± 0.1	1.67 ± 0.15	85.0	0.255 ± 0.064	0.393 ± 0.046	0.351

[a] A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95
[b] Unbiased estimate (Nei, 1978)
[c] seedlings
[d] juveniles
[e] adults
[f] PECB-A + PEI-NS
[g] PESM-NP + PESM-NSV
**$p < 0.01$

Table 5. Estimates of gene diversity parameters in *Cryptocarya moschata* Nees populations. H_T, total gene diversity; H_S, average gene diversity within populations; D_{ST}, average gene diversity among populations; G_{ST}, genetic differentiation between populations

Populations				
Adults (4)[a]	0.332	0.297 ± 0.044	0.035 ± 0.019	0.107**
Juveniles (3)	0.225	0.206 ± 0.050	0.019 ± 0.017	0.084**
Southern Region vs. Northern Region (2)[b]	0.338	0.322 ± 0.043	0.017 ± 0.017	0.049**
PECB-A vs. PEI-NS	0.353	0.321 ± 0.043	0.033 ± 0.026	0.093**
PESM-NP vs. PESM-NSV	0.287	0.273 ± 0.046	0.014 ± 0.009	0.049

[a] ( ) number of groups in comparison
[b] Southern Region = PECB-A + PEI-NS; Northern Region = PESM-NP + PESM-NSV
(χ^2 test: ** $p<0.01$)

populations has shown that population PECB-D1 was completely different from both PECB-D2 and PECB-D3, which were similar to each other. This may indicate that these results could be promoted by a combination of the two feeding behaviours of "muriquis" within sampled populations, with distinctive predominance of each one according to the distance from fruit source trees. Alternatively, PECB-D2 and PECB-D3 populations could probably be originated from the same local trees fed by these animals in an event of short distance dispersion, where the feeding party stayed in the same place during many hours or even camped out for several days (see Strier, 1987, 1989; Moraes, 1992). Whereas, PECB-D1 would be composed of seeds ingested from trees of other sites in an event of wide distance dispersion or by seeds of another local

site through short dispersion, since these populations were considerably far from each other. In both cases, PECB-D1 population presented lower heterozigosity than the others, and it was responsible for the significant divergence found. In this way, the role "muriquis" may play in structuring local demes of *C. moschata* could be relevant if those juveniles were not environmentally selected and recruited to be new reproductive individuals.

For adults, the coefficient of gene differentiation ($\hat{G}_{ST}$) among populations was 0.107, while among regions it was 0.049. Within regions, the coefficients of differentiation among populations were 0.093 and 0.049 in southern and northern regions, respectively (Table 5). Significant level of differentiation was found between populations PECB-A and PEI-NS, which are contiguous and relatively close, but are separated by a large physical barrier (Agudos Grandes Mountains), besides their environmental differences mainly due to altitude. For populations PESM-NP and PESM-NSV, the differentiation was non significant, and this genetic similarity between them was coherent due to their proximity and absence of physical isolation. Despite the fact that the coefficient of gene differentiation for adult populations of *C. moschata* was highly significant, its value was lower than those calculated for distinct ecological groups by Loveless & Hamrick (1984). However, according to Loveless & Hamrick (1987) this level is not so small as it might be expected for a species with great potential of gene dispersion, and it is comparable to what would be expected for groups of plants having a half-sib family structure (Moraes et al., 1999). The divergence among populations of *C. moschata* is similar to those found for predominantly outcrossed (0.118 ± 0.036), long-lived (0.077 ± 0.027), and polycarpic (0.105 ± 0.036) species (Loveless & Hamrick, 1984). When compared to values of other tropical tree species, this value is very close to those found for most species investigated (Loveless & Hamrick, 1987; Moran et al., 1989; Murawski & Bawa, 1994; Chase et al., 1995).

The average within-population gene diversity (0.297) we found for adult populations of *C. moschata* was close to that found in other studies for predominantely outcrossed (0.214 ± 0.034), with general entomophilous pollination (0.244 ± 0.066), with endozoocoric seed dispersal (0.213 ± 0.136), and late successional species (0.264 ± 0.053) (Loveless & Hamrick, 1984). The same value, when compared more specifically with those found for other tropical tree species, is close to that of *Rinorea sylvatica* (Violaceae), and *Sorocea affinis* (Moraceae; Loveless & Hamrick, 1987); and is also similar to that of *Stemonoporus oblongifolius* (Dipterocarpaceae; Murawski & Bawa, 1994). It is also close to the average value found for tropical species (0.240; Loveless & Hamrick, 1987).

Genetic distance

Table 6 shows Nei's (1972) genetic distance matrix for populations of adults of *C. moschata*, *C. aschersoniana*, and *Cryptocarya* sp. Figure 1 portrays the hierarchical structure of genetic relationships among the six populations of this genus. According to the proportion of permuted data sets that result in the formation of a node seen in the original data set, from bootstrapping over loci, the cluster grouping *C. moschata* adult populations is the only node with increased confidence, in the

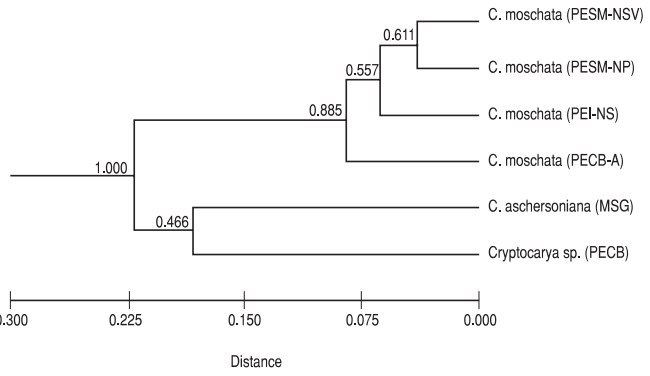

Figure 1. UPGMA cluster analysis based on Nei's genetic distances (*D*), showing the proportion of similar replicates of the original tree for 10,000 permutations. Data from 20 isozyme loci analysed in six natural populations of *Cryptocarya* spp. Cophenetic correlation: r = 0.932; Mantel test from 1,000 random permutations: p (random Z ³ observed Z) = 0.003.

tree formed by the original data. However, only six loci supported this cluster, indicating that its relative strength is not great, since there is a high heterogeneity among these loci, as described by Moraes et al. (1999). Although five loci produced tied trees, the cophenetic correlation (r = 0.932) was highly significant, indicating that the results are not misleading. However, since Nei's genetic distance assumes that populations must be in a mutation-drift equilibrium, and populations from PEI-NS and MSG (*C. aschersoniana*) were not at this equilibrium (results not shown; computed according Cornuet & Luikart, 1996 and Piry et al., 1999), this dendrogram should be cautiously considered. Additionally, the detection of bottleneck principally in MSG population is important because it can increase the risk of its extinction, since it is a fragmented and relatively isolated population.

The average genetic distance between *C. moschata* adult populations was 0.07 ± 0.03, which is above the average found in many researches on conspecific populations (; Gottlieb, 1977). Even considering that these populations were geographically distant from each other, their genetic differentiation is pronounced, as pointed out before. The average genetic distances between populations from northern and southern coastal regions were 0.040 ± 0.026 and 0.102 ± 0.044, respectively, while the genetic distance between both regions was 0.049 ± 0.026. This indicates again a higher similarity between the northern populations and the other two from the south, whose gene flow might be less pronounced due to a physical obstacle.

The average genetic distance between adult populations of *C. moschata*, *C. aschersoniana*, and *Cryptocarya* sp. was 0.221 ± 0.054 (I = 0.802), whereas it was 0.154 ± 0.055 (I = 0.857) between *C. moschata* and *C. aschersoniana* populations, and 0.157 ± 0.055 (I = 0.855) between *C. moschata* and *Cryptocarya* sp. populations. According to Nei (1987), for the majority of interspecific divergence studied cases, the value of I

Table 6. Matrix of Nei's (1972) genetic distance (*D*) for six natural adult populations of *Cryptocarya* spp. (below diagonal) and geographic distances in kilometers (above diagonal).

Population	PECB-A	PEI-NS	PESM-NP	PESM-NSV	*C. aschersoniana*	*Cryptocarya* sp.
PECB-A	0.000	25	310	280	184	0
PEI-NS	0.102	0.000	350	336	215	25
PESM-NP	0.076	0.065	0.000	30	240	310
PESM-NSV	0.077	0.059	0.039	0.000	210	280
C. aschersoniana	0.190	0.234	0.293	0.302	0.000	184
Cryptocarya sp.	0.152	0.232	0.182	0.186	0.184	0.000

ranged from 0.8 (D = 0.22) to 0.2 (D = 1.6). In this way, the present values obtained from isozymes support morphological and wood anatomy evidence (Richter, 1981) that *C. moschata* and *C. aschersoniana* are closely related species. However, due to the small number of individuals and populations of *C. aschersoniana* and *Cryptocarya* sp. analysed, a deeper study have been conducted to confirm these results and to explain the real taxonomical status of *Cryptocarya* sp., since the genetic distance between both species was not consistent.

In the present study, no relationship of genetic and geographical distance exists within *C. moschata* adult populations. There is one significant outlier (PECB-A and PEI-NS distance) which if excluded results in a positive correlation between genetic and geographical distance (r^2 = 0.718), indicating a trend to isolation by distance, as found by Moraes et al. (1999) through simple linear regression between log( ) and log(geographic distance). Geographical distances between populations ranged from 25 to 350 km.

According to Ritland (1989) two alternative patterns of hierarchical structure are possible in a dendrogram. The first is a continuous structure wherein internal branches are small. The second structure is a discrete type, in which some internal branches of the dendrogram are large due to higher order groupings. The former structure can arise from a network of small populations linked by low, constant rates of gene flow for a long period of time. The latter can result from: (i) long-term isolation of higher order groups from each other, with gene flow occurring within groups; or (ii) founding effects, where each higher order group of populations is founded from a different source population. The second pattern probably occurs between populations of *C. moschata*, although it is impossible in this case to distinguish between gene flow and phylogeny.

Acknowledgments

We thank Professor Paulo Sodero Martins (in memoriam) for his invaluable support; Instituto Florestal de São Paulo, Fundação Florestal, and Fundação José Pedro de Oliveira for permission to collect the species. Funding was provided by CAPES.

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