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Mendelian inheritance, genetic linkage, and genotypic disequilibrium at nine microsatellite loci of *Cariniana legalis* (Mart.) O. Kuntze

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ABSTRACT. *Cariniana legalis* is one of the largest tropical trees with a wide distribution in the Brazilian Atlantic rainforest. We investigated the Mendelian inheritance, genetic linkage, and genotypic disequilibrium at seven microsatellite loci specifically isolated for *C. legalis*, and at two previously developed heterologous microsatellite loci. Forty to 100 open-pollinated seeds were collected from 22 seed-trees in two populations. Using the Bonferroni correction, no remarkable deviations from the expected Mendelian segregation, linkage, or genotypic disequilibrium were detected in the nine loci studied. Only 3.7% of the tests were significant for investigations of the Mendelian proportions. On the other hand, only 2.8% of tests for linkage detection showed significance. In addition, among all pairwise tests used for investigating linkage disequilibrium, significance was found in 9.7% of the locus pairs. Our results show clear evidence that

the nine simple sequence repeat loci can be used without restriction in genetic diversity, mating system, and parentage analyses.

Key words: Brazilian Atlantic forest; Conservation genetics; Microsatellite; Population genetics; Tropical tree species

INTRODUCTION

Cariniana legalis (Mart.) O. Kuntze grows naturally throughout southeastern and northeastern Brazil, and is one of the most important historically harvested timber species. Its wood is light and is used in civil construction only for internal rooms, as well as in furniture manufacturing, because it is not very resistant to wood decay attacks. *C. legalis* is an endangered tropical tree species (FAO, 1996) that is pollinated by bees with wind-dispersed seeds (Carvalho, 2003). This species is endemic to the Atlantic Forest in Brazil and has a low population density (<1 tree/ha). Effective genetic conservation of a species requires knowledge of its mating system, genetic diversity, spatial genetic structure, and gene flow. Microsatellite markers or simple sequence repeats (SSRs) are suitable for such studies due to their very high polymorphism in terms of number of alleles (Ashley, 2010). However, for SSR markers to be used as genetic markers, it is necessary to know if their inheritance follows Mendelian rules (Brondani et al., 1998; Tarazi et al., 2010), as well as if loci are linked. Such information is particularly necessary for studies of genetic diversity, intra-population spatial genetic structure, mating systems, and gene flow because multilocus estimates are used and population genetic models are based on assumptions of Mendelian inheritance, absence of genetic linkage, and linkage equilibrium. Thus, studies related to Mendelian inheritance, absence of genetic linkage, and linkage equilibrium should be evaluated and reported when novel SSRs are developed. These were the aims of the present study. We investigated these genetic properties in seven microsatellite loci isolated from *C. legalis* by Tambarussi et al. (2013) and two heterologous microsatellite markers previously developed by Guidugli et al. (2010).

MATERIAL AND METHODS

Sampling

Open-pollinated seeds were collected from 15 seed-trees at the Floresta Estadual de Ibicatu (22° 46' S, 47° 43' W, 540 m) and from seven seed-trees in Mogi-Guaçu (22° 16' S, 47° 11' W, 568 m), both located in São Paulo State, Brazil. In Ibicatu, 40 seeds were collected per seed-tree and in Mogi-Guaçu, 50 seeds from five seed-trees, and 100 seeds from two seed-trees were collected. All fruits were directly collected from the canopy of the trees to ensure that all seeds were siblings. Cambium tissue was also collected from the trunk of the seed-trees for DNA analysis. We also collected cambium tissue from another 40 adult trees in Ibicatu and a further 19 trees in Mogi-Guaçu.

Microsatellite analysis

From all adult trees, deoxyribonucleic acid (DNA) was extracted from 100 mg adult

stem bark material per tree using AnalytikJena DNA isolation kits. Seeds were germinated in vermiculite until the cotyledons emerged, and then DNA was extracted from 15- to 20-day-old seedlings using the method of Doyle and Doyle (1987).

Nine primers were used in this study. Seven of the primers were developed by Tambarussi et al. (2013) and two (Ce07 and Ce18) were developed by Guidugli et al. (2010). Microsatellite loci were amplified with polymerase chain reaction in a 15-µL final volume using GoTaq® Colorless Master Mix containing 7.5 µL 2X GoTaq® Colorless Master Mix, 10 µM each primer, forward and reverse, 3.0 µL nuclease-free water, and 7.5 ng template DNA. The amplification program for all primers consisted of an initial denaturing step at 94°C for 1 min; followed by 35 cycles each of amplification at 94°C for 1 min, 1 min at the specific annealing temperature of each primer pair (Tambarussi et al., 2013), and 72°C for 1 min; and a final elongation step at 72°C for 10 min. Amplifications were performed using a Mastercycler (Eppendorf, Hamburg, Germany). The amplification products (2-µL total reaction volume) were separated on a Fragment Analyzer™ Automated CE System (Advanced Analytical Technologies, Inc. [AATI], Ames, IA, USA) using the dsDNA Reagent Kit, 35-500 bp. Raw data were analyzed using the PROSize version 2.0 software (AATI).

Analysis of inheritance

We used the Gillet and Hattemer (1989) method to investigate the Mendelian inheritance of the *C. legalis* SSR loci. This method compares the genotype of a heterozygous maternal tree with the segregation of its open-pollinated progeny. This method assumes that the loci have regular segregation and its alleles follow classic Mendelian inheritance patterns, which is based on three main requirements: i) regular meiotic segregation during production of ovules; ii) random fertilization of ovules by each type of pollen; iii) no selection occurred between the moment of fertilization and genotyping of the seeds. The model also assumes that there is a co-dominant relationship among all alleles. The method further requires that the following conditions are met: 1) all progeny of a tree must possess a maternal allele, and 2) in cases of heterozygous parent trees (e.g., A_iA_j, i ≠ j): a) among offspring, each individual must possess an allele of the maternal tree, A_i or A_j; b) the number of heterozygous progeny A_iA_j (n_{ij}) must equal the sum of homozygous progeny A_iA_i (n_{ii}) and A_jA_j (n_{jj}), or $n_{ij} = n_i + n_j$; and c) the number of heterozygous progeny A_iA_k (n_{ik}) must equal the number of heterozygous progeny A_jA_k (n_{jk}), or $n_{ik} = n_{jk}$, where $k \neq$ i, j. The phenotypes observed in each heterozygous seed-tree were compared with the expected 1:1 segregation pattern by means of a maximum likelihood *G*-test (Sokal and Rohlf, 1981) based on the following formula (Equation 1):

$$G = 2\left[n_i \ln\left(\frac{n_i}{E(n)}\right) + n_j \ln\left(\frac{n_j}{E(n)}\right)\right] \qquad \text{(Equation 1)}$$

where n_i and n_j are the observed number of genotypes containing alleles A_i and A_j, respectively, ln is the natural logarithm, and $E(n)$ is the expected number of genotypes for the alleles A_i and A_j based on Equation 2:

$$E(n) = 0.5\,(n_i + n_j) \qquad \text{(Equation 2)}$$

The *G*-test determines if the deviation between the observed and expected segregation is statistically significant or if deviations may be explained by chance. We also applied the Bonferroni correction for multiple comparisons (95%, $\alpha = 0.05$) to avoid false positives.

Analysis of genetic linkage between pairwise loci

To confirm the independence of allele segregation among different loci, we carried out a test of linkage between pairwise loci using genetic information from parent trees that were doubly heterozygous for two loci, and observed segregation in their progeny. In this case, the null hypothesis (H_O) is regular Mendelian segregation of 1:1:1:1. The hypothesis of regular segregation between pairwise loci was accepted or discarded based on a maximum likelihood *G*-test (Sokal and Rohlf, 1981), shown in Equation 3, performed for each progeny. For each cell the expected frequency under the null hypothesis of segregation 1:1:1:1 was calculated as:

$$G = 2\left[n_{ik} \ln\left(\frac{n_{ik}}{E(n)}\right) + n_{il} \ln\left(\frac{n_{il}}{E(n)}\right) + n_{jk} \ln\left(\frac{n_{jk}}{E(n)}\right) + n_{jl} \ln\left(\frac{n_{jl}}{E(n)}\right)\right] \quad \text{(Equation 3)}$$

where, n_{ik}, n_{il}, n_{jk}, and n_{jl} are the observed number of phenotypes A_iB_k, A_iB_l, A_jB_k, and A_jB_l, respectively, $E(n)$ is the expected number of genotypes A_iB_k, A_iB_l, A_jB_k, and A_jB_l, respectively, ln is the natural logarithm, and $E(n)$ is calculated as in Equation 4:

$$E(n) = 0.25\ (n_{ik} + n_{il} + n_{jk} + n_{jl}) \quad \text{(Equation 4)}$$

We again applied the Bonferroni correction for multiple comparisons (95%, $\alpha = 0.05$) to avoid false positives.

Analysis of linkage disequilibrium

The genotypic disequilibrium test was performed only for adult trees, since genotypic disequilibrium is obviously expected in progeny arrays because all descendants always receive a maternal allele, which generates an "apparent genotypic imbalance". The genotypic disequilibrium test was carried out using the FSTAT program (Goudet, 1995). The H_O was tested and the probability of the test was used to determine the imbalance between all pairwise loci. For the avoidance of false positives, we used a Bonferroni correction at 95% probability ($\alpha = 0.05$).

RESULTS

The results showed a significant deviation from the expected 1:1 Mendelian segregation pattern in only 22 cases of 589 tests (3.7%) (Table 1). For the Ce07, Cle12, and Cle04 loci, no deviation was observed. In the other loci, some deviations were detected in different progenies.

Table 1. Mendelian inheritance tests for nine microsatellite loci in *Cariniana legalis*.

Loci	Seed-trees	Genotypes	n1	$n_{ij} : n_i + n_j$	G_1	n2	$n_{ik} : n_{jk}$	G_2
Ce07	J04	170/186	40	20:20	0.00	0	NE	0.00
	J16	180/186	24	6:18	6.27	15	6:9	0.60
	J22	170/186	34	20:18	1.06	3	1:2	0.34
	J23	176/184	24	5:19	8.70	16	8:8	0.00
	J27	168/186	26	6:20	7.95	9	3:6	1.09
	J41	172/182	25	7:18	5.01	12	3:9	3.13
	J49	180/186	25	7:18	2.01	14	13:1	12.2
	J61	178/202	31	25:6	12.51	8	5:3	0.50
	J67	184/202	27	15:12	0.33	13	7:6	0.07
	J70	168/184	18	10:8	0.22	21	8:13	1.02
	1M	176/184	23	18:5	7.79	20	16:4	7.70
	2M	164/184	17	11:6	1.49	22	13:9	0.73
	3M	166/174	22	13:9	0.73	22	18:4	9.63
	4M	160/180	21	10:11	0.04	23	11:12	0.04
	5M	170/176	29	15:14	0.03	12	3:9	3.13
	6M	162/182	60	37:23	3.30	40	20:20	0.00
Cle09	J16	160/174	20	19:1	19.79*	20	9:11	0.20
	J23	160/176	30	19:11	2.15	10	5:5	0.00
	J27	178/186	12	8:4	1.36	28	21:7	7.32
	J28	160/176	26	16:10	1.39	14	5:9	1.15
	J30	176/182	6	0:6	NE	31	22:9	5.62
	J41	154/168	26	18:8	3.94	14	14:0	NE
	J49	152/158	22	17:5	6.91	18	15:3	8.73
	J61	170/180	16	13:3	6.70	24	23:1	24.91*
	J67	154/170	38	17:21	0.42	1	0:1	NE
	J70	152/168	26	11:15	0.61	14	8:6	0.28
	1M	168/198	34	17:17	0.00	13	12:1	10.97
	4M	150/166	39	14:25	3.14	6	0:6	NE
	5M	152/166	40	22:18	0.40	4	1:3	1.04
	7M	150/158	78	43:35	0.82	21	12:9	0.43

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Table 1. Continued.

Loci	Seed-trees	Genotypes	n1	$n_{ij} : n_i + n_j$	G_1	n2	$n_{ik} : n_{jk}$	G_2
Cle10	J04	160/166	35	30:5	19.81*	5	5:0	NE
	J06	160/166	30	17:13	0.53	9	9:0	NE
	J16	156/162	19	16:3	9.75	21	5:16	6.05
	J22	160/168	20	12:8	0.80	20	20:0	NE
	J23	162/168	26	22:4	13.72	13	6:7	0.07
	J27	162/168	24	20:4	11.64	16	10:6	1.01
	J28	160/168	31	25:6	12.51	9	9:0	NE
	J29	162/170	23	18:5	7.80	16	11:5	2.30
	J30	160/166	27	21:6	8.82	13	8:5	0.69
	J36	160/166	25	12:13	0.04	15	11:4	3.39
	J41	152/166	28	21:7	7.32	11	6:5	0.09
	J49	150/156	19	19:0	NE	20	15:5	5.23
	J61	150/164	26	9:17	2.50	14	7:7	0.00
	J67	152/166	22	11:11	0.00	16	16:0	NE
	J70	150/166	13	3:10	3.97	27	23:4	14.78
	1M	150/154	39	24:15	2.09	11	8:3	2.35
	2M	146/156	29	15:14	0.03	17	9:8	0.05
	3M	150/164	39	30:9	11.92	10	7:3	1.64
	4M	150/164	40	32:8	15.41*	10	6:4	0.40
	5M	146/162	27	11:16	0.93	22	8:14	1.65
	6M	150/166	49	27:22	0.51	48	36:12	12.55
	7M	150/156	77	52:25	9.67	22	11:11	0.00
Cle12	J16	202/216	16	9:7	0.25	24	11:13	0.17
	J22	202/216	18	8:10	0.22	21	6:15	3.90
	J28	204/210	15	2:13	9.01	26	18:8	3.94
	J30	206/220	13	9:4	1.97	27	14:13	0.03
	J36	202/216	21	4:17	8.66	19	13:6	2.64
	J41	198/204	9	1:8	6.19	30	15:15	0.00
	J49	198/216	8	5:3	0.50	31	20:11	2.65
	J61	202/218	25	9:16	1.98	14	12:2	7.92
	J67	202/220	33	18:15	0.27	7	1:6	3.90
	J70	200/230	24	16:8	2.71	16	3:13	6.73
	1M	214/220	38	26:12	5.28	10	4:6	0.40
	2M	220/230	26	19:7	5.75	23	20:3	14.07
	3M	202/216	27	12:15	0.33	23	4:19	10.63
	4M	200/230	20	9:11	0.20	30	16:14	0.13
	5M	196/222	16	10:6	1.01	34	14:20	1.06
	6M	202/230	62	33:29	0.25	38	17:21	0.42
	7M	200/214	68	31:37	0.53	31	15:16	0.03

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Table 1. Continued.

Loci	Seed-trees	Genotypes	n1	$n_{ij} : n_i + n_j$	G_1	n2	$n_{ik} : n_{jk}$	G_2
Cle04	J04	268/272	27	14:13	0.03	10	6:4	0.40
	J06	268/272	22	8:14	1.65	17	8:9	0.05
	J16	270/288	13	4:9	1.97	26	15:11	0.61
	J22	266/274	9	1:8	6.19	29	13:16	0.31
	J23	270/284	17	3:14	7.72	21	18:3	11.89
	J27	270/284	22	3:19	12.97	17	13:4	5.01
	J28	268/276	11	3:8	2.35	29	14:15	0.03
	J29	270/284	15	5:11	1.69	24	23:1	24.96*
	J30	270/274	28	12:16	0.57	12	8:4	1.35
	J36	268/274	22	8:14	1.65	18	11:7	0.89
	J41	286/290	20	2:18	14.72	19	8:11	0.47
	J49	272/288	2	0:2	NE	38	20:18	0.10
	J61	276/294	15	14:1	13.45	25	10:15	1.00
	J67	286/300	24	14:10	0.67	16	12:4	4.18
	J70	286/290	14	5:9	1.15	25	9:16	1.98
	1M	280/300	34	24:10	5.93	15	11:4	3.30
	2M	278/290	27	24:3	18.59*	23	9:14	1.09
	3M	272/286	35	9:26	8.61	11	1:10	8.54
	4M	278/288	25	12:13	0.04	25	5:20	6.63
	5M	284/294	29	26:3	20.91*	18	11:7	0.89
	6M	278/300	17	12:5	2.96	72	35:37	0.05
	7M	270/284	50	19:31	2.90	46	5:41	32.1*
Cle08	J06	156/174	22	4:18	9.64	18	12:6	2.03
	J22	176/180	7	2:5	1.33	33	27:6	14.40
	J23	152/176	25	3:22	16.31*	12	1:11	9.75
	J27	156/162	12	6:6	0.00	26	21:5	10.59
	J28	156/162	11	3:8	2.36	29	15:14	0.03
	J30	156/166	22	4:18	9.64	19	5:14	4.43
	J36	156/176	23	11:12	0.04	16	2:14	10.12
	J41	156/160	33	17:16	0.03	7	7:0	NE
	J49	172/178	14	2:12	7.93	25	16:9	1.98
	J61	154/172	24	11:13	0.17	13	11:2	6.85
	J67	152/168	24	9:15	1.52	14	5:9	1.15
	J70	158/172	18	13:5	3.69	20	14:6	3.29
	1M	148/164	24	16:8	2.71	10	4:6	0.40
	2M	148/154	18	15:3	8.73	24	1:23	24.95*
	3M	154/162	13	7:6	0.07	37	21:16	0.67
	4M	156/160	16	4:12	4.18	33	17:16	0.03
	5M	156/158	16	11:5	2.30	35	15:20	0.71
	6M	150/156	31	3:28	23.26*	58	10:48	27.08*
	7M	154/170	54	39:15	11.04	44	14:30	5.95

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Table 1. Continued.

Loci	Seed-trees	Genotypes	n1	$n_{ij} : n_i + n_j$	G_1	n2	$n_{ik} : n_{jk}$	G_2
Cle01	J22	160/166	22	22:0	NE	15	4:11	3.39
	J23	170/182	20	11:9	0.20	20	17:3	10.82
	J28	162/170	18	8:10	0.23	22	12:10	0.18
	J30	162/170	23	19:4	10.63	16	9:7	0.25
	J36	168/180	23	10:13	0.39	16	5:11	2.31
	J41	160/166	23	8:15	2.17	18	3:15	8.73
	J49	166/168	15	3:12	5.78	24	16:8	2.72
	J61	178/184	20	13:7	1.83	20	16:4	7.71
	J67	172/180	25	16:9	1.99	15	8:7	0.07
	J70	170/178	16	9:7	0.25	21	14:7	2.38
	1M	176/184	26	18:8	3.94	21	18:3	11.88
	2M	170/232	21	16:5	6.05	29	11:18	1.70
	3M	170/176	31	23:8	7.57	18	5:13	3.68
	4M	158/166	18	17:1	17.22*	30	10:20	3.39
	5M	176/180	26	21:5	10.58	24	20:4	11.64
	6M	162/176	51	38:13	12.80	44	22:22	0.00
	7M	156/162	44	28:16	3.31	53	17:36	3.20
Cle05	J04	190/202	22	16:6	4.72	18	18:0	NE
	J06	190/202	33	21:12	2.49	5	4:1	1.92
	J16	190/196	39	38:1	44.76*	1	1:0	NE
	J22	190/196	38	33:5	23.09*	2	2:0	NE
	J23	190/196	39	30:9	11.93	1	0:1	NE
	J27	190/196	39	30:9	11.93	1	0:1	NE
	J28	190/200	24	22:2	19.50*	16	7:9	0.25
	J29	190/226	11	9:2	4.82	29	18:11	1.70
	J30	188/194	29	21:8	6.04	11	8:3	2.35
	J36	188/194	17	13:4	5.02	22	14:8	1.65
	J41	188/196	11	3:8	2.36	27	14:13	0.03
	J49	186/190	16	13:3	6.74	19	4:15	6.78
	J61	188/202	23	19:4	10.63	17	12:5	2.96
	J67	188/202	29	19:10	2.84	11	8:3	2.35
	J70	188/202	19	14:5	4.44	20	14:6	3.29
	1M	186/200	32	30:2	29.39*	7	4:3	0.14
	2M	186/200	28	24:4	15.84*	14	10:4	2.65
	3M	184/186	17	0:17	NE	29	9:20	4.27
	4M	182/196	29	21:8	6.04	16	11:5	2.30
	5M	186/190	38	21:17	0.42	9	6:3	1.01
	6M	180/226	40	29:11	8.39	58	37:21	4.47
	7M	186/196	56	51:5	43.93*	41	23:18	0.61

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Table 1. Continued.

Loci	Seed-trees	Genotypes	n1	$n_{ij} : n_i + n_j$	G_1	n2	$n_{ik} : n_{jk}$	G_2
Ce18	J04	166/170	15	11:4	3.39	24	12:12	0.00
	J06	170/182	35	21:14	1.41	5	5:0	NE
	J16	164/172	21	6:15	3.99	19	13:6	2.64
	J22	166/218	21	10:11	0.05	19	16:3	9.77
	J27	166/226	15	6:9	0.60	25	23:2	20.72*
	J28	162/172	17	7:10	0.53	23	13:10	0.39
	J29	162/172	25	16:9	1.99	14	8:6	0.29
	J30	162/214	18	7:11	0.89	23	21:2	18.29
	J36	162/172	17	11:6	1.49	22	16:6	4.72
	J49	164/172	25	17:8	3.31	14	10:4	2.66
	J61	162/170	24	19:5	8.70	18	11:7	0.89
	J67	166/176	17	11:6	1.49	23	19:4	10.63
	J70	162/218	25	11:14	0.36	15	9:6	0.60
	1M	158/212	23	18:5	7.79	22	17:5	6.91
	2M	166/218	22	21:1	22.36*	24	16:8	2.71
	3M	166/180	30	21:9	4.93	16	11:5	2.30
	4M	164/184	21	17:4	8.66	23	10:13	0.39
	5M	166/218	28	23:5	12.53	19	10:9	0.05
	6M	162/218	30	23:7	8.99	65	42:23	5.63
	7M	160/228	58	53:5	46.33*	40	20:20	0.00

n1 and 2 = sample size; G_1 and G_2 = maximum likelihood G statistics for the hypothesis of $n_{ij} = n_{ii} + n_{jj}$ and $n_{ik} = n_{jk}$, respectively, with one degree of freedom. *Significance after Bonferroni's correction for $\alpha = 0.05$ (χ^2table = 15.14). NE = not estimated.

The *G*-test was used to compare the observed values with those expected under the null hypothesis for the 1:1:1:1 segregation of genotypes between two heterozygous SSR loci. After the Bonferroni correction, only 2.8% of the 594 linkage tests performed (Table 2) were significant. However, in all cases in which significant linkage was observed, it occurred in different pairs of loci of different sampled progenies. Moreover, in the largest group of sampled progenies (N = 100), all pairwise loci adjusted to the expected 1:1:1:1 Mendelian segregation. On the other hand, the majority of progeny adhered to the expected 1:1:1:1 Mendelian inheritance for the same pairs of loci analyzed.

Genotypic disequilibrium in molecular markers is one of the basic assumptions for use in genetic diversity and structure, mating system, and paternity analyses. After Bonferroni correction, the results showed no significant evidence of genotypic disequilibrium between pairwise loci. Only 9.7% of pairwise loci showed linkage (Table 3). This was most likely a consequence of the small sample size within families. Combining this result with that observed in the test of linkage between loci, it appears that this imbalance was not associated with physical linkage between the loci.

DISCUSSION

These few cases of deviation of Mendelian segregation can be influenced by sex-linkage, physical association with genes under strong selection, centers of recombination, transposable elements, or processes during meiosis such as non-disjunction or meiotic drive (Selkoe and Toonen, 2006). Some observed deviations were also likely caused by the reduced number of seedlings per progeny (ranging from 40 to100).

Again, it is happened in null hypothesis for the 1:1:1:1 segregation. With the reduced number of seedlings per progenies, deviations were expected by chance. This was apparent by the smaller deviations obtained for Mogi-Guaçu (N = 50 to 100 per seed-tree) when compared with the Ibicatu result (N = 40) samples. This suggests that the deviations found in groups of smaller sample size were sampling artifacts. Tarazi et al. (2010), using 20 seeds per progeny collected from 28 seed-trees, found two loci with 20% significant genetic linkage in *Copaifera langsdorffii*. Carneiro et al. (2012) observed similar results in *Hymenaea courbaril* with sample sizes ranging from 13 to 20 seeds per seed-tree. Both authors suggested that the small number of progenies were the likely cause of these observed deviations.

These results are expected in studies involving species with relatively large numbers of chromosomes and a small number of markers. In such situations, the probability of finding markers localized in a given chromosome is small. In general, *Cariniana* species have X = 17 chromosomes, and nine microsatellite markers were used in the present study. It is also important to mention that the majority of investigations involving wild species have been carried out using six locus markers. In only 10% of such studies were more than 10 loci used (Koskinen et al., 2004). Therefore, our study was based on more loci than are commonly used.

Also, genotypic disequilibrium can be generated by self-pollination, correlated mating, mating among relatives, genetic bottleneck effects, founder effects, and natural and artificial selection. The bottleneck effect is a possible explanation for the observed deviations in the Ibicatu population, which recently underwent strong forest fragmentation. Belaj et al. (2007) observed that among a total of 308 tests for linkage disequilibrium between pairs of loci, 6% were significant. It is known that population structure increases linkage disequilibrium in the

Table 2. Maximum likelihood G-test for testing the hypothesis of independent segregation between pairs of microsatellite loci (1:1:1:1) in *Cariniana legalis*.

Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G
Ce07xCle09	J16	12.95	Ce07xCle12	J16	14.47	Ce07xCle04	6M	1.52	Ce07xCle01	6M	0.56	Ce07xCe18	3M	5.62
Ce07xCle09	J23	7.66	Ce07xCle12	J22	2.83	Ce07xCle04	7M	5.00	Ce07xCle01	7M	3.47	Ce07xCe18	4M	3.12
Ce07xCle09	J27	12.38	Ce07xCle12	J41	10.08	Ce07xCle08	J22	11.88	Ce07xCle05	J04	4.63	Ce07xCe18	5M	4.60
Ce07xCle09	J41	8.37	Ce07xCle12	J49	11.91	Ce07xCle08	J23	17.86	Ce07xCle05	J16	13.17	Ce07xCe18	6M	3.04
Ce07xCle09	J49	9.67	Ce07xCle12	J61	11.22	Ce07xCle08	J27	15.04	Ce07xCle05	J22	5.48	Ce07xCe18	7M	2.65
Ce07xCle09	J61	9.37	Ce07xCle12	J67	4.91	Ce07xCle08	J41	18.71	Ce07xCle05	J23	11.43	Cle09xCle10	J16	5.15
Ce07xCle09	J67	3.36	Ce07xCle12	J70	2.73	Ce07xCle08	J49	6.40	Ce07xCle05	J27	5.94	Cle09xCle10	J23	0.28
Ce07xCle09	J70	13.11	Ce07xCle12	1M	1.74	Ce07xCle08	J61	5.67	Ce07xCle05	J41	8.34	Cle09xCle10	J27	8.60
Ce07xCle09	1M	2.73	Ce07xCle12	2M	13.60	Ce07xCle08	J67	7.77	Ce07xCle05	J49	9.15	Cle09xCle10	J28	2.41
Ce07xCle09	4M	12.80	Ce07xCle12	3M	2.87	Ce07xCle08	J70	2.67	Ce07xCle05	J61	4.88	Cle09xCle10	J30	8.50
Ce07xCle09	5M	11.57	Ce07xCle12	4M	6.79	Ce07xCle08	1M	1.42	Ce07xCle05	J67	1.98	Cle09xCle10	J41	12.59
Ce07xCle09	7M	1.29	Ce07xCle12	5M	5.22	Ce07xCle08	2M	4.76	Ce07xCle05	J70	14.05	Cle09xCle10	J49	11.31
Ce07xCle10	J04	4.26	Ce07xCle12	6M	0.29	Ce07xCle08	3M	6.26	Ce07xCle05	1M	0.49	Cle09xCle10	J61	28.07*
Ce07xCle10	J16	17.65	Ce07xCle12	7M	0.31	Ce07xCle08	4M	0.37	Ce07xCle05	2M	2.89	Cle09xCle10	J67	6.62
Ce07xCle10	J22	23.76*	Ce07xCle04	J04	7.09	Ce07xCle08	5M	7.31	Ce07xCle05	3M	1.70	Cle09xCle10	J70	11.24
Ce07xCle10	J23	6.29	Ce07xCle04	J16	8.72	Ce07xCle08	6M	8.71	Ce07xCle05	4M	2.85	Cle09xCle10	1M	2.72
Ce07xCle10	J27	2.62	Ce07xCle04	J22	2.89	Ce07xCle08	7M	3.79	Ce07xCle05	5M	4.08	Cle09xCle10	4M	13.43
Ce07xCle10	J41	10.90	Ce07xCle04	J23	18.23	Ce07xCle01	J22	2.92	Ce07xCle05	6M	2.09	Cle09xCle10	5M	2.75
Ce07xCle10	J49	14.92	Ce07xCle04	J27	11.01	Ce07xCle01	J23	15.70	Ce07xCle05	7M	1.15	Cle09xCle10	7M	0.45
Ce07xCle10	J61	6.58	Ce07xCle04	J41	11.19	Ce07xCle01	J41	16.05	Ce07xCe18	J04	8.09	Cle09xCle12	J16	8.06
Ce07xCle10	J67	5.26	Ce07xCle04	J49	2.47	Ce07xCle01	J49	9.81	Ce07xCe18	J16	17.04	Cle09xCle12	J28	29.58*
Ce07xCle10	J70	1.17	Ce07xCle04	J61	2.02	Ce07xCle01	J61	4.66	Ce07xCe18	J22	2.60	Cle09xCle12	J30	5.11
Ce07xCle10	1M	2.20	Ce07xCle04	J67	6.12	Ce07xCle01	J67	5.08	Ce07xCe18	J27	15.81	Cle09xCle12	J41	0.76
Ce07xCle10	2M	3.71	Ce07xCle04	J70	3.00	Ce07xCle01	J70	3.71	Ce07xCe18	J49	9.97	Cle09xCle12	J49	4.47
Ce07xCle10	3M	6.5	Ce07xCle04	1M	5.84	Ce07xCle01	1M	12.70	Ce07xCe18	J61	7.17	Cle09xCle12	J61	33.60*
Ce07xCle10	4M	3.26	Ce07xCle04	2M	5.53	Ce07xCle01	2M	5.87	Ce07xCe18	J67	9.70	Cle09xCle12	J67	4.96
Ce07xCle10	5M	3.42	Ce07xCle04	3M	9.57	Ce07xCle01	3M	7.59	Ce07xCe18	J70	2.08	Cle09xCle12	J70	4.13
Ce07xCle10	6M	2.55	Ce07xCle04	4M	1.54	Ce07xCle01	4M	2.10	Ce07xCe18	1M	8.91	Cle09xCle12	1M	1.53
Ce07xCle10	7M	0.85	Ce07xCle04	5M	9.59	Ce07xCle01	5M	13.19	Ce07xCe18	2M	4.30	Cle09xCle12	4M	6.36

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Table 2. Continued.

Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G
Cle09xCle12	5M	1.62	Cle09xCle01	J23	3.18	Cle09xCe18	J30	4.11	Cle10xCle04	J22	9.46	Cle10xCle08	J67	5.76
Cle09xCle12	7M	2.44	Cle09xCle01	J28	0.66	Cle09xCe18	J49	5.79	Cle10xCle04	J23	13.48	Cle10xCle08	J70	18.33
Cle09xCle04	J16	1.64	Cle09xCle01	J30	0.85	Cle09xCe18	J61	12.97	Cle10xCle04	J27	13.38	Cle10xCle08	1M	2.80
Cle09xCle04	J23	26.50*	Cle09xCle01	J41	5.98	Cle09xCe18	J67	8.75	Cle10xCle04	J28	5.18	Cle10xCle08	2M	9.64
Cle09xCle04	J27	13.50	Cle09xCle01	J49	8.22	Cle09xCe18	J70	3.22	Cle10xCle04	J29	2.30	Cle10xCle08	3M	2.81
Cle09xCle04	J28	1.31	Cle09xCle01	J61	11.35	Cle09xCe18	1M	9.11	Cle10xCle04	J30	1.46	Cle10xCle08	4M	0.36
Cle09xCle04	J30	14.49	Cle09xCle01	J67	3.41	Cle09xCe18	4M	6.69	Cle10xCle04	J36	9.32	Cle10xCle08	5M	0.55
Cle09xCle04	J41	1.73	Cle09xCle01	J70	6.63	Cle09xCe18	5M	4.45	Cle10xCle04	J41	3.53	Cle10xCle08	6M	5.86
Cle09xCle04	J49	3.26	Cle09xCle01	1M	7.36	Cle09xCe18	6M	1.65	Cle10xCle04	J49	4.14	Cle10xCle08	7M	2.76
Cle09xCle04	J61	10.23	Cle09xCle01	4M	15.80	Cle10xCle12	J16	4.54	Cle10xCle04	J61	2.93	Cle10xCle01	J22	16.78
Cle09xCle04	J67	6.15	Cle09xCle01	5M	6.06	Cle10xCle12	J22	22.94*	Cle10xCle04	J67	4.55	Cle10xCle01	J23	2.41
Cle09xCle04	J70	2.50	Cle09xCle01	7M	9.11	Cle10xCle12	J28	10.14	Cle10xCle04	J70	12.76	Cle10xCle01	J28	6.20
Cle09xCle04	1M	5.06	Cle09xCle05	J16	0.03	Cle10xCle12	J30	1.74	Cle10xCle04	1M	5.06	Cle10xCle01	J30	0.60
Cle09xCle04	4M	7.14	Cle09xCle05	J23	1.41	Cle10xCle12	J36	7.59	Cle10xCle04	2M	1.26	Cle10xCle01	J36	10.96
Cle09xCle04	5M	6.03	Cle09xCle05	J27	15.48	Cle10xCle12	J41	3.30	Cle10xCle04	3M	21.11	Cle10xCle01	J41	10.34
Cle09xCle04	7M	7.60	Cle09xCle05	J28	0.42	Cle10xCle12	J49	4.41	Cle10xCle04	4M	4.26	Cle10xCle01	J49	5.73
Cle09xCle08	J23	25.94*	Cle09xCle05	J30	3.20	Cle10xCle12	J61	17.76	Cle10xCle04	5M	4.30	Cle10xCle01	J61	8.12
Cle09xCle08	J27	18.44	Cle09xCle05	J41	2.26	Cle10xCle12	J67	11.15	Cle10xCle04	6M	3.48	Cle10xCle01	J67	5.06
Cle09xCle08	J28	0.32	Cle09xCle05	J49	7.53	Cle10xCle12	J70	19.02	Cle10xCle04	7M	4.85	Cle10xCle01	J70	11.89
Cle09xCle08	J30	11.77	Cle09xCle05	J61	18.18	Cle10xCle12	1M	0.86	Cle10xCle08	J06	4.26	Cle10xCle01	1M	4.04
Cle09xCle08	J41	7.21	Cle09xCle05	J67	3.40	Cle10xCle12	2M	8.23	Cle10xCle08	J22	30.61*	Cle10xCle01	2M	5.16
Cle09xCle08	J49	17.37	Cle09xCle05	J70	0.59	Cle10xCle12	3M	14.26	Cle10xCle08	J23	18.78	Cle10xCle01	3M	1.22
Cle09xCle08	J61	12.72	Cle09xCle05	1M	1.81	Cle10xCle12	4M	7.50	Cle10xCle08	J27	11.67	Cle10xCle01	4M	4.86
Cle09xCle08	J67	13.84	Cle09xCle05	4M	10.57	Cle10xCle12	5M	0.38	Cle10xCle08	J28	1.26	Cle10xCle01	5M	8.33
Cle09xCle08	J70	4.48	Cle09xCle05	5M	2.34	Cle10xCle12	6M	5.85	Cle10xCle08	J30	1.34	Cle10xCle01	6M	3.82
Cle09xCle08	1M	3.15	Cle09xCle05	7M	1.52	Cle10xCle12	7M	1.40	Cle10xCle08	J36	9.26	Cle10xCle01	7M	5.67
Cle09xCle08	4M	7.17	Cle09xCe18	J16	5.35	Cle10xCle04	J04	0.42	Cle10xCle08	J41	2.28	Cle10xCle05	J04	12.95
Cle09xCle08	5M	4.51	Cle09xCe18	J27	23.92*	Cle10xCle04	J06	1.25	Cle10xCle08	J49	9.34	Cle10xCle05	J06	4.31
Cle09xCle08	7M	2.41	Cle09xCe18	J28	2.01	Cle10xCle04	J16	1.22	Cle10xCle08	J61	10.34	Cle10xCle05	J16	7.10

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Table 2. Continued.

Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G
Cle10xCle05	J22	21.19	Cle10xCe18	J61	7.34	Cle12xCle08	J30	1.24	Cle12xCle01	7M	5.84	Cle12xCe18	3M	7.20
Cle10xCle05	J23	3.88	Cle10xCe18	J67	9.97	Cle12xCle08	J36	10.79	Cle12xCle05	J16	0.60	Cle12xCe18	4M	5.09
Cle10xCle05	J27	5.27	Cle10xCe18	J70	16.53	Cle12xCle08	J41	3.84	Cle12xCle05	J22	2.08	Cle12xCe18	5M	0.90
Cle10xCle05	J28	3.33	Cle10xCe18	1M	6.88	Cle12xCle08	J49	5.58	Cle12xCle05	J28	5.68	Cle12xCe18	6M	3.27
Cle10xCle05	J29	2.40	Cle10xCe18	2M	0.89	Cle12xCle08	J61	16.32	Cle12xCle05	J30	6.13	Cle12xCe18	7M	2.21
Cle10xCle05	J30	3.72	Cle10xCe18	3M	5.80	Cle12xCle08	J67	13.65	Cle12xCle05	J36	7.17	Cle04xCle08	J06	3.25
Cle10xCle05	J36	5.82	Cle10xCe18	4M	3.11	Cle12xCle08	J70	2.31	Cle12xCle05	J41	2.55	Cle04xCle08	J22	13.90
Cle10xCle05	J41	0.62	Cle10xCe18	5M	1.63	Cle12xCle08	1M	1.42	Cle12xCle05	J49	12.83	Cle04xCle08	J23	26.05*
Cle10xCle05	J49	11.72	Cle10xCe18	6M	9.72	Cle12xCle08	2M	18.68	Cle12xCle05	J61	14.64	Cle04xCle08	J27	12.67
Cle10xCle05	J61	12.00	Cle10xCe18	7M	1.99	Cle12xCle08	3M	3.03	Cle12xCle05	J67	4.20	Cle04xCle08	J28	4.04
Cle10xCle05	J67	4.29	Cle12xCle04	J16	1.51	Cle12xCle08	4M	4.34	Cle12xCle05	J70	1.05	Cle04xCle08	J30	0.91
Cle10xCle05	J70	12.85	Cle12xCle04	J22	2.55	Cle12xCle08	5M	2.00	Cle12xCle05	1M	0.57	Cle04xCle08	J36	7.18
Cle10xCle05	1M	1.57	Cle12xCle04	J28	2.99	Cle12xCle08	6M	7.67	Cle12xCle05	2M	13.35	Cle04xCle08	J41	12.95
Cle10xCle05	2M	2.89	Cle12xCle04	J30	2.89	Cle12xCle08	7M	4.50	Cle12xCle05	3M	3.71	Cle04xCle08	J49	2.52
Cle10xCle05	3M	3.39	Cle12xCle04	J36	6.71	Cle12xCle01	J22	3.76	Cle12xCle05	4M	7.03	Cle04xCle08	J61	9.70
Cle10xCle05	4M	2.48	Cle12xCle04	J41	6.26	Cle12xCle01	J28	4.82	Cle12xCle05	5M	0.97	Cle04xCle08	J67	5.82
Cle10xCle05	5M	2.52	Cle12xCle04	J49	2.86	Cle12xCle01	J30	0.96	Cle12xCle05	6M	3.01	Cle04xCle08	J70	3.20
Cle10xCle05	6M	1.08	Cle12xCle04	J61	16.98	Cle12xCle01	J36	4.77	Cle12xCle05	7M	1.16	Cle04xCle08	1M	2.67
Cle10xCle05	7M	0.20	Cle12xCle04	J67	8.43	Cle12xCle01	J41	14.18	Cle12xCe18	J16	4.40	Cle04xCle08	2M	14.92
Cle10xCe18	J04	2.01	Cle12xCle04	J70	3.96	Cle12xCle01	J49	6.93	Cle12xCe18	J22	13.38	Cle04xCle08	3M	8.88
Cle10xCe18	J06	5.29	Cle12xCle04	1M	7.11	Cle12xCle01	J61	18.01	Cle12xCe18	J28	4.58	Cle04xCle08	4M	6.04
Cle10xCe18	J16	4.55	Cle12xCle04	2M	12.19	Cle12xCle01	J67	3.27	Cle12xCe18	J30	9.43	Cle04xCle08	5M	3.34
Cle10xCe18	J22	27.58*	Cle12xCle04	3M	13.40	Cle12xCle01	J70	4.18	Cle12xCe18	J36	12.00	Cle04xCle08	6M	2.30
Cle10xCe18	J27	33.14*	Cle12xCle04	4M	13.35	Cle12xCle01	1M	10.37	Cle12xCe18	J49	5.38	Cle04xCle08	7M	6.45
Cle10xCe18	J28	6.04	Cle12xCle04	5M	1.41	Cle12xCle01	2M	9.83	Cle12xCe18	J61	16.70	Cle04xCle01	J22	0.38
Cle10xCe18	J29	1.27	Cle12xCle04	6M	0.59	Cle12xCle01	3M	7.18	Cle12xCe18	J67	12.40	Cle04xCle01	J23	21.41*
Cle10xCe18	J30	12.11	Cle12xCle04	7M	7.78	Cle12xCle01	4M	5.26	Cle12xCe18	J70	4.23	Cle04xCle01	J28	5.98
Cle10xCe18	J36	11.77	Cle12xCle08	J22	14.36	Cle12xCle01	5M	5.39	Cle12xCe18	1M	4.01	Cle04xCle01	J30	3.54
Cle10xCe18	J49	4.46	Cle12xCle08	J28	1.75	Cle12xCle01	6M	0.49	Cle12xCe18	2M	10.02	Cle04xCle01	J36	2.33

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Table 2. Continued.

Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G	Loci	Seed-trees	G
Cle04xCle01	J41	10.18	Cle04xCle05	3M	13.95	Cle08xCle01	J36	6.13	Cle08xCle05	5M	1.01	Cle01xCle05	J70	2.71
Cle04xCle01	J49	1.70	Cle04xCle05	4M	7.20	Cle08xCle01	J41	7.33	Cle08xCle05	6M	5.69	Cle01xCle05	1M	6.59
Cle04xCle01	J61	2.62	Cle04xCle05	5M	2.56	Cle08xCle01	J49	5.47	Cle08xCle05	7M	1.43	Cle01xCle05	2M	3.40
Cle04xCle01	J67	3.62	Cle04xCle05	6M	4.37	Cle08xCle01	J61	14.35	Cle08xCe18	J06	4.03	Cle01xCle05	3M	4.93
Cle04xCle01	J70	3.53	Cle04xCle05	7M	9.68	Cle08xCle01	J67	3.77	Cle08xCe18	J22	20.45	Cle01xCle05	4M	8.76
Cle04xCle01	1M	10.92	Cle04xCe18	J04	6.46	Cle08xCle01	J70	5.91	Cle08xCe18	J27	NE	Cle01xCle05	5M	6.74
Cle04xCle01	2M	1.88	Cle04xCe18	J06	3.13	Cle08xCle01	1M	7.16	Cle08xCe18	J28	4.99	Cle01xCle05	6M	3.15
Cle04xCle01	3M	15.31	Cle04xCe18	J16	6.79	Cle08xCle01	2M	14.01	Cle08xCe18	J30	12.59	Cle01xCle05	7M	1.75
Cle04xCle01	4M	6.83	Cle04xCe18	J22	9.70	Cle08xCle01	3M	2.79	Cle08xCe18	J36	5.59	Cle01xCe18	J22	18.08
Cle04xCle01	5M	7.49	Cle04xCe18	J27	22.58*	Cle08xCle01	4M	0.90	Cle08xCe18	J49	10.21	Cle01xCe18	J28	4.60
Cle04xCle01	6M	0.84	Cle04xCe18	J28	4.96	Cle08xCle01	5M	6.25	Cle08xCe18	J61	2.87	Cle01xCe18	J30	8.89
Cle04xCle01	7M	8.24	Cle04xCe18	J29	9.58	Cle08xCle01	6M	13.33	Cle08xCe18	J67	8.06	Cle01xCe18	J36	4.66
Cle04xCle05	J04	6.37	Cle04xCe18	J30	8.46	Cle08xCle01	7M	6.00	Cle08xCe18	J70	2.87	Cle01xCe18	J49	8.20
Cle04xCle05	J06	1.47	Cle04xCe18	J36	1.33	Cle08xCle05	J06	6.37	Cle08xCe18	1M	7.60	Cle01xCe18	J61	4.56
Cle04xCle05	J16	1.29	Cle04xCe18	J49	0.51	Cle08xCle05	J22	23.00*	Cle08xCe18	2M	12.14	Cle01xCe18	J67	7.13
Cle04xCle05	J22	1.46	Cle04xCe18	J61	4.02	Cle08xCle05	J23	24.01*	Cle08xCe18	3M	4.27	Cle01xCe18	J70	4.77
Cle04xCle05	J23	16.27	Cle04xCe18	J67	12.13	Cle08xCle05	J27	13.44	Cle08xCe18	4M	5.28	Cle01xCe18	1M	6.94
Cle04xCle05	J27	17.35	Cle04xCe18	J70	4.15	Cle08xCle05	J28	1.11	Cle08xCe18	5M	1.52	Cle01xCe18	2M	1.58
Cle04xCle05	J28	0.94	Cle04xCe18	1M	8.41	Cle08xCle05	J30	3.26	Cle08xCe18	6M	12.08	Cle01xCe18	3M	1.32
Cle04xCle05	J29	5.32	Cle04xCe18	2M	3.73	Cle08xCle05	J36	9.68	Cle08xCe18	7M	2.38	Cle01xCe18	4M	4.61
Cle04xCle05	J30	2.51	Cle04xCe18	3M	21.08	Cle08xCle05	J41	0.86	Cle01xCle05	J22	3.51	Cle01xCe18	5M	7.39
Cle04xCle05	J36	6.30	Cle04xCe18	4M	5.51	Cle08xCle05	J49	7.62	Cle01xCle05	J23	5.14	Cle01xCe18	6M	5.57
Cle04xCle05	J41	9.19	Cle04xCe18	5M	2.10	Cle08xCle05	J61	6.58	Cle01xCle05	J28	0.11	Cle01xCe18	7M	7.80
Cle04xCle05	J49	3.20	Cle04xCe18	6M	1.93	Cle08xCle05	J67	4.24	Cle01xCle05	J30	4.45	Cle05xCe18	J04	6.46
Cle04xCle05	J61	2.72	Cle04xCe18	7M	9.43	Cle08xCle05	J70	8.01	Cle01xCle05	J36	9.18	Cle05xCe18	J06	5.02
Cle04xCle05	J67	10.10	Cle08xCle01	J22	14.79	Cle08xCle05	1M	1.38	Cle01xCle05	J41	6.97	Cle05xCe18	J16	10.92
Cle04xCle05	J70	0.52	Cle08xCle01	J23	20.25	Cle08xCle05	2M	13.94	Cle01xCle05	J49	10.42	Cle05xCe18	J22	17.30
Cle04xCle05	1M	3.56	Cle08xCle01	J28	2.29	Cle08xCle05	3M	4.11	Cle01xCle05	J61	5.76	Cle05xCe18	J27	25.77*
Cle04xCle05	2M	2.85	Cle08xCle01	J30	2.43	Cle08xCle05	4M	3.88	Cle01xCle05	J67	1.25	Cle05xCe18	J28	0.29
Cle05xCe18	J49	5.91	Cle05xCe18	J70	1.52	Cle05xCe18	3M	6.56	Cle05xCe18	6M	5.83	Cle05xCe18	J29	2.47
Cle05xCe18	J61	3.08	Cle05xCe18	1M	8.03	Cle05xCe18	4M	2.63	Cle05xCe18	7M	1.35	Cle05xCe18	J30	13.74
Cle05xCe18	J67	8.30	Cle05xCe18	2M	5.20	Cle05xCe18	5M	2.11				Cle05xCe18	J36	8.55

*Significance after Bonferroni's correction for $\alpha = 0.05$, 0.00047 (χ^2 table = 21.45). G = G-test for three degrees of freedom. NE = not estimated.

Table 3. Genotypic disequilibrium between pairwise microsatellite loci in adult trees of *Cariniana legalis*.

Pairwise loci	Locations	
	Ibicatu	Mogi-Guaçu
Ce07xCle09	0.14861	0.39444
Ce07xCle10	0.07847	1.0
Ce07xCle12	0.44792	1.0
Ce07xCle04	0.35139	1.0
Ce07xCle08	0.44236	1.0
Ce07xCle01	0.05972	0.20625
Ce07xCle05	0.02014	1.0
Ce07xCe18	0.03056	0.06736
Cle09xCle10	0.00069*	0.01875
Cle09xCle12	0.00069*	0.24931
Cle09xCle04	0.07361	1.0
Cle09xCle08	0.09722	1.0
Cle09xCle01	0.00694	0.10208
Cle09xCle05	0.00069*	0.19444
Cle09xCe18	0.07153	0.21181
Cle10xCle12	0.06319	0.61667
Cle10xCle04	0.00069*	0.12083
Cle10xCle08	0.20972	1.0
Cle10xCle01	0.01736	1.0
Cle10xCle05	0.00069*	1.0
Cle10xCe18	0.00417	0.02986
Cle12xCle04	0.03542	1.0
Cle12xCle08	0.07917	0.28194
Cle12xCle01	0.47222	0.45972
Cle12xCle05	0.00069*	0.00208
Cle12xCe18	0.46458	0.33472
Cle04xCle08	0.16458	1.0
Cle04xCle01	0.09931	1.0
Cle04xCle05	0.01458	1.0
Cle04xCe18	0.68611	0.39444
Cle08xCle01	0.33889	0.16944
Cle08xCle05	0.16389	0.24861
Cle08xCe18	0.80417	0.24861
Cle01xCle05	0.00069*	1.0
Cle01xCe18	0.79722	0.29444
Cle05xCe18	0.32014	0.78403

The values represent the probability of genotypic disequilibrium after 1000 permutations of alleles among individuals. Probability after Bonferroni's corrections: $P = 0.00069$ ($\alpha = 0.05$).

genome. Guidugli et al. (2009) found significant genotypic disequilibrium in only one pair of SSR loci for *C. estrellensis*. However, Tarazi et al. (2010) observed genotypic disequilibrium between all 28 combinations in pairs of SSR loci in *C. langsdorffii*.

The seven microsatellite loci and the two heterologous microsatellite markers developed for *C. legalis* presented a Mendelian inheritance pattern, no genetic linkage, and negligible genotypic disequilibrium. Therefore, this analysis indicated that this set of SSR loci could be used without restriction in studies on the genetic diversity and structure, mating system, and parentage analysis of *C. legalis*.

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REFERENCES

Ashley MV (2010). Plant parentage, pollination, and dispersal: How DNA microsatellites have altered the landscape. *Crit. Rev. Plant Sci.* 29: 148-161.

Belaj A, Muñoz-Diez C, Baldoni L, Porceddu A, et al. (2007). Genetic diversity and population structure of wild olives from the North-western Mediterranean assessed by SSR markers. *Ann. Bot.* 100: 449-458.

Brondani RPV, Brondani C, Tarchini R and Grattapaglia D (1998). Development, characterization and mapping of microsatellite markers in *Eucalyptus grandis* and *E. urophylla*. *Theor. Appl. Genet.* 97: 816-827.

Carneiro FS, Lacerda AE, Lemes MR, Gribel R, et al. (2012). Mendelian inheritance, linkage and genotypic disequilibrium in microsatellite loci isolated from *Hymenaea courbaril* (Leguminosae). *Genet. Mol. Res.* 11: 1942-1948.

Carvalho PER (2003). Espécies Florestais Brasileiras: Recomendações Silviculturais, Potencialidades e Uso da Madeira. EMBRAPA - CNPF, Colombo.

Doyle JJ and Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry* 19: 11-15.

FAO (1996). Panel of Experts on Forest Gene Resources. Ninth Session. Food and Agriculture Organization of the United Nation, Roma.

Gillet E and Hattemer HH (1989). Genetic analysis of isoenzyme phenotypes using single tree progenies. *Heredity* 63: 135-141.

Goudet J (1995). FSTAT (Version 2.9.3.2.): a computer program to calculate F-statistics. *J. Hered.* 86: 485-486.

Guidugli MC, Campos T, Sousa ACB, Feres JM, et al. (2009). Development and characterization of 15 microsatellite loci for *Cariniana estrellensis* and transferability to *Cariniana legalis*, two endangered tropical tree species. *Conserv. Genet.* 10: 1001-1004.

Guidugli MC, Accoroni KAG, Mestriner MA, Contel EPB, et al. (2010). Genetic characterization of 12 heterologous microsatellite markers for the giant tropical tree *Cariniana legalis* (Lecythidaceae). *Genet. Mol. Biol.* 33: 131-134.

Koskinen MT, Hirvonen H, Landry PA and Primmer CR (2004). The benefits of increasing the number of microsatellites utilized in genetic population studies: an empirical perspective. *Hereditas* 141: 61-67.

Selkoe1 KA and Toonen RJ (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol. Lett*. 9: 615-629.

Sokal RR and Rohlf FJ (1981). Biometry: The Principles and Practice of Statistics in Biological Research. Copyright Ltd., New York.

Tambarussi EV, Sebbenn AM, Moreno MA, Ferraz EM, et al. (2013). Microsatellite markers for *Cariniana legalis* (Lecythidaceae) and their transferability to *C. estrellensis*. *Appl. Plant Sci.* 1: 1200493.

Tarazi R, Sebbenn AM, Mollinari M and Vencovsky R (2010). Mendelian inheritance, linkage and linkage disequilibrium in microsatellite loci of *Copaifera langsdorffii* Desf. *Conserv. Genet. Resour.* 2: 201-204.