Short Communication

Mendelian inheritance, genetic linkage, and genotypic disequilibrium for nine microsatellite loci in *Cariniana estrellensis* (Raddi) Kuntze (Lecythidaceae)

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Genet. Mol. Res. 16 (2): gmr16029653
Received February 23, 2017
Accepted March 24, 2017
Published May 4, 2017
DOI http://dx.doi.org/10.4238/gmr16029653

ABSTRACT. *Cariniana estrellensis* is one of the largest trees found in Brazilian tropical forests. The species is typical of advanced stages of succession, characteristic of climax forests, and essential in genetic conservation and environmental restoration plans. In this study, we assessed Mendelian inheritance, genetic linkage, and genotypic disequilibrium in nine microsatellite loci for a *C. estrellensis* population. We sampled and genotyped 285 adult trees and collected seeds from 20 trees in a fragmented forest landscape in Brazil. Based on maternal genotypes and their seeds, we found no deviation from the expected 1:1 Mendelian segregation and no genetic linkage between pairwise loci. However, for adults, genotypic disequilibrium was detected for four pairs of loci, suggesting that this result was not caused by genetic linkage. Based on these results, we analyzed microsatellite loci that are suitable for use in population genetic studies assessing genetic diversity, mating system, and gene flow in *C. estrellensis* populations.

Key words: Forest fragment; Genetic conservation; Jequitibá-branco; Population genetics; Tropical trees

INTRODUCTION

Cariniana estrellensis (Raddi) Kuntze (Lecythidaceae), or jequitibá-branco, has a wide geographic range distributed across Brazil, Bolivia, Paraguay, and Peru. The species is a priority for genetic conservation due to its ecological (reforestation) and commercial (wood and pulp) importance. It is currently threatened with extinction due to intense exploitation of the species as a timber resource (FAO, 2002). The species is hermaphroditic and pollinated mainly by bees of the genus *Melipona* and *Trigona*. Its winged seeds are dispersed by wind and periods of flowering and fruiting vary greatly across its range (Prance and Mori, 1979; Carvalho, 2003; Leite, 2007).

Microsatellite markers (simple sequence repeats, SSR) are a useful tool to analyze the genetics of forest species due to their high degree of polymorphism in terms of numbers of alleles. However, in order to use molecular markers as genetic markers, it is important to determine whether their inheritance follows the rules of Mendelian segregation, and whether the loci are genetically linked (Tambarussi et al., 2013; Manoel et al., 2015; Moraes et al., 2016). Studies assessing linkage among loci are also necessary because the detected loci are used to calculate averages among loci in population genetic studies; therefore, linked loci can create bias in the estimates (Guidugli et al., 2010). To enable the analysis of genetic diversity and structure, mating system, and gene flow for *C. estrellensis* herein we assess the Mendelian inheritance, genetic linkage, and genotypic disequilibrium for nine microsatellite loci developed for the species.

MATERIAL AND METHODS

The study was based on samples collected from a highly fragmented forest landscape situated in a transition zone between the Savanna and Atlantic Forest biomes. The study area covers 448.2 ha and is located in the city of Bataguassu (Mato Grosso do Sul State, Brazil), alongside the Pardo River (21°38'00"S, 52°14'02"W). All 285 adult trees found in the area were sampled and 32 seeds were collected from each of 20 selected seed trees. For the molecular analyses, we used foliar or cambial tissue from adult trees and foliar samples from seeds germinated in a nursery.

Multilocus genotyping of the *C. estrellensis* samples was performed at the HERÉDITAS/GENOMAX laboratory. We used nine microsatellite loci chosen from the 15 loci previously developed for the species (Guidugli et al., 2009). The loci were analyzed in an ABI 3100XL automatic sequencer. Based on these genotypes, a multilocus profile was defined that allows for the identification of each sample individually and enables the analyses of population genetic parameters.

The study of microsatellite locus inheritance was based on the method described by Gillet and Hattemer (1989), which compares the genotype of a heterozygous maternal tree with the segregation of its open-pollinated progenies. This method assumes that all loci have regular segregation and their alleles follow Mendelian inheritance patterns based on the following conditions: i) regular meiotic segregation during ovule production; ii) random ovule fertilization by a type of pollen; and iii) no selection between the moment of fertilization and the genotyping of seeds. The model also assumes a co-dominant relationship between all alleles. The method further requires that all progeny of a tree must possess a maternal allele, and in cases of a heterozygous mother tree (e.g., A_iA_j, $i \neq j$, the following are required: a) each individual within progeny must have one allele of the maternal tree, A_i or A_j; b) the number of heterozygous progeny A_iA_j (n_{ij}) must be equal to the sum of the number of homozygous progeny A_iA_i (n_{ii}) and A_jA_j (n_{jj}): $n_{ij} = n_{ii} + n_{jj}$; and c) the number of heterozygous progeny A_iA_k (n_{ik}) must be equal to the number of heterozygous progeny A_jA_k (n_{jk}), or $n_{ik} = n_{jk}$, in other words $k \neq i, j$. The observed segregation of each progeny of the heterozygous maternal tree for a given locus was statistically compared to that expected for the segregation hypothesis of 1:1, using the *G*-test (Sokal and Rohlf, 1981):

$$G = 2\left[n_{ij} \ln\left(\frac{n_{ij}}{E(n1)} \right) + (n_{ii} + n_{ij}) \ln\left(\frac{(n_{ii} + n_{jj})}{E(n1)} \right) \right] \qquad \text{(Equation 1)}$$

where ln is the natural logarithm, $E(n1)$ is the expected number of offspring genotypes A_iA_j (n_{ij}) and $A_iA_i + A_jA_j$ ($n_{ii} + n_{jj}$): $E(n1) = 0.5(n_{ij} + n_i + n_j)$, or:

$$G = 2\left[n_{ik} \ln\left(\frac{n_{ik}}{E(n2)} \right) + n_{jk} \ln\left(\frac{n_{jk}}{E(n2)} \right) \right] \qquad \text{(Equation 2)}$$

where $E(n2)$ is the expected number of genotypes for alleles A_iA_k (n_{ik}) and A_jA_k (n_{jk}): $E(n2) = 0.5(n_{ik} + n_{jk})$. To avoid false positives, the *G*-test was determined only when $n1$ and $n2$ was ≥ 10, and deviation from the *G*-test between the observed and expected segregation was determined as statistically significant using the Bonferroni correction for multiple comparisons (95%, $\alpha = 0.05$).

To determine if the loci were genetically linked, a test was carried out between pairs of loci using genetic information from mother trees that were doubly heterozygous for two loci (A_iA_j, B_lB_m). The segregation was recorded in their progeny. In this case, the null hypothesis (H_O) was regular Mendelian segregation of 1:1:1:1. The regular segregation hypothesis between pairs of loci was accepted or rejected based on a maximum likelihood *G*-test (Sokal and Rohlf, 1981), performed for each progeny:

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

where n_{il}, n_{im}, n_{jl}, and n_{jm} are the observed numbers of the phenotypes A_iB_l, A_iB_m, A_jB_l, and A_jB_m, respectively, and $E(n)$ is the expected number of each genotype A_iB_l, A_iB_m, A_jB_l, and A_jB_m, calculated by $E(n) = 0.25\ (n_{il} + n_{im} + n_{jl} + n_{jm})$. We again applied the Bonferroni correction for multiple comparisons (95%, $\alpha = 0.05$) to avoid false positives.

The genotypic disequilibrium test between pairwise loci was only performed with adult samples. Estimates of gene frequencies based on open-pollinated progeny arrays are biased because each progeny has at least one maternal allele, resulting in a genotypic disequilibrium. This analysis was carried out using the FSTAT software (Goudet, 1995). The probabilities of the significance test were obtained by permutation of alleles among individuals, associated with the Bonferroni correction for multiple comparisons (95%, $\alpha = 0.05$).

RESULTS

After Bonferroni correction, we found no deviation from 1:1 Mendelian segregation for the nine loci analyzed for *C. estrellensis* heterozygous trees (Table 1). Furthermore, after Bonferroni correction, we detected no deviation from 1:1:1:1 Mendelian segregation between pairwise loci, indicating that the nine loci analyzed in this study are not linked (Table 2). However, significant genotypic disequilibrium was detected between four pairs of loci among adult trees after Bonferroni correction: Ces01 x Ces02, Ces01 x Ces04, Ces01 x Ces11, and Ces04 x Ces11 (Table 3).

The P values represent the probability of genotypic disequilibrium after 1440 permutations of alleles among individuals. Value at which results are deemed significant after Bonferroni correction: $P = 0.00069$ ($\alpha = 0.05$).

DISCUSSION

Confirmation of Mendelian segregation for individual loci was confirmed based on the expected 1:1 Mendelian segregation test. Independent segregation of alleles between different loci was performed through the linkage test based on 1:1:1:1 segregation, using genetic information of doubly heterozygous mother trees and observed segregation in progenies. We found that the nine loci assessed herein present Mendelian segregation and are not linked. Thus, these molecular markers developed by Guidugli et al. (2010) can be considered as genetic markers and our results support the hypothesis that they are not located in the same chromosome linkage group. However, genotypic disequilibrium was detected between four pairs of loci for adults. Genotypic disequilibrium is largely caused by genetic linkage, natural and artificial selection, genetic bottleneck, founder effect, and genetic drift (Hartl and Clark, 2010). Among these, genetic bottleneck and genetic drift can be the result of forest fragmentation due to decreases in effective population size, resulting in a limited number of pollen donors participating in reproduction. In studying a different population of the same species, Guidugli et al. (2010) found no significant genotypic disequilibrium between the same pairwise loci assessed herein. Thus, the genotypic disequilibrium that we detected may be attributed to genetic drift caused by forest fragmentation in the study region. Studies on other tree species have also found an absence of genetic linkage with a presence of genotypic disequilibrium, including *Araucaria angustifolia* (Medina-Macedo et al., 2014), *Copaifera langsdorffii* (Tarazi et al., 2010), *Cariniana legalis* (Tambarussi et al., 2013), and *Genipa americana* (Manoel et al., 2015). The nine microsatellite loci evaluated in this study exhibit Mendelian inheritance, are

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

Locus/mother	Mother genotype	n_1	n_{ij}: n_{ii} + n_{jj}	G_1	n_2	n_{ik}:n_{jk}	G_2
Ces01							
1	158/162	6	0:6	NE	26	11:15	0.62
5	148/152	23	11:12	0.04	10	8:2	3.85
15	148/152	23	9:14	1.09	9	1:8	NE
22	152/156	23	4:19	10.63	9	9:0	NE
31	152/156	27	8:19	4.61	5	1:4	NE
66	148/152	31	16:15	0.03	1	0:1	NE
91	152/156	23	5:18	7.80	9	9:0	NE
101	146/156	22	12:10	0.18	10	1:9	7.36
123	146/152	29	17:12	0.86	3	0:3	NE
125	146/150	30	11:19	2.15	2	1:1	NE
136	148/152	23	15:8	2.16	9	0:9	NE
Ces02							
15	170/186	24	12:12	0.00	8	4:4	NE
19	182/186	22	17:5	0.18	10	8:2	3.85
21	170/182	28	9:19	3.65	4	4:0	NE
22	170/182	22	14:8	1.65	10	6:4	0.40
31	170/182	17	10:7	0.53	15	9:6	0.60
35	170/186	25	14:11	0.36	7	6:1	NE
45	170/182	25	15:10	1.00	7	4:3	NE
66	170/186	32	10:22	4.61	0	0:0	NE
96	170/186	22	11:11	0.00	10	7:3	1.64
101	182/186	12	6:6	0.00	20	13:7	1.82
119	170/186	26	12:14	0.15	6	3:3	NE
123	170/186	32	17:15	0.12	0	0:0	NE
125	170/186	32	11:21	3.17	0	0:0	NE
136	170/186	32	16:16	0.00	0	0:0	NE
225	182/186	21	9:12	0.43	11	6:5	0.09
Ces04							
1	184/204	17	8:9	0.05	15	7:8	0.06
5	204/212	13	7:6	0.07	19	10:9	0.05
15	182/216	10	8:2	3.85	22	11:11	0.00
19	182/204	19	12:7	1.33	13	2:11	6.85
21	206/216	5	1:4	NE	27	16:11	0.93
22	186/212	1	0:1	NE	31	17:14	0.29
31	186/214	15	3:12	5.78	17	12:5	2.97
35	212/216	11	6:5	0.09	21	9:12	0.43
45	182/214	11	6:5	0.09	21	14:7	2.37
64	204/216	5	5:0	NE	27	13:14	0.03
66	204/212	4	1:3	NE	28	16:12	0.57
88	178/216	15	8:7	0.06	17	12:5	2.97
91	182/186	5	3:2	NE	27	15:12	0.33
96	188/214	4	2:2	NE	28	13:15	0.14
101	214/216	15	8:7	0.06	17	7:10	0.53
119	182/216	17	8:9	0.05	15	8:7	0.06
123	204/216	3	3:0	NE	29	13:16	0.31
125	182/216	0	0:0	NE	32	14:18	0.50
136	182/200	6	3:3	NE	26	14:12	0.15
225	208/214	0	0:0	NE	32	20:12	2.02
Ces09							
1	179/183	32	16:16	0.00	0	0:0	NE
15	177/179	26	11:15	0.61	6	1:5	NE
19	177/179	21	8:13	1.20	0	0:0	NE
22	179/183	25	17:8	3.31	7	4:3	NE
35	179/183	32	14:18	0.50	0	0:0	NE
45	179/183	23	13:10	0.39	9	8:1	NE
64	177/179	26	11:15	0.61	6	5:1	NE
66	179/183	27	11:16	0.93	5	3:2	NE
88	177/183	11	4:7	0.82	21	9:12	0.43
96	177/183	5	3:2	NE	27	13:14	0.03
101	179/183	26	10:16	1.39	6	4:2	NE
123	179/183	28	14:14	0.00	4	2:2	NE
225	179/183	32	16:16	0.00	0	0:0	NE
Ces10							
21	197/199	29	14:15	0.03	3	0:3	NE
35	197/199	32	9:23	6.33	0	0:0	NE
91	197/199	32	14:18	0.50	0	0:0	NE
96	197/199	29	9:20	4.27	3	0:3	NE
101	197/199	32	14:18	0.50	0	0:0	NE
119	197/199	32	20:12	2.02	0	0:0	NE
123	197/199	27	14:13	0.03	5	4:1	NE
125	199/203	11	5:6	0.09	21	11:10	0.04
136	197/199	24	12:12	0.00	8	5:3	NE

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Table 1. Continued.

Locus/mother	Mother genotype	n_1	n_{ij}: $n_{ii} + n_{jj}$	G_1	n_2	n_{ik}:n_{jk}	G_2
Ces11							
1	206/212	15	3:12	5.78	17	9:8	0.05
5	206/210	20	13:7	1.82	12	9:3	3.13
19	208/232	14	6:8	0.28	18	12:6	2.03
21	206/232	14	6:8	0.28	18	12:6	2.03
22	230/232	15	3:12	5.78	17	8:9	0.05
31	206/230	12	6:6	0.00	20	8:12	0.80
35	210/234	6	3:3	NE	26	8:18	3.94
45	206/208	16	8:8	0.00	16	7:9	0.25
64	206/232	8	4:4	NE	24	9:15	1.51
66	206/208	18	10:8	0.22	14	7:7	0.00
88	206/218	8	2:6	NE	24	10:14	0.66
91	208/212	9	4:5	NE	23	11:12	0.04
123	206/208	25	11:14	0.36	7	4:3	NE
125	206/208	21	8:13	1.20	11	10:1	8.54
136	206/208	32	18:14	0.50	0	0:0	NE
Ces13							
1	130/142	22	14:8	1.65	10	3:7	1.64
5	140/148	13	3:10	3.97	19	12:7	1.33
15	130/142	19	13:6	2.64	13	4:9	1.97
19	130/142	22	11:11	0.00	10	7:3	1.64
21	138/142	24	14:10	0.66	8	0:8	NE
22	142/148	6	3:3	NE	26	15:11	0.61
31	130/148	4	3:1	NE	28	14:14	0.00
45	140/142	20	7:13	1.82	12	5:7	0.33
64	130/142	12	4:8	1.35	20	12:8	0.80
66	130/148	4	2:2	NE	28	14:14	0.00
88	142/148	12	3:9	3.13	20	11:9	0.20
91	138/142	18	14:4	5.88	14	5:9	1.15
101	140/148	7	3:4	NE	25	12:13	0.04
119	142/148	18	11:7	0.89	14	11:3	4.85
123	138/142	15	6:9	0.60	17	6:11	1.49
125	138/148	21	13:8	1.20	11	5:6	0.09
136	138/140	23	8:15	2.16	9	5:4	NE
225	140/142	21	5:16	6.05	11	10:1	8.54
Ces14							
15	182/184	19	8:11	0.47	13	4:9	1.97
31	184/188	24	5:19	8.70	8	2:6	NE
45	182/184	24	8:16	2.71	8	2:6	NE
88	182/184	25	12:13	0.04	7	3:4	NE
91	184/188	32	14:18	0.50	0	0:0	NE
96	182/184	30	13:17	0.53	2	1:1	NE
125	182/184	22	9:13	0.73	10	4:6	0.40
136	184/188	26	13:13	0.00	6	4:2	NE
225	182/184	32	11:21	3.17	0	0:0	NE
Ces18							
1	168/180	11	6:5	0.09	21	4:17	8.66
15	174/180	13	2:11	6.85	19	14:5	4.43
19	168/170	25	9:16	1.98	7	0:7	NE
35	168/170	28	12:16	0.57	4	2:2	NE
45	168/180	11	6:5	0.09	21	9:12	0.43
64	168/180	9	2:7	NE	23	9:14	1.09
66	170/180	15	3:12	5.78	17	5:12	2.97
88	170/180	30	14:16	0.13	2	1:1	NE
91	168/170	27	11:16	0.93	5	1:4	NE
96	166/174	9	4:5	NE	23	10:13	0.39
119	174/178	8	3:5	NE	24	21:3	15.18*
123	166/168	17	5:12	2.97	14	9:5	1.15
125	166/170	22	13:9	0.73	10	5:5	0.00
136	168/180	13	5:8	0.70	19	14:5	4.43
225	170/176	16	13:3	6.73	16	7:9	0.25

n_1 and n_2 are the sample size; G_1 and G_2 are the maximum likelihood *G* statistics for the hypothesis of n_{ij}: $n_{ii} + n_{jj}$ and n_{ik}: n_{ik}, respectively, for one degree of freedom. *Significance after Bonferroni correction for $\alpha = 0.05$ ($\chi^2 = 12.18$). NE is not estimated due to a sample size of less than ten progeny.

Table 2. Values of maximum likelihood *G*-test for the hypothesis of independent segregation between pairwise loci (1:1:1:1) of *Cariniana estrellensis*.

	Ces1 x Ces2	Ces1 x Ces4	Ces1 x Ces9	Ces1 x Ces10	Ces1 x Ces11	Ces1 x Ces13	Ces1 x Ces14	Ces1 x Ces18
1	0.35 (15)	5.12 (1)	6.45 (1)	0.35 (91)	8.32 (1)	1.14 (1)	4.76 (15)	6.64 (1)
2	6.92 (22)	1.32 (5)	10.08 (15)	3.16 (101)	5.22 (5)	1.68 (5)	1.59 (31)	5.38 (15)
3	1.60 (31)	0.40 (15)	10.20 (22)	0.26 (123)	10.30 (22)	2.37 (15)	1.60 (91)	1.74 (66)
4	5.74 (66)	5.42 (22)	1.84 (66)	3.23 (125)	5.38 (31)	7.91 (22)	8.02 (125)	2.16 (91)
5	3.50 (101)	2.08 (31)	5.83 (101)	8.37 (136)	2.46 (66)	0.45 (31)	11.51 (136)	2.16 (101)
6	4.28 (123)	0.23 (66)	6.36 (123)		2.46 (91)	1.27 (66)		2.35 (123)
7	1.74 (125)	0.40 (91)			7.32 (123)	3.87 (91)		8.02 (125)
8	3.31 (136)	1.60 (101)			6.64 (125)	0.84 (101)		11.34 (136)
9		0.35 (123)			8.03 (136)	0.90 (123)		
10		1.73 (125)				3.00 (125)		
11		6.49 (136)				10.12 (136)		
	Ces2 x Ces4	Ces2 x Ces9	Ces2 x Ces10	Ces2 x Ces11	Ces2 x Ces13	Ces2 x Ces14	C2 x C18	Ces4 x Ces09
1	0.29 (15)	10.08 (15)	0.33 (21)	1.12 (19)	1.92 (5)	6.51 (15)	3.06 (15)	5.17 (1)
2	3.38 (19)	12.51 (19)	7.63 (35)	8.22 (21)	1.12 (19)	2.17 (31)	4.04 (19)	9.70 (15)
3	2.81 (21)	0.92 (22)	4.00 (6)	7.10 (22)	8.76 (21)	13.12 (45)	6.68 (35)	14.04 (19)
4	1.40 (22)	3.26 (35)	7.61 (101)	1.19 (31)	1.19 (22)	5.15 (96)	5.67 (45)	1.27 (35)
5	3.89 (31)	3.92 (45)	1.71 (119)	7.14 (35)	1.79 (31)	11.51 (125)	3.69 (66)	7.17 (45)
6	3.50 (35)	3.25 (66)	3.75 (123)	3.71 (45)	5.94 (45)	2.49 (136)	2.02 (96)	2.56 (64)
7	4.16 (45)	0.23 (96)	2.21 (125)	5.08 (66)	2.19 (66)	2.67 (225)	5.66 (119)	3.66 (66)
8	3.13 (66)	7.71 (101)	0.55 (136)	6.14 (123)	3.75 (101)		7.33 (123)	0.44 (66)
9	0.94 (96)	1.94 (123)		0.35 (125)	4.14 (119)		0.97 (125)	1.11 (96)
10	6.17 (101)	1.64 (125)		0.76 (136)	2.49 (123)		15.97 (136)	13.09 (101)
11	2.67 (119)				0.67 (125)		0.05 (225)	6.03 (123)
12	3.98 (123)				1.34 (136)			7.51 (225)
13	0.80 (125)				0.52 (225)			
14	1.51 (136)				0.52 (225)			
15	0.44 (225)							
	Ces4 x Ces10	Ces4 x Ces11	Ces4 x Ces13	Ces4 x Ces14	Ces4 x Ces18	Ces9 x Ces10	Ces9 x Ces11	Ces9 x Ces13
1	3.69 (21)	6.34 (1)	3.00 (1)	7.06 (15)	3.71 (1)	7.79 (35)	6.85 (1)	4.21 (1)
2	9.54 (35)	1.94 (5)	2.43 (5)	1.01 (31)	1.63 (15)	3.89 (96)	16.80 (19)	9.80 (15)
3	0.98 (91)	5.05 (15)	1.50 (19)	2.89 (45)	5.61 (19)	2.74 (101)	4.19 (22)	7.67 (19)
4	3.82 (96)	5.42 (21)	10.13 (21)	0.72 (88)	3.23 (35)	6.22 (123)	2.17 (35)	2.04 (22)
5	2.96 (101)	5.20 (22)	2.13 (22)	0.58 (91)	2.69 (45)		8.28 (45)	0.17 (35)
6	0.30 (119)	1.53 (131)	4.59 (31)	1.97 (6)	2.70 (64)		2.64 (64)	3.54 (45)
7	1.35 (123)	1.90 (35)	0.76 (45)	6.27 (119)	2.81 (66)		0.44 (66)	4.36 (64)
8	1.94 (125)	0.33 (45)	0.65 (64)	5.67 (125)	1.52 (88)		0.11 (88)	1.74 (66)
9	0.17 (136)	1.25 (64)	0.98 (66)	2.32 (136)	1.38 (91)		0.06 (96)	1.56 (88)
10		0.18 (66)	0.18 (88)	5.27 (225)	1.78 (96)		8.51 (123)	9.34 (101)
11		0.00 (88)	0.40 (91)		6.84 (119)			4.47 (123)
12		0.50 (91)	6.20 (101)		6.54 (123)			4.15 (225)
13		0.24 (96)	1.84 (119)		1.12 (125)			
14		5.23 (123)	4.75 (123)		9.26 (136)			
15		0.58 (125)	0.48 (125)		4.99 (225)			
16		1.65 (136)	4.07 (136)					
17			3.54 (225)					
	Ces9 x Ces14	Ces9 x Ces8	Ces10 x Ces11	Ces10 x Ces13	Ces10 x Ces14	Ces10 x Ces18	Ces11 x Ces13	Ces11 x Ces14
1	14.29 (15)	6.31 (1)	2.53 (21)	9.19 (21)	0.90 (91)	12.5 (35)	3.53 (1)	1.76 (31)
2	4.57 (45)	8.02 (15)	7.68 (35)	1.68 (91)	7.38 (96)	2.47 (91)	1.90 (5)	2.54 (45)
3	0.38 (88)	10.04 (19)	0.06 (91)	1.37 (101)	2.49 (125)	4.30 (96)	2.56 (19)	3.23 (88)
4	3.70 (96)	2.85 (35)	7.20 (123)	5.43 (119)	4.82 (136)	4.77 (119)	8.73 (21)	1.83 (91)
5	10.42 (225)	5.99 (45)	3.04 (125)	1.47 (123)		3.88 (123)	8.57 (22)	7.89 (125)
6		1.79 (64)	1.00 (136)	2.15 (125)		3.93 (125)	0.90 (31)	3.78 (136)
7		3.81 (66)		4.15 (136)		7.02 (136)	1.90 (45)	
8		1.46 (88)					2.79 (64)	
9		2.36 (96)					1.30 (66)	
10		5.84 (123)					0.30 (88)	
11		7.48 (225)					5.42 (91)	
12							4.75 (123)	
13							2.00 (125)	
14							1.73 (136)	
	Ces11 x Ces18	Ces13 x Ces14	Ces13 x Ces18	Ces14 x Ces18				
1	4.99 (1)	7.69 (15)	3.40 (1)	7.52 (15)				
2	2.82 (19)	1.71 (31)	0.06 (5)	3.74 (45)				
3	2.84 (35)	7.29 (45)	3.08 (15)	0.63 (88)				
4	2.72 (45)	1.48 (88)	4.14 (19)	5.94 (91)				

Continued on next page

Table 2. Continued.

	Ces1 x Ces2	Ces1 x Ces4	Ces1 x Ces9	Ces1 x Ces10	Ces1 x Ces11	Ces1 x Ces13	Ces1 x Ces14	Ces1 x Ces18
5	2.14 (64)	0.79 (91)	143 (22)	3.23 (96)				
6	1.91 (66)	8.56 (125)	3.21 (45)	11.50 (125)				
7	1.68 (88)	4.49 (136)	0.50 (64)	11.96 (136)				
8	2.25 (91)	2.17 (225)	2.12 (66)					
9	4.49 (123)		1.82 (88)					
10	0.59 (125)		1.85 (91)					
11	5.79 (136)		7.13 (119)					
12			5.60 (123)					
13			1.63 (125)					
14			5.70 (136)					
15			1.52 (225)					

*Significance after Bonferroni correction for $\alpha = 0.05$ ($\chi^2 = 20.73$). G = G-test for three degrees of freedom.

Table 3. Genotypic disequilibrium between pairwise microsatellite loci for *Cariniana estrellensis* adult trees.

Pairwise loci	P value	Pairwise loci	P value
Ces01 x Ces02	0.00069	Ces04 x Ces13	0.00278
Ces01 x Ces04	0.00069	Ces04 x Ces14	0.10833
Ces01 x Ces09	0.20833	Ces04 x Ces18	0.12847
Ces01 x Ces10	0.00139	Ces09 x Ces10	0.60556
Ces01 x Ces11	0.00069	Ces09 x Ces11	0.84514
Ces01 x Ces13	0.03611	Ces09 x Ces13	0.09653
Ces01 x Ces14	0.26458	Ces09 x Ces14	0.32431
Ces01 x Ces18	0.06042	Ces09 x Ces18	0.00556
Ces02 x Ces04	0.08681	Ces10 x Ces11	0.43750
Ces02 x Ces09	0.61111	Ces10 x Ces13	0.01111
Ces02 x Ces10	0.19931	Ces10 x Ces14	0.08264
Ces02 x Ces11	0.00139	Ces10 x Ces18	0.85000
Ces02 x Ces13	0.09028	Ces11 x Ces13	0.01181
Ces02 x Ces14	0.03403	Ces11 x Ces14	0.17361
Ces02 x Ces18	0.06597	Ces11 x Ces18	0.41667
Ces04 x Ces09	0.16319	Ces13 x Ces14	0.02222
Ces04 x Ces10	0.20486	Ces13 x Ces18	0.00417
Ces04 x Ces11	0.00069	Ces14 x Ces18	0.52431

not linked, and segregate independently. These loci are therefore suitable for population genetic analyses, which can generate precise estimates of genetic diversity, spatial genetic structure, mating system, and contemporary gene flow for *C. estrellensis*.

ACKNOWLEDGMENTS

Research supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; Project #2014/02675-8) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors would like to thank FAPESP for financial support provided to T.Y.K. Kubota (Scholarship #2014/02675-8) and CNPq for research funding to A.M. Sebbenn. We also extend a special thank to Universidade Estadual Paulista/UNESP and to Dr. Evelyn Nimmo for her correction of the English in the manuscript.

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