Genetics and Molecular Research

Environmental variations drive polyploid evolution in neotropical *Eugenia* species (Myrtaceae)

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ABSTRACT. Polyploidy is one of the most important mechanisms of speciation and diversification in plant evolution. Polyploidy results in genetic variation among individuals of the same species and even between populations, and may be responsible for differences in environmental tolerance between populations of the same species. This study determined chromosome numbers of *Eugenia* L. (Myrtaceae, x = 11) for 26 populations of 14 species by conventional cytogenetic techniques. Nine species (13 populations) were diploid (2n = 2x = 22), but diploid and/or polyploid cytotypes were found in the other five species (13 populations), with 2n = 33, 2n = 44, and 2n = 55. Data on chromosome number/ploidy level for other *Eugenia* species/populations were collected from the literature and included in this cytogeographic

analysis. For each collection point (32 species and 62 populations), environmental variables were recorded using georeferencing techniques through the DIVA-GIS v.7.5 program. Environmental variables such as temperature, altitude, rainfall, solar radiation, soil type, and vegetation were analyzed with the R program, using Mann-Whitney and chi-square tests, principal component analysis, and graphic analyses, such as scatterplots, boxplots, and barplot. Polyploid and diploid populations had different spatial distribution patterns and were found in areas subjected to different environmental conditions. Polyploid individuals were collected from locations with more adverse environmental conditions, usually at higher elevations than the diploid individuals. Polyploidy allows species to occur at locations with varying environmental conditions. As diploidy and polyploidy occur under different environmental conditions, species with cytotypes exhibit wide environmental tolerance.

Key words: Chromosomes; Karyotype; Neotropics; Myrtaceae; Cytogeography; Polyploidy

INTRODUCTION

The existence of a given population in a particular region is determined by the tolerance of individuals within the population to a set of biotic or abiotic factors and environmental conditions that affect at least one stage of its development (Begon et al., 2007). Abiotic factors include climatic, edaphic, and hydrological factors. Conversely, biotic factors include interactions between organisms/species in an ecosystem, namely predation, parasitism, or competition (Begon et al., 2007).

The environmental tolerance range (ETR) varies between the minimum and maximum values for each environmental factor (Begon et al., 2007). The ETR may be narrower or wider depending on the species and the related environmental factors (IBGE, 2004). In this way, species with wide tolerance to various environmental factors tend to present wide geographical and ecological distribution (Aguiar and Gaglianone, 2012). ETR is not constant for all individuals, and genetic variability permits the existence of individuals that respond differently to a certain environmental factor within a population. This is better illustrated in heterogeneous habitats, in which several individuals present different adaptive strategies or phenotypical plasticity (distinct phenotypes) (Via et al., 1995).

Polyploidy promotes genetic variability between individuals or populations of a given species (Schifino-Wittmann, 2004), and co-operates with differences in ETR. Polyploidy is considered to be an important evolutionary force promoting sympatric speciation and diversification in plants, and occurs in up to 80% of angiosperms (Otto and Whitton, 2000). Polyploids are able to colonize pioneer habitats, which would permit their occurrence in different environments to the diploid parents. Sometimes, cytotypes of the same species occur in distinct geographical regions (Levin, 2002) that present ecological differences such as temperature, rainfall, and radiation levels (Otto and Whitton, 2000; Schifino-Wittmann, 2004). Polyploids may arise as the result of autopolyploidy or allopolyploidy (Schifino-Wittmann, 2004). Risso-Pascotto et al. (2006) described a probable allopolyploid (2n = 6x

= 54) in *Brachiaria brizantha* (Poaceae), with a high percentage of meiotic abnormalities. This cytotype could have resulted from chromosomal doubling of a triploid derived from interspecific hydridization.

In fact, the effect of polyploidy in ecological processes is a relevant issue for ecologists, botanists, and geneticists. Johnson et al. (2003) noted that little is known about the mechanisms that maintain cytotype separation, despite their importance. The way in which polyploidy affects the tolerance of individuals to environmental factors and the geographical pattern of diploids and polyploids remain unclear (Johnson et al., 2003; Soltis et al., 2010). Researchers reinforce the importance of quantitative and statistical inferences to provide evidence of the differences between diploids and polyploids (Johnson et al., 2003).

Polyploidy affects important ecological characters in species, such as pollination, geographical distribution, life form, and growth form (Thompson et al., 2004). Due to this, we speculate that ETR would be influenced by polyploidy. Abiotic factors such as temperature and water stress modify the frequency of unreduced gametes (Ramsey and Schemske, 1998). However, it is unknown whether there is a pattern of environmental conditions that are related to the occurrence of polyploidy, or which environmental conditions influence the persistence and natural distribution of polyploids (Johnson et al., 2003). The study of widely distributed species enables us to understand how polyploidy influences the diversification and geographical distribution of closed related taxa, permitting the determination of a pattern of local environmental conditions in which the polyploids are found.

Eugenia L., which has approximately 1009 species (Govaerts et al., 2016) occurring from Mexico and the Caribbean to Argentina, is considered to be the second most diverse genus in neotropical Myrtaceae (Govaerts et al., 2016; Sobral et al., 2016) and one of the most speciose genera from tropical America. Approximately 384 species are native to Brazil (Sobral et al., 2016). Only 24 *Eugenia* species (2.3% of the species diversity) have known chromosome numbers, which is a small amount considering the diversity of the genus. Analyses of the morphology of mitotic chromosomes and chromosome behavior during meiosis are scarce for Myrtaceae (Costa and Forni-Martins, 2007; Costa, 2009). Studies have noted the polyploid cytotypes in *Eugenia*, *Myrcia* DC. ex. Guill. and *Psidium* L. with 2x, 3x, 4x, and 6x populations (Costa and Forni-Martins, 2006a,b, 2007; Costa et al., 2008; Costa, 2009). Polyploid cytotypes are known for innumerous species, as the invasive species *Euphorbia heterophylla* L. (Euphorbiaceae), with 2n = 14, 26, 28, 32, or 56 (Aarestrup et al., 2008).

In this context, the present study aimed to determine the pattern of environmental conditions and the distribution of ploidy in 32 species of *Eugenia* from 62 different collections distributed in eastern Brazil (Table 1). More than two populations were analyzed for some species, such as *E. punicifolia* (Kunth) DC., *E. dysenterica* DC., *E. hyemalis* Cambess., *E. klotzschiana* O. Berg, and *E. pitanga* (O. Berg) Kiaersk. (Table 1).

We hypothesized that polyploidy extends the tolerance of species, thus allowing the occurrence of species at different sites with wide variations in environmental conditions. We predict that polyploid species occur in environments of higher altitude, high temperature variability, low rainfall (low water availability), chemically poor, newly formed soils, and high levels of radiation.

The specific objectives were: i) to determine the chromosome number of populations collected from different vegetation types under different conditions of rainfall, temperature, altitude, and soil types, aiming to correlate environmental patterns with chromosome numbers; and ii) to determine similarities in environmental factors at the sites of diploid and polyploid populations.

Table 1. Summary of environmental conditions associated with the 62 georeferenced populations of 32 *Eugenia* species collected in this study and available in the literature.

Species (populations)	Chromosome number/Ploidy level	Altitude (m)	Variation of AAT (°C)	AARain (mm^3)	AARad ($kW \cdot h \cdot m^2$ per day)	Soil type	Vegetation type	References
E. aurata Cambess. (Pop1)	2n = 2x = 22	616	12.03	113.42	6123.27	AVA	AT	Forni-Martins and Martins (2000)
E. aurata Cambess. (Pop1)	2n = 4x = 44	800	10.98	114.67	5792.58	AVA	S	This study
E. bimarginata DC. (Pop1)	2n = 3x = 32	800	10.98	114.67	5792.58	AVA	S	Forni-Martins and Martins (2000)
E. brasiliensis Lam. (Pop1)	2n = 2x = 22	734	10.98	114.67	5934.78	AVA	S	Costa and Forni-Martins (2006a)
E. brasiliensis Lam. (Pop2)	2n = 2x = 22	575	12.63	111.00	5874.27	AVA	FES	Costa and Forni-Martins (2006a)
E. crenata Vell. (Pop1)	2n = 2x = 22	25	7.88	116.75	5109.82	AVA	FOD	Costa et al. (2008)
E. cyclophyla O. Berg (Pop1)	2n = 2x = 22	24	6.50	114.67	4437.05	AVA	FOD	Franco-Cairo et al. (2009)
E. dysenterica DC. (Pop1)	2n = 3x = 33	1400	11.55	129.67	5755.35	NL	S	Costa and Forni-Martins (2006a)
E. dysenterica DC. (Pop2)	2n = 2x = 22	1039	11.11	135.08	5510.97	CH	S	Costa and Forni-Martins (2006)
E. dysenterica DC. (Pop3)	2n = 2x = 22	1039	11.11	135.08	5510.97	CH	S	This study
E. florida DC. (Pop1)	2n = 2x = 22	700	12.13	117.83	5975.21	AVA	S	Costa (2009)
E. hirta O. Berg (Pop1)	2n = 4x = 44	4	8.68	177.67	4122.14	AVA	FES	Amorin et al. (2012)
E. hyemalis Cambess. (Pop1)	2n = 2x = 22	1066.8	11.88	128.50	5862.55	AVA	S	Costa et al. (2008)
E. hyemalis Cambess. (Pop2)	2n = 2x = 22	1200	10.63	127.75	5522.50	AVA	FOD	Costa and Forni-Martins (2006a)
E. hyemalis Cambess. (Pop3)	2n = 4x = 44	1400	11.55	129.67	5755.35	NL	S	Costa and Forni-Martins (2006a)
E. involucrata DC. (Pop1)	2n = 2x = 22	603	11.66	109.58	5934.78	AVA	AT	Costa and Forni-Martins (2006a)
E. involucrata DC. (Pop2)	2n = 2x = 22	603	11.66	109.58	5934.78	AVA	AT	This study
E. klotzschiana O. Berg (Pop1)	2n = 2x = 22	800	10.98	114.67	5896.33	AVA	S	Costa and Forni-Martins (2006a)
E. klotzschiana O. Berg (Pop2)	2n = 3x = 33	800	10.98	114.67	5896.33	AVA	S	Costa and Forni-Martins (2006a)
E. klotzschiana O. Berg (Pop3)	2n = 2x = 22	1130	11.33	137.50	5539.99	CH	S	This study
E. linearifolia O. Berg (Pop1)	2n = 2x = 22	917	11.97	82.17	5773.82	AVA	FED	Costa (2009)
E. luschnathiana (O. Berg) KIotzsch ex B.D Jacks. (Pop1)	2n = 2x = 22	15	6.53	151.00	4059.91	AVA	FES	Pedrosa et al (1999)
E. mosenii (Kausel) Sobral (Pop1)	2n = 2x = 22	742	10.20	114.50	4461.09	AVA	AT	Costa and Forni-Martins (2006a)
E. mosenii (Kausel) Sobral (Pop2)	2n = 4x = 44	4	9.46	205.67	4508.70	CH	FOD	This study
E. multicostata D. Legrand (Pop1)	2n = 2x = 22	4	9.46	205.67	4385.57	CH	FOD	Costa et al. (2008)
E. pitanga (O. Berg) Kiaersk (Pop1)	2n = 2x = 22	578	12.03	113.42	6123.27	AVA	AT	Costa and Forni-Martins (2006a)
E. pitanga (O. Berg) Kiaersk (Pop2)	2n = 4x = 44	578	12.03	113.42	6123.27	AVA	AT	This study
E. pitanga (O. Berg) Kiaersk (Pop3)	2n = 2x = 22	575	12.63	111.00	5874.27	CH	FES	Costa and Forni-Martins (2006a)/This study
E. punicifolia (Kunth) DC. (Pop1)	2n = 2x = 22	734	10.98	114.67	5896.33	AVA	S	Costa and Forni-Martins (2007)
E. punicifolia (Kunth) DC. (Pop2)	2n = 4x = 44	1066.8	11.88	128.50	5755.35	NL	S	This study
E. punicifolia (Kunth) DC. (Pop3)	2n = 3x = 33	1207.6	10.94	116.67	5921.53	CH	RV	This study
E. punicifolia (Kunth) DC. (Pop4)	2n = 4x = 44	1207.6	10.94	116.67	5921.53	CH	RV	This study
E. punicifolia (Kunth) DC. (Pop5)	2n = 3x = 33	546	12.63	112.17	5893.71	AVA	FES	Costa and Forni-Martins (2006a)
E. punicifolia (Kunth) DC. (Pop6)	2n = 3x = 33	730	12.43	103.67	5768.02	CH	SGL	This study
E. punicifolia (Kunth) DC. (Pop7)	2n = 4x = 44	730	12.43	103.67	5768.02	CH	SGL	This study
E. punicifolia (Kunth) DC. (Pop8)	2n = 4x = 44	1106	11.76	74.50	5529.15	AVA	AT	This study
E. punicifolia (Kunth) DC. (Pop9)	2n = 4x = 44	1113	11.16	69.75	5307.68	AVA	AT	This study
E. punicifolia (Kunth) DC. (Pop10)	2n = 3x = 33	895	10.58	71.50	6103.26	AVA	S	This study
E. punicifolia (Kunth) DC. (Pop11)	2n = 4x = 44	700	10.76	79.25	5536.57	AVA	SEA	This study
E. punicifolia (Kunth) DC. (Pop12)	2n = 2x = 22	736	12.08	112.25	5772.82	AVA	S	Costa and Forni-Martins (2006a)
E. punicifolia (Kunth) DC. (Pop13)	2n = 4x = 44	4	8.68	177.67	4122.14	AVA	FES	Amorin et al. (2012)
E. pyriformis Cambess. (Pop1)	2n = 3x = 33	580	12.61	112.17	5893.71	AVA	FES	Costa and Forni-Martins (2006a)
E. stictopetala DC. (Pop1)	2n = 2x = 22	700	10.76	79.25	5536.57	AVA	SEA	This study

Continued on next page

Table 1. Continued.

Species (populations)	Chromosome number/Ploidy level	Altitude (m)	Variation of AAT (°C)	AARain (mm^3)	AARad ($kW \cdot h \cdot m^2$ per day)	Soil type	Vegetation type	References
E. stigmatosa DC. (Pop1)	2n = 2x = 22	700	11.66	109.58	5934.78	AVA	AT	Costa et al. (2008)
E. tumescens B. S. Amorim & M. Alves (Pop1)	2n = 2x = 22	4	7.94	143.17	4189.96	AVA	FES	Amorin et al. (2012)
E. umbrosa O. Berg (Pop1)	2n = 2x = 22	4	7.94	143.17	4189.96	AVA	FES	Amorin et al. (2012)
E. uniflora L. (Pop1)	2n = 2x = 22	580	11.73	108.92	5934.78	AVA	AT	This study
E. uniflora L. (Pop2)	2n = 2x = 22	580	11.73	108.92	5934.78	AVA	AT	This study
E. uniflora L. (Pop3)	2n = 2x = 22	600	11.66	109.58	5934.78	AVA	AT	This study
E. uniflora L. (Pop4)	2n = 2x = 22	55	8.03	98.42	5109.82	AVA	FOD	This study
E. uniflora L. (Pop5)	2n = 2x = 22	734	10.98	114.67	5896.33	AVA	S	Costa et al. (2008)
E. uniflora L. (Pop6)	2n = 2x = 22	15	6.53	151.00	4059.91	AVA	FES	Costa (2009)
E. uniflora L. (Pop7)	2n = 2x = 22	4	7.94	143.17	4189.96	AVA	FES	Amorin et al. (2012)
Eugenia sp1 (Pop1)	2n = 2x = 22	700	11.66	109.58	5934.78	AVA	AT	This study
Eugenia sp2 (Pop1)	2n = 2x = 22	4	11.50	109.83	5934.78	AVA	AT	Costa (2009)
Eugenia sp3 (Pop1)	2n = 2x = 22	4	11.50	109.83	5934.78	AVA	AT	Costa (2009)
Eugenia sp4 (Pop1)	2n = 2x = 22	4	9.46	205.67	4385.57	CH	FOD	Costa (2009)
Eugenia sp5 (Pop1)	2n = 2x = 22	4	9.46	205.67	4385.57	CH	FOD	Costa (2009)
Eugenia sp6 (Pop1)	2n = 5x = 55	700	12.13	117.83	5881.24	AVA	S	This study
Eugenia sp7 (Pop1)	2n = 2x = 22	4	9.46	205.67	4385.57	CH	FOD	This study
Eugenia sp8 (Pop1)	2n = 2x = 22	4	9.46	205.67	4385.57	CH	FOD	This study
Eugenia sp9 (Pop1)	2n = 2x = 22	4	9.46	205.67	4385.57	CH	FOD	This study

Soil: AVA = lixisol, CH = cambisol, NL = leptosol. Vegetation: S = savanna, FES = semideciduous seasonal forest, FOD = dense rain forest, AT = area of ecological tension, RV = altomontanos refuges, SGL = woody-grassy savanna, SEA = wooded steppe savanna, FED = deciduous forest.

MATERIAL AND METHODS

Material collection

Biological material (roots or seeds) was collected from the eastern region of Brazil, including areas in the states of Ceará, Bahia, Minas Gerais, São Paulo, Rio de Janeiro, and Federal District. Voucher materials of these collections are deposited in the Herbarium UEC (Universidade Estadual de Campinas) and in the Herbarium EAC (Universidade Federal do Ceará).

Chromosome counts and literature review

To obtain mitotic metaphases, seeds were germinated at 28°-30°C. Root tips were pretreated with 2 mM 8-hydroxyquinoline for 24 h, at 8°C and fixed in Farmer's solution (Costa and Forni-Martins, 2006, 2007). For slide preparation, samples were frozen (-20°C) and stained using the Giemsa technique (Guerra, 1983). The slides were examined by light microscopy, and meiotic and mitotic cells with good chromosome condensation and spreading were photographed with a photomicroscope. We searched the literature for chromosome numbers/ploidy levels of other *Eugenia* species/populations (Table 1).

Collection and analysis of environmental variables

For all the populations analyzed here and obtained from the literature review, we collected data on ploidy levels and produced maps of ploidy distribution using the DIVA-GIS software 7.5 (Hijmans et al., 2012). For each collection site, we recorded temperature, rainfall, and altitude data.

Climate information available in DIVA-GIS for each population was organized in Excel spreadsheets for statistical analyses. Solar radiation data were extracted from shapes plotted in DIVA-GIS available at the National Organization for Environmental Data System (PROBE) site (http://sonda.ccst.inpe.br/publicacoes/atlas_solar.html). Similarly, data on soil and vegetation were extracted from shapes plotted in DIVA-GIS available at the Brazilian Institute of Geography and Statistics (IBGE) site (http://mapas.ibge.gov.br/tematicos/).

The recorded environmental variables were analyzed in R. Data on environmental variables were divided into two groups, namely, those associated with polyploid and diploid populations. Polyploids and diploids were examined to determine the similarity of environmental conditions under which they occur. Non-parametric tests were used to determine the significance of these differences, since the data were not normally distributed (Morettin and Bussab, 2010).

The Wilcoxon-Mann-Whitney test was used to compare mean and median altitude, annual rainfall, rainfall in the rainy and dry periods, annual direct radiation and radiation in the seasons, annual temperature variation (maximum and minimum temperature), and temperature variation in summer, fall, winter, and spring. Chi-squared test was used to construct a graph showing the frequency distribution of soil types of polyploids and diploids and to determine the significance of any differences.

We applied principal component analysis (PCA) to determine the environmental conditions most correlated with the occurrence of polyploids and diploids.

RESULTS

Chromosome counts and ploidy distribution

The chromosome numbers of 26 populations of 14 different *Eugenia* species were determined (Table 1 and Figure 1). There were 13 polyploid populations, of which three showed a ploidy level equal to 2n = 3x = 33, nine populations showed a ploidy level equal to 2n = 4x = 44, and one population was pentaploid (2n = 5x = 55). Note that two populations of *E. punicifolia* showed polyploid sympatric speciation (pop5 and pop6). There were 13 diploid populations with chromosome number equal to 2n = 2x = 22 (Table 1).

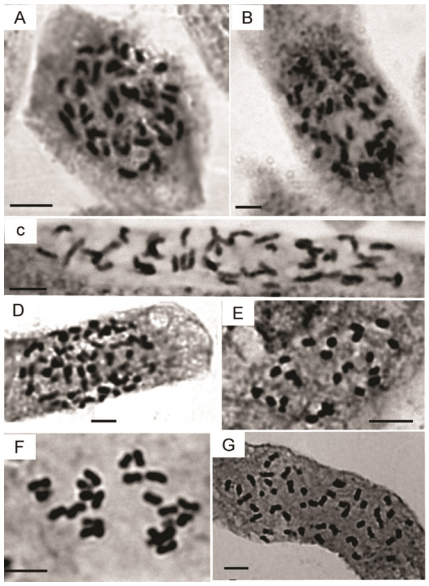

Figure 1. Mitotic metaphase of *Eugenia* species. **A.** *Eugenia punicifolia* (3x). **B.** *E. pitanga* (4x). **C.** *E. punicifolia* (4x). **D.** *Eugenia* sp6 (5x). **E.** *E. uniflora* (2x). **F.** *E. stictopetala* (2x). **G.** *E. mosenii.*

Our data, along with that published in the literature, show that the following *Eugenia* species had diploid (2x) and polyploid cytotypes (4x): *E. aurata* O. Berg, *E. hyemalis*, *E. mosenii* (Kausel) Sobral, and *E. pitanga*. *E. dysenterica* and *E. klotzschiana* presented cytotypes 2x and 3x, while *E. punicifolia* had three cytotypes (2x, 3x, and 4x). A further four species showed only one polyploid number: *E. bimarginata* (2n =3x-1 = 32), *E. pyriformis* (2n = 3x = 33), *E. hirta* (2n = 4x = 44), and *Eugenia* sp6 (2n = 5x = 55) (Table 1).

Maps showing the geographical distribution of polyploids and diploids generated in DIVA-GIS are shown in Figure 2. A total of 62 populations of 32 *Eugenia* species are plotted.

Polyploid cytotypes showed a distinct geographical distribution to diploid cytotypes (Figure 2), occurring more frequently in high-altitude areas. Some populations showed diploid and polyploid cytotypes in sympatry, such as *E. klotzschiana* and *E. pitanga*. In those populations, we identified individuals with diploid and polyploid chromosome numbers within the same population. Given the high number of populations, *E. punicifolia* was analyzed separately.

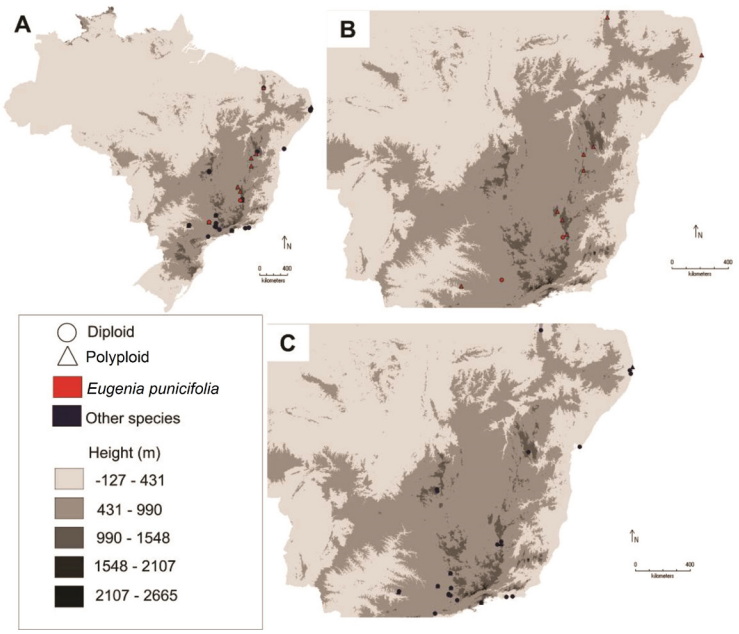

Figure 2. Distribution map of populations of *Eugenia* species. **A.** Diploid populations are represented by circles and polyploid populations are represented by triangles. **B.** Populations of *E. punicifolia*; diploid populations are represented by red circles and polyploid populations are represented by red triangles. **C.** Populations of other *Eugenia* species; diploid populations are represented by blue circles and polyploid populations by blue triangles.

Environmental variables x ploidy levels

The pattern of environmental factors under which polyploids occur was significantly different from that of diploids, as described in Table 1. Here, we present the results for each environmental feature.

Altitude

Altitude data for polyploid and diploid populations were compared. The central tendency (median and mean) of altitude of polyploid populations was significantly different and greater than the median altitude of diploids ($W = 240$, $P < 0.05$). The boxplot in Figure 3 shows the median value and the distribution of altitude values for diploid and polyploid populations.

Annual temperature variation

Variation in the annual temperature (maximum minus the minimum temperature of each month for each population) was compared between diploids and polyploids. Variation in the annual temperature at sites of polyploid occurrence was significantly different and higher than that of diploids ($W = 67002.5$, $P < 0.05$). A boxplot of the data on annual temperature variation illustrates this difference (Figure 4A).

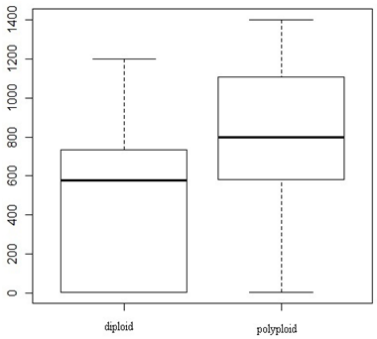

Figure 3. Boxplot of altitude of diploids and polyploids from all populations of *Eugenia* sampled in this study.

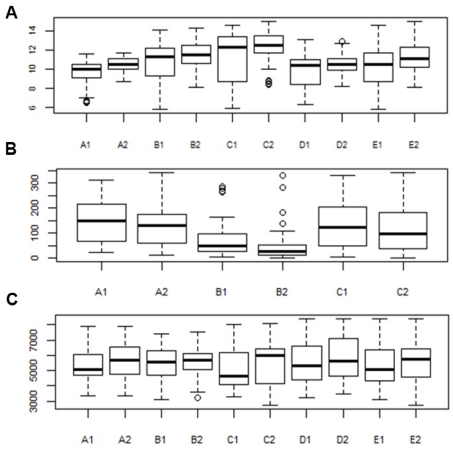

Figure 4. Boxplot showing temperature variation (**A**) over the seasons (A = summer; B = autumn; C = winter; D = spring) and along the year (E). Numbers represent the ploidy level (1 = diploid; 2 = polyploid); **B.** rainfall in the rainy (A) and dry (B) periods and along the year (C). Numbers represent the ploidy level (1 = diploid; 2 = polyploid). **C.** Direct solar radiation over the seasons (A = summer; B = autumn; C = winter; D = spring) and along the year (E). Numbers represent the ploidy level (1 = diploid; 2 = polyploid).

Annual rainfall

Annual rainfall (rainfall data for each month throughout the year) was compared between diploids and polyploids. There were significant differences in the annual rainfall between groups ($W = 67152.5$, $P < 0.05$). Figure 4B shows that polyploids experience lower rainfall throughout the year.

Rainfall in the rainy period

Rainfall in the rainy and dry periods was analyzed to consider variations in rainfall throughout the year (Figure 4B). Comparison of rainfall in the rainy period between diploids and polyploids indicated there were no significant differences ($W = 7733.5$, $P = 0.05307$). The rainiest period corresponded to the summer months.

Rainfall in the dry period

Rainfall in the dry period was significantly lower for polyploids ($W = 9430.5$, $P < 0.05$). The driest period corresponded to the winter months (Figure 4B).

Annual direct solar radiation

Solar radiation reaching the Earth surface, or direct radiation, was analyzed and values throughout the year were compared between diploids and polyploids. Differences between the groups were significant ($W = 50329.5$, $P < 0.05$). The boxplot shows that polyploid populations receive higher levels of radiation throughout the year (Figure 4C).

Soil classes

Soil classes were analyzed herein as an environmental variable. Soil types on which diploids occurred were lixisol and cambisol (Table 1 and Figure 5A). Diploid populations of *Eugenia* occurred on lixisol (77.5%) and cambisol (22.5%). Polyploids were found on three classes of soil, lixisol (61%), cambisol (24%), and leptosol (14%) (Table 1 and Figure 5A).

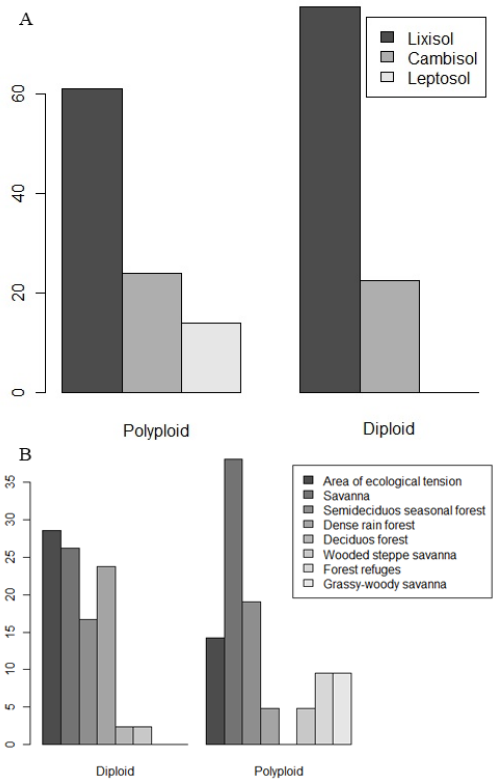

Figure 5. Barplot of percentage of diploid and polyploid populations (**A**) occurring on different soil types, and percentage of vegetation types of diploid and polyploid populations **(B)**.

The frequency observed for each soil class was compared between diploids and polyploids. The differences between the expected and observed frequencies of polyploids and diploids were significant for the class leptosol (chi-squared = 14, $P < 0.05$). This difference is visualized in the barplot for the leptosol class (Figure 5A).

Vegetation types

The types of vegetation in which *Eugenia* species occur and the frequency of diploid and polyploid distribution are presented in Table 1 and Figure 5B. The difference between the observed and expected frequency between polyploids and diploids was significant for the following vegetation types: savanna (chi-squared = 4.0114, $P < 0.05$), area of ecological tension (transition areas) (chi-squared = 4.1831, $P < 0.05$), dense rain forest (chi-squared = 13.7654, $P < 0.05$), grassy-woody savanna (Chi-squared = 9.52, $P < 0.05$), and highland refuges (chi-squared = 9.52, $P < 0.05$). Polyploids were more frequent in savannas, whereas diploids were mainly collected in transition areas between savanna and tropical rain forest.

Figure 6 illustrates the distribution of all analyzed data (Figure 6). The relationship between ploidy and those variables described above are presented graphically. The effect of altitude on the other variables is also shown. At high altitudes, temperature and rainfall decrease while radiation increases (Figure 6). The PCA separated the populations into four groups (Figure 7A). The groups were formed according to the quantitative environmental variables analyzed. Populations closest geographically and under similar environmental conditions were located within the same quadrant (Figure 7B).

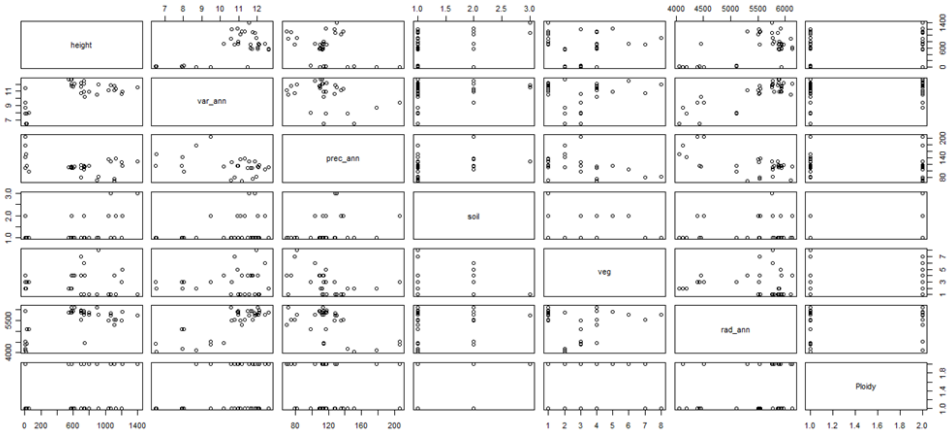

Figure 6. Scatterplot of the matrix containing data on environmental variables. Annual rainfall (prec_ann), annual temperature variation (var_ann), vegetation types (veg), annual radiation (rad_ann). Qualitative variables: Ploidy: (1) diploid - 2x (2) polyploidy - 3x, 4x or 5x. Vegetation: 1) savanna, 2) semideciduous seasonal forest, 3) dense rain forest, 4) area of ecological tension, 5) forest refuges, 6) grassy-woody savanna, 7) wooded steppe savanna, 8) deciduous forest. Soil type: 1) lixisol, 2) cambisol, 3) leptosol.

Quantitative environmental variables influenced the distribution of *Eugenia* species the most, since axis 1 was formed by those variables and explained 60% of the data variability. In the PCA ordination graph, the most similar populations were located close to each other. The distribution of polyploids was mainly associated with altitude (Figure 8).

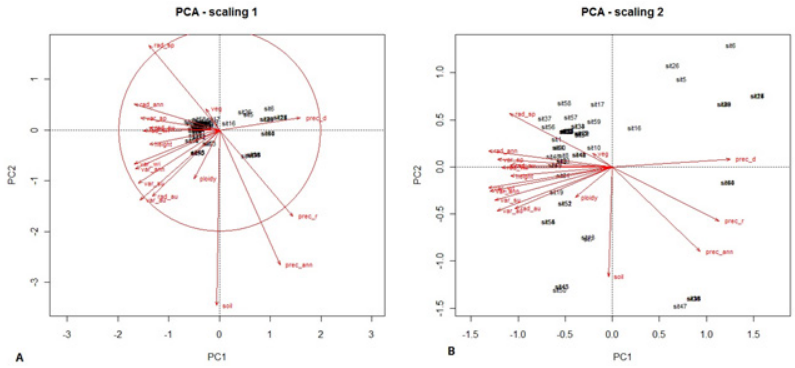

Figure 7. Principal component analysis (PCA): **A.** Separation of *Eugenia* populations into four groups. **B.** Populations with associated variables.

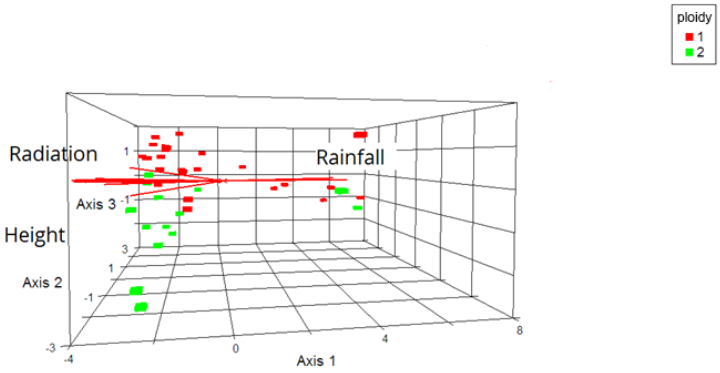

Figure 8. Arrangement of *Eugenia* populations (represented by parallelepipeds) according to the variables (represented by red lines). Ploidy level: (1) diploid, (2) polyploid.

DISCUSSION

We recorded only one unpublished chromosome number, 2n = 2x = 22 for *E. stictopetala* DC. (Table 1), and we found several records of ploidy levels until no recorded as for species *E. aurata*, *E. mosenii*, and *E. punicifolia* (Table 1). For some populations, the records are consistent with the findings of Costa and Forni-Martins (2006, 2007).

Generally, polyploid and diploid populations exhibited distinct spatial distribution patterns, which were related to different environmental conditions. The species that showed polyploid cytotypes, mainly *E. punicifolia*, have a wide geographical distribution, and occur in different types of soil and vegetation, at high altitudes and high radiation, and under a wide range of temperature and rainfall conditions. Polyploid cytotypes of other species occurred in regions differing in at least one environmental factor in relation to the diploid cytotypes.

The occurrence of polyploid cytotypes in sites with environmental conditions different from those found where diploids occur, indicates that polyploids exhibit environmental tolerance for those factors, unlike diploids of certain species. Thus, in general, considering the diploid and polyploid cytotypes, this species has increased environmental tolerance due to polyploidy.

Broad environmental tolerance allows the species to colonize and settle in different locations, which tends to expand the geographical distribution of the species. Populations subjected to different selection pressures start to accumulate genetic and phenotypic differences. This diversification process, via the production of polyploid cytotypes, is responsible for the classification of cytotypes into infraspecific categories, due to difficulties in species identification (Schifino-Wittmann, 2004).

Researchers use the term "robust" to describe the ability of polyploid individuals to colonize and settle under conditions that are adverse for diploid individuals of the same species (de Wet, 1980). Conditions are considered adverse if they do not allow the establishment of diploids. The genetic robustness of polyploids is explained by the high genetic variability in these individuals due to the high number of chromosomes. In addition, chromosomes of different species can sometimes become activated in the genome (Osborn et al., 2003; Soltis et al., 2010).

The high genetic variability responsible for polyploid "robustness" results in wide ecological tolerance. This explanation can be applied to species of *Eugenia*. Polyploidy increased the environmental tolerance of the species for at least one environmental factor. *E. punicifolia* had the largest number of polyploid cytotypes; for this species, polyploidy resulted in the formation of triploid and tetraploid cytotypes. It is not coincidental that this was the most diverse species in relation to environmental conditions under which its populations occurred. *E. punicifolia* was found in seven vegetation types, three soil types, at altitudes from 4 m to over 1300 m. The populations were found in sites where temperature varied by more than 12°C, on average, and both in drier (77 mm^3) and wetter (177 mm^3) regions.

In addition, this species is difficult to identify, given the morphological variation among individuals of the same species, resulting in many synonyms (Sobral et al., 2016). The species has polyploid populations, which exhibit morphological differences and occur in different places, resulting in its wide distribution in Brazil. Thus, it is likely that polyploidy was involved in the evolutionary history of this species. Morphological differences between different ploidy levels resulted from genetic differences. However, it remains unclear how such differences affect the spatial distribution of cytotypes (Johnson et al., 2003). The explanation suggested herein is that ecological and genetic variations contribute to the establishment of polyploids (Husband, 2000), thus permitting the spread of species with different levels of ploidy. The mechanism is that seen previously, termed as diversification via the formation of polyploid cytotypes.

The genetic variability of polyploids, translated ecologically into broad environmental tolerance, enabled the establishment and maintenance of populations in regions with different environmental conditions. Different environmental factors represent different selection pressures that act on the population, which along with high genetic variability, are responsible for their diversification (Begon et al., 2007). *E. punicifolia* illustrates the pattern found in the analysis of 62 populations of *Eugenia* in this study, which supports the initial hypothesis.

Altitude

We noted differences in altitude between diploid and polyploid populations, since the climatic conditions of high altitude regions are different from those of low-altitude regions (Azócar et al., 2007). In this way, if the altitudes differ, other environmental factors would also differ. This shows that polyploids occur at higher altitudes than diploids. At high altitudes, environmental conditions are considered to be more severe (Mocochinski, 2006), and the ability to survive these environmental factors differs from low altitude regions. Consequently, for a

species occurring at low altitudes to survive and establish in high altitude regions, it must have broad environmental tolerance to environmental factors that change depending on the altitude.

Temperature

Temperature is an environmental condition that changes at high altitudes (Sakai and Larcher, 1987). In high altitude regions, the minimum temperature is low, and there is little variation between seasons. Conversely, there is large variation in daily temperature (Table 1). Thus, altitude is an important variable with respect to the distribution limits of plant species since not all species can support rapid changes in temperature or lower temperatures. Polyploid populations that occur in areas of high altitude support larger variations in temperature than diploid populations. Polyploidy increased the limits of temperature supported by species by extending the tolerance to low temperatures and daily temperature variations. The increase in thermal amplitude may influence several physiological processes, such as photosynthesis, with effects on the ecological characteristics of the species, including the time of germination (Mondo et al., 2010). The effect observed in this study was that the polyploid cytotypes, when found at a high altitude, expanded the geographical distribution of the species.

Rainfall

Rainfall governs plant development. Many plant species do not tolerate water stress in response to both excess and lack of water in the soil (Sá et al., 2004). Thus, the amount and distribution of rainfall throughout the year in a region limits the establishment of plant species. Polyploid populations can occur under conditions of lower rainfall than diploid populations. In both dry and rainy periods, the amount of rainfall was significantly lower where polyploids occurred. The lowest amount of rainfall is positively correlated to lower water availability for plants (Krupek and Fritz, 2011). For plants to survive under conditions of low water availability, several physiological, morphological, and developmental changes are triggered (Nepomuceno et al., 2001). Those changes derive from the plant's genetic repertoire and as polyploids have high genetic variability, they are able to tolerate environments where rainfall is lower. Thus, polyploid populations were found in places with greater water scarcity compared with diploid populations, which increases the tolerance of species to this environmental factor.

Radiation

The level of radiation influences the distribution of plant species, since light energy is converted into chemical energy, which drives metabolic processes in the plant (Zeiger and Taiz, 2006). Plants differ in their tolerance to radiation levels, which is understandable in view of their importance to plant survival (Zeiger and Taiz, 2006). Additionally, some plants exist that tolerate larger or smaller levels of radiation. Researchers use the terms sun plants and shade plants to refer to the ability of individuals to survive under higher or lower levels of light energy, respectively. Increased tolerance to radiation is an adaptive trait that be advantageous for the species. In the present study, the amount of direct radiation received by polyploid populations was significantly greater than that received by diploid populations. This indicates that polyploid cytotypes are able to withstand higher levels of radiation, which extends the limits of environmental tolerance to this factor. The ability to support higher levels of radiation

allows polyploids to establish in open areas, where the amount of light reaching the plants is higher, such as grasslands and fields, which was confirmed in the present study.

Soil types

Plants take nutrients and water from the soil. However, soils differ in their physical and chemical structure, the amount and size of the clumps, and in the proportion of chemical elements, organic matter, and humus. These factors alter the amount and availability of nutrients and water. Consequently, plants have adaptations and specializations permitting their occurrence in certain types of soil (Benites et al., 2003). Comparison between the expected and observed frequencies of polyploid and diploid populations on different soil types indicated that they differ in relation to the class leptosol. Leptosols are a class of underdeveloped soils in relation to pedogenetic processes (Jacomine, 2009). In this sense, the mechanical structure of this soil and its physical and chemical characteristics differ in relation to cambisol and lixisol.

The soil thickness does not exceed 0.50 m (Mocochinski, 2006), which may limit root growth, especially tap roots. The concentration of nutrients, such as phosphorus, and water availability may be low, which also limits plant growth. Thus, the conditions provided by this type of soil restrict the establishment of plant species and may be considered adverse (Benites et al., 2003). The establishment of plants on leptosol requires the adaptation of individuals to specific conditions. Such adaptations are present in polyploids, which were the only plants found in this type of soil. Thus, polyploidy increased the environmental tolerance to soil, permitting the occurrence of species in different types of soil.

Type of vegetation

Plants make up different vegetation types, which differ in the dominance of life forms, density of individuals, leaf lifespan, structure, and physiognomy (Coutinho, 2005). Those differences reflect the main limiting physical factors such as climate, soil, and fire (Coutinho, 2005). Polyploid populations of *Eugenia* have been able to establish in vegetation types different to those used by diploid cytotypes. In cases where diploids and polyploids occurred in the same type of vegetation, there were differences in the altitude, soil type, or amount of radiation. In this way, the occurrence of a species in more than one type of vegetation is indicative of wide environmental tolerance, particularly if the types of vegetation are found in environments that differ with respect to other factors that influence plant establishment.

Polyploid cytotypes were more frequent in savannas. Considering the occurrence frequencies in savanna (38.09%), wooded steppe savanna (9.52%), and grassy-woody savanna (9.52%), about 57% of polyploid cytotypes were found in this environment. The observed frequencies of polyploids and diploids differed significantly from the expected frequencies. Polyploids occur in savanna more frequently than would be expected by chance, as well as in grassy-woody savanna vegetation in which only polyploid populations were collected.

Diploid populations occur more frequently in areas of ecological tension between savanna and rain forest (27.5%). Areas of ecological tension are regions containing two types of vegetation (IBGE, 2004). These areas are common in the Cerrado biome, considered to be a mosaic of vegetation types, representing the area from where most of the diploid species were collected. The observed frequency of diploids collected in areas of ecological tension and dense rain forest were significantly higher than the observed frequency of polyploids.

The division of groups in the PCA reflects the region of occurrence of the population, thus populations present in regions with similar environmental conditions are grouped together. Both the diploid and polyploid populations collected in the northeastern region, and in coastal regions, were influenced by rainfall. Populations collected in the northeast were influenced by rainfall during the dry period, and populations collected on the coast were grouped by similarities in annual rainfall and in the rainy period. Polyploid populations collected in the southeastern and central-western region, were grouped by altitude, while diploid populations collected in this region responded mainly to similarities in radiation.

Moreover, environmental variables affecting each group correspond to the most prominent characteristic of each region, which showed similar variance between components. The northeast region presents low rainfall and long periods of drought, such that rainfall in the dry period was the main variable influencing the components of this group. Polyploids of southeastern and central-western regions were collected at high altitudes, consequently, this was the variable most closely associated with these populations.

Populations located near the coast, in dense rain forests, have high levels of rainfall. In this way, the annual rainfall and that in the rainy period were the variables most associated with populations of this group. We found diploid populations collected in the southeastern and central-western regions in forested areas, which determine the entry of radiation; therefore, this was the variable that most influenced the representatives of this group.

Environmental distribution pattern

Diploids were found in vegetation types typical of milder climates, with high rainfall levels, low temperature variation, deep soil types, and appropriate structural, chemical, and physical conditions for large trees. Large vegetation affects the levels of radiation reaching the surface. Forested areas have low albedo (surface reflection coefficient), which decreases the amount of radiation that reaches the surface and is reflected. As described, radiation received by diploids was lower than that received by polyploids. This was due to increased cloud cover and rainfall in those areas (Pereira et al., 2006). Therefore, environmental factors affecting sites where diploids occur are mild.

Conversely, polyploids were found in savanna vegetation, in which plants are of smaller size and the trees are more scattered, contributing to a higher incidence of radiation on the surface, in addition to the altitude increase. The climate was more adverse, the temperature variation between seasons and over the year was greater, reaching 15°C in a few months. Water availability was lower given the low level of rainfall, the slope of the area, typical of mountain regions and the soil type, especially for the absence of mechanical structure for water storage, such as cambisol and lixisol.

Even cambisol and lixisol in high slope regions, such as populations collected at high altitudes, are leached and eroded by rainwater, leading to a lack of nutrients and water, and a change in the shape and size of the clumps causing defects in the mechanical structure of the soil. Given the high genetic variability, polyploidy established in environments where conditions are more adverse. From an ecological point of view, polyploidy extended the limits of environmental tolerance of the species.

Spatial distribution pattern

The spatial distribution pattern of polyploids followed an altitudinal gradient. To understand this result, it is sufficient to know that the climate changes according to altitude, that is, the higher the altitude, the more adverse the conditions. As polyploids are more robust than diploids, they tolerate these environmental conditions, increasing their frequency with increasing altitude. Additionally, climate changes in a similar way, depending on both altitudinal and latitudinal gradients. In this sense, the spatial distribution of polyploids worldwide can be understood by their environmental tolerance. The environmental tolerance may also explain the separation between cytotypes of species.

In conclusion, polyploidy is very frequent in *Eugenia* (x = 11), and there are both diploid (2n = 2x = 22) and polyploid (2n = 3x = 33, 2n =4x = 44 and 2n = 5x = 55) species. Some species showed diploid and polyploid cytotypes, such as *E. aurata*, *E. hyemalis*, *E. mooseni,* and *E. pitanga* (2x and 4x), *E. dysenterica* and *E. klotzschiana* (2x and 3x), and *E. punicifolia* (2x, 3x, and 4x). The cytogeographical analysis applied to 32 species and 62 populations revealed a different spatial distribution pattern and the existence of different environmental conditions according to the level of ploidy. Polyploidy allows the occurrence of species at locations where environmental conditions have a wide range of variation. Polyploid individuals are usually found at higher altitudes and under more adverse environmental conditions (large temperature variation, low amount of rainfall and great water scarcity, large level of radiation, underdeveloped soils, and open vegetation, such as savanas). Thus, species with diploid and polyploid cytotypes had a broader environmental tolerance, and occurred under different environmental conditions, altitude, soil types. and vegetation.

Conflicts of interest

The authors declare no conflict of interest.

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