Demography, and genetic and morphological variability of the endangered *Sophronitis sincorana* (Orchidaceae) in the Chapada Diamantina, Brazil

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Received October 3, 2006; accepted April 13, 2007 Published online: July 2, 2007 © Springer-Verlag 2007

Abstract. We carried out a demographic study and evaluated the genetic and morphological variability in five populations of the endangered Sophronitis sincorana (Orchidaceae) endemic to Northeastern Brazil, based on allozyme and morphometric analyses. Plant density was approximately 0.5 plants/m^2 , and the projected total number of plants was approximately 50,000 individuals. However, fruit set and recruitment of individuals are rare. The genetic variability was very high in all populations $(P = 100, A = 3.0-3.5, H_e = 0.33-0.48)$, and all populations presented similar values of morphological variability. Low genetic and morphological structuring were found in the species $(F_{ST} = 0.053, A_{MRPP} = 0.018)$. The elevated coefficient of endogamy encountered in populations of S. sincorana indicates the occurrence of structuring within the populations. The lack of correlation between morphological and genetic variation in this species indicates that none of the markers examined should be used separately for either conservation purposes.

Key words: Allozyme, campo rupestre, Chapada Diamantina, conservation, morphometrics, Orchidaceae, *Sophronitis sincorana*, variability.

Introduction

"Campo rupestre" vegetation occurs in the Northeastern and Southeastern regions of Brazil, mainly in the states of Bahia and Minas Gerais. The low, principally herbaceous vegetation form of campo rupestre that grows on sandy and rocky soils, as well as the herbaceous-shrubby form that grows on rock outcrops (principally quartzite, sandstones, and gneiss) are almost exclusively found at elevations above 900 m (Giulietti and Pirani 1988, Giulietti et al. 1997). Campo rupestre vegetation has one of the highest degrees of speciesrichness and endemism among the numerous vegetations forms found in Brazil, and the Chapada Diamantina is one of the major areas of occurrence of this ecosystem (Giulietti and Pirani 1988, Giulietti et al. 1997). Due to the discontinuity of the mountain ranges and rock outcrops in the Chapada Diamantina, many species (especially rupicolous ones) are encountered in scattered populations. Apparently, the discontinuous nature of the plant populations has led to the high diversity and endemism found there (Giulietti and Pirani 1988, Borba et al. 2001a, Jesus et al. 2001).

Among the angiosperm families that occur in the campo rupestre, the Orchidaceae, Eriocaulaceae, and Cactaceae are among those with the greatest diversity and highest numbers of endemic species, and can be considered families typical of this formation (Giulietti and Pirani 1988, Giulietti et al. 1997). Additionally, these families share in common high ornamental and commercial values for many of their component species. While many species of Orchidaceae and Cactaceae are cultivated for commercial purposes, collecting from natural sources is still very common in Brazil (and in many parts of the world), in spite of prohibitions of international commerce of species collected in the wild (Appendix II of the Convention on International Trade in Endangered Species - CITES). Many species of Eriocaulaceae are commercially collected as straw-flowers although specifically prohibited by the Brazilian national environmental agency (IBAMA). The internal and external commerce in dried flowering stalks of these species is very lucrative, but rarely have attempts been made at cultivation (Giulietti et al. 1988). The extraction and commercialization of native species of campo rupestre plants now constitutes an extinction risk for some species with restricted distribution in the campo rupestre, especially the Orchidaceae, Eriocaulaceae, and Cactaceae.

Sophronitis sincorana (Schltr.) Van den Berg & M.W.Chase was originally described from the Serra do Sincorá Range in the Chapada Diamantina at the beginning of the 20th century (formerly as *Laelia sincorana* Schltr.), although it was re-located only about two decades ago, in spite of numerous excursions by orchid collectors from Brazil and abroad (Duveen 1983, Withner 1990). Shortly after their re-discovery, these plants were heavily collected for commercial purposes, which lead to the near-extinction of the known populations. More recently, additional small populations have been encountered in Abaíra and Mucugê.

In spite of prohibitions of its collection or sale, it is often sold locally and exported to other regions (occasionally in larger scales when commercial buyers are involved). As the populations are sparse and retain a reduced number of individuals, and the species' capacity for recolonization is very slow, there is an immediate risk of extinction of the species that demands immediate intervention in both managing and protecting the remaining populations.

According to Fawk and Holsinger (1991), to conserve biological diversity, conservation programs must be guided by the biology of the species they seek to preserve, and population dynamics and the genetics of rare species are key areas of research in biological conservation. Actually, genetics and demography are crucial to success in long-term management of any species, and maintaining genetic diversity in endangered species has become a central theme in conservation planning (Fawk and Holsinger 1991).

The present work focused on studies of the demography, genetic variability using allozymes, and morphological variability using multivariate morphometric analysis of populations of Sophronitis sincorana in the Chapada Diamantina. Variability was quantified within the populations, as well as the partitioning of this variability among the different populations. Genetic variability was correlated with morphological variability. This study was part of a project directed towards the conservation and management of threatened species of the Orchidaceae, Eriocaulaceae, and Cactaceae in the Chapada Diamantina, Bahia state, Brazil, and involved studies of the demography, biology, variability, propagation, and ethnobiology of these plants.

Materials and methods

Demography. Sophronitis sincorana is found in reasonably distinct populations in the campo rupestre vegetation on the western border of the Serra do Sincorá Range, almost exclusively in the region of Mucugê, and above 1,000 meters in altitude (Fig. 1). These areas were identified in the



Fig. 1. Individual and habitat of *Sophronitis sincorana* (Orchidaceae) in the Serra do Sincorá Range (region of Guiné), municipality of Mucugê, Chapada Diamantina, Brazil. The individuals grow along the top of the extreme western scarp of the mountain range

field and subsequently mapped using GIS technology. In order to quantify the populations of S. sincorana in the region of the Chapada Diamantina, Bahia state, five areas with relatively dense populations of this species were sampled using transects (Serra da Tesoura 1, Serra da Tesoura 2, Serra da Bacia, Guiné, and Beco). Additionally, a large area around the town of Mucugê was surveyed in order to delineate isolated populations of this species. Large populations were quantified in the following manner: transects were established along the long axis of fields of S. sincorana (the populations normally occupy the crests of the mountains or rock surfaces that follow the generally N-S alignment of the Sincorá Range) and these transects further divided into 10 m plots that were two meters wide. All plants within the plots were counted. Plants were divided into three categories according to their development: seedlings (only one or two pseudobulbs); immature plants (without vestiges of inflorescences); mature plants (presence of flowers/fruits or vestiges of inflorescences). After sampling along the principal axis, perpendicular or random transects were established to sample the width of the fields (which were often quite irregular). As these fields were usually quite narrow, below the accuracy range of a hand-held GPS, their areas were directly calculated based on the length of the transects. Some populations were extremely small, allowing the direct counting of essentially all of the individuals present. In the case of other relatively small, isolated populations, their positions were noted with a GPS and their population density (within a 5 m diameter area, $\sim 80 \text{ m}^2$) estimated and classified according to the following categories: 0 plants = Absent; 1-5 plants = Rare; 6-15 plants = Infrequent; 16-50 plants = Frequent; 51 + plants = Abundant.

Plant material. Plants of *S. sincorana* used for the genetic and morphometric analyses were sampled from the same populations used in the demographic study. Only four of the five populations were used in the morphometric analysis as one of them (Serra da Bacia) was not found flowering. The distance between the populations ranged from 0.6 to 46.3 km. In order to avoid collecting more than one ramet per genet, two samples were never taken in a same rock or phorophyte. A total of 143 individuals were sampled in the genetic analysis, and 93 individuals in the morphometric analysis. Leaves (for the genetic analysis) and flowers (for morphometric analysis) were collected and immediately stored in liquid nitrogen and 70% ethanol, respectively. Voucher specimens are deposited in the herbarium of Universidade Estadual de Feira de Santana (HUEFS; *E.L. Borba et al. 2106*). The exact locations of the populations are being omitted due to the threat of unauthorized collecting.

Allozyme study. Sections of leaf tissue were crushed in 0.5 mL of grinding buffer (100 mL Tris/ HCl 0.1 mol/L pH 7.0; 6.846 g saccharose; 0.6 g PVP [polyvinylpyrrolidone]; 0.0372 g EDTA [ethylenediaminetetraacetate]; 0.145 g BSA [bovine albumin]; 0.13 g DIECA [sodium diethylcarbamate]; 0.6 g BORAX and 100 μ L β -mercaptoethanol; modified from Sun and Ganders 1990). The extracts were absorbed on to Whatman #3 paper and then applied to a Sigma starch gel. Three buffer systems were used: system 1-electrode: boric acid 0.3 mol/L, NaOH 0.06 mol/L, pH 8.0; gel: Tris 0.01 mol/L, pH 8.5; modified from Shaw and Prasad (1970); system 2-electrode: histidine 0.065 mol/L adjusted to pH 6.5 with citric acid; gel: electrode buffer diluted 1:4; modified from Stuber et al. (1977); system 3-electrode: lithium hydroxide 0.05 mol/L, boric acid 0.0935 mol/L, EDTA 0.0059 mol/L, pH 8.0; gel: electrode solution diluted 1:10; modified from Ridgway et al. (1970). Standard horizontal electrophoresis was performed until the inner marker (bromophenol blue) moved 9 cm from the application site, using the following running conditions: system 1- 25 mA and 13 mA; system 2- 150 V; system 3- 25 mA. Five enzymatic systems gave sufficient resolution for reading: buffer system 1: esterase (EST; EC 3.1.1.1); buffer system 2: malate dehydrogenase (MDH; EC 1.1.1.37), diaphorase (DIA; EC 1.8.1.4); buffer system 3: phosphoglucomutase (PGM; EC 2.7.5.1), phosphoglucoisomerase (PGI; EC 5.3.1.9). The staining procedures were similar to, but slightly modified from, Brune et al. (1998; EST, DIA), Corrias et al. (1991; PGI), and Soltis et al. (1983; PGM, MDH).

Enzymatic systems showing more than one locus were numbered in ascending order starting from the locus with the lowest mobility. The alleles were numbered according their mobility relative to the allele of a standard individual present in all gels. The allelic frequencies were determined by manually counting the banding patterns of the homozygotes and heterozygotes stained in the gels. Genetic variability for all populations was estimated using the following parameters: proportion of polymorphic loci (P; 0.95 criteria); mean number of alleles per locus (A); observed (H_0) and expected $(H_{\rm e})$ mean heterozygosity per locus. Departures from the expected mean heterozygosity under Hardy-Weinberg (HW) equilibrium were tested using χ^2 with a correction for small samples, according to Levene (1949). Partitioning of genetic diversity among conspecific populations was estimated by F statistics (the inbreeding coefficient F_{IS} measures the reduction in heterozygosity due to nonrandom mating within a population; F_{ST} measures the differentiation among populations; Wright 1978). Cluster analysis was performed with the genetic distance matrix (Nei 1978; unbiased genetic distance) of the populations using UPGMA (Sneath and Sokal 1973). All analyses were performed using the BIOSYS 1.0 software package (Swofford and Selander 1989), except for the cluster analysis, which was performed using Statistica 6.1 (StatSoft 2003).

Morphometric analysis. Twenty-six continuous and discontinuous flower characters were measured (Table 1, Fig. 2). A discriminant analysis was conducted for all characters. The standardized coefficients for canonical variables resulting from discriminant analysis were used to identify characteristics that most significantly contribute to the resulting patterns. Cluster analysis of the populations was carried out using the Mahalanobis generalized distance calculation from the pooled residual covariance within group matrix, and UPGMA (unweighted pair-group method of arithmetical averages) as the clustering algorithm. The median values of the Mahalanobis generalized distance from the individuals to the centroid of their population (D2_m) was calculated, and a nonparametric variance analysis was performed using the Kruskall-Wallis test. A multi-response permutation procedure (MRPP) analysis was used to calculate the average within-group distance (mean Euclidean distance - ED) for all populations and the chance-corrected within-group agreement (A_{MRPP}) among populations. $D2_m$ and ED values were used as measures of morphological variability, and A_{MRPP} as a measurement of morphological differentiation of conspecific populations and correlated to F_{ST} (Borba et al. 2002; Goldman et al. 2004; Lambert et al. 2006a, b). The two indexes of morphological variability are essentially different,

as $D2_m$ is more affected by form and ED is more affected by size of the characters (Lambert et al. 2006a, b). We carried out a variance analysis of all populations, for each variable separately (one-way ANOVA). The significance of the differences between the means was tested using a Tukey's test (p < 0.05). Discriminant analysis and cluster analysis were carried out using Statistica 6.0, the MRPP was run using PCOrd 4.10 (McCune and Mefford 1999), and variance analysis performed using BioEstat 3.0 (Ayres et al. 2003).

Correlation analysis. An analysis of nonparametric Spearman rank correlation between genetic variability (H_e) and morphological variability (mean Euclidean distance, ED; and median Mahalanobis distance, D2_m) was performed. These analyses were run in the BioEstat 3.0. The Mahalanobis generalized distance matrix was compared with matrices of Nei's (1978) genetic distance and geographic distances of populations using the Mantel test with the Monte Carlo option of PCOrd (1000 randomizations). The pair-wise geographical distances between the populations were computed using FITOPAC 1.0 (Shepherd 1995).

Results

Demography. Sophronitis sincorana grows almost exclusively along the extreme western scarp of the Chapada Diamantina National Park (Serra do Sincorá Range) near the town of Mucugê. Populations diminish rapidly both north and south of this point, and penetrate at most 3.5 km eastwards from that scarp. In general, the individual populations are not very large, but are relatively widely spread throughout the mountains. Seventy-four areas (sub-populations) were delimited in the region near Mucugê, plus a very small area in the municipality of Ibicoara, to the south. Near Mucugê and Ibicoara, S. sincorana normally occurs as an epiphyte on *Vellozia* sp. (Velloziaceae), though it is also often rupicolous. In the region known as Beco, Vellozia sp. is rare, and almost the entire population of S. sincorana is rupicolous, growing on the open rock surfaces at the western-most extent of the cliffs. Table 2 presents a summary of the five areas of S. sincorana near Mucugê surveyed in

| ological characters used in the morphometric analysis of four populations of Sophronitis sincorana occurring in the Chapada | azil. Measures presented in centimeters; data presented as average \pm standard deviation (minimum-maximum) |
|---|---|
| 1. Morphole | ntina, Brazi |
| ble J | ama |

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| Table 1. Morphological chan Diamantina, Brazil. Measure | racters used in the morphon as presented in centimeters; o | netric analysis of four popul: data presented as average \pm : | lations of <i>Sophronitis sincor</i> , standard deviation (minimu | <i>ana</i> occurring in the Chapada m-maximum) |
|--|---|---|---|--|
| Character | Beco | Guiné | Serra da Tesoura 1 | Serra da Tesoura 2 |
| Dorsal sepal | | | | |
| 1- Length | $5.1 \pm 0.7 \ (3.5 - 6.5)$ | $5.3 \pm 0.4 \ (3.9 - 6.1)$ | $5.1\pm0.5~(4.4{-}6.2)$ | $4.9 \pm 0.6 \ (3.4 - 6.1)$ |
| 2- Width at $1/3^*$ | $1.5 \pm 0.2 \; (1.2 - 1.8)$ | $1.5 \pm 0.1 \; (1.1 - 1.7)$ | $1.4 \pm 0.2 \ (1.1 - 1.7)$ | $1.4 \pm 0.1 \ (1.2 - 1.6)$ |
| 3- Width at $2/3^{*\dagger}$ | $1.5 \pm 0.1 \; (1.2 - 1.8)^{ m a}$ | $1.4 \pm 0.1 (1.2 {-} 1.7)^{ m a}$ | $1.4 \pm 0.2 (1.1 - 1.8)^{ m ab}$ | $1.3 \pm 0.1 \ (1.1 - 1.6)^{b}$ |
| 4- Largest width [†] | $1.5 \pm 0.2 \; (1.2 - 1.8)^{ m ab}$ | $1.6\pm0.1~(1.3{-}1.8)^{ m a}$ | $1.5 \pm 0.2 (1.2 - 1.9)^{ m ab}$ | $1.4 \pm 0.1 \ (1.2 - 1.6)^{\rm b}$ |
| 5- Apex angle | $89.4 \pm 9.9 \ (74.0 - 105.0)$ | $89.6\pm16.1\ (59.0{-}120.0)$ | $90.1 \pm 11.9 \ (64.0 - 110.0)$ | $82.4 \pm 8.7 \ (65.0 - 98.0)$ |
| Lateral sepals | | | | |
| 6- Length | $5.0 \pm 0.7 \ (3.4 - 6.5)$ | $5.3 \pm 0.5 \ (3.8 - 6.1)$ | 5.2 ± 0.5 (4.4–6.3) | $5.0 \pm 0.6 \; (3.5 - 6.2)$ |
| 7- Width at 1/3* | $1.4 \pm 0.2 \ (1.0 - 1.7)$ | $1.4 \pm 0.2 \; (1.0 - 1.8)$ | 1.4 ± 0.2 $(1.1 - 1.8)$ | $1.4 \pm 0.2 \; (1.1 - 1.7)$ |
| 8- Width at 2/3* | $1.2 \pm 0.2 \ (0.9 - 1.4)$ | $1.3 \pm 0.2 \; (1.0 - 1.9)$ | $1.3 \pm 0.2 \; (1.0 - 1.6)$ | $1.3 \pm 0.2 \ (0.9 - 1.6)$ |
| 9- Largest width | $1.4 \pm 0.2 \; (1.1 - 1.7)$ | $1.5 \pm 0.2 \ (1.0 - 1.8)$ | $1.5 \pm 0.2 \ (1.2 - 1.8)$ | $1.4 \pm 0.2 \ (1.1 - 1.8)$ |
| 10- Apex angle | $84.7 \pm 8.6 \ (73.0 - 107.0)$ | $82.6 \pm 14.5 \ (54.0 - 109.0)$ | $80.7 \pm 11.3 \ (58.0 - 104.0)$ | $80.4 \pm 12.7 \ (54.0 - 112.0)$ |
| Petal | | | | |
| 11- Length | $5.0 \pm 0.7 \; (3.7 - 6.6)$ | $5.3 \pm 0.5 \; (3.9 - 6.1)$ | $5.1 \pm 0.5 \ (4.3 - 6.3)$ | $5.0 \pm 0.6 \; (3.4 - 6.1)$ |
| 12- Width at $1/3^*$ | $2.5 \pm 0.3 \; (1.8 - 3.2)$ | $2.6 \pm 0.3 \ (2.1 - 3.4)$ | $2.6 \pm 0.3 \ (2.2 - 3.2)$ | $2.4 \pm 0.4 \ (1.7 - 3.0)$ |
| 13- Width at 2/3* | $2.3 \pm 0.2 \ (1.9 - 2.8)$ | $2.4 \pm 0.4 \; (1.6 - 3.2)$ | $2.2 \pm 0.3 (1.7 - 2.9)$ | $2.2 \pm 0.4 \ (1.2 - 2.8)$ |
| 14- Largest width | $2.8 \pm 0.3 \ (2.0 - 3.3)$ | $2.9 \pm 0.3 \ (2.2 - 3.6)$ | $2.8 \pm 0.3 \ (2.3 - 3.5)$ | $2.7 \pm 0.4 \ (1.9 - 3.3)$ |
| 15- Apex angle | $128.0 \pm 8.7 \ (105.0 - 140.0)$ | $117.4 \pm 19.2 \ (86.0 - 162.0)$ | $119.2 \pm 18.3 \ (65.0 - 147.0)$ | $118.7 \pm 17.1 \ (74.0 - 147.0)$ |
| Lip | | | | |
| 16- Length | $4.7 \pm 0.4 \; (3.5 - 5.6)$ | $4.8 \pm 0.4 \ (3.6 - 5.4)$ | $4.6 \pm 0.4 \; (4.0 - 5.6)$ | $4.7 \pm 0.4 \ (3.4 - 5.7)$ |
| 17- Width | $3.8 \pm 0.3 \ (3.1 - 4.5)$ | $4.0 \pm 0.4 \; (3.3 - 5.0)$ | $3.9 \pm 0.3 \ (3.5 - 4.5)$ | $3.7 \pm 0.6 \ (2.0 - 4.6)$ |
| 18- Width at the base | $2.1 \pm 0.3 \; (1.1 - 2.8)$ | $2.3 \pm 0.3 \ (1.5 - 2.8)$ | $2.2 \pm 0.3 \ (1.7 - 3.0)$ | $2.1 \pm 0.3 \ (1.3 - 2.5)$ |
| of mid lobe | | | | |
| 19- Largest width of the mid lobe | $2.2 \pm 0.3 \ (1.1 - 2.6)$ | $2.4 \pm 0.3 \; (1.6 - 2.9)$ | $2.3 \pm 0.3 \ (1.8 - 3.2)$ | 2.2 ± 0.3 (1.3-2.6) |
| 20- Angle between lip base and anices of lateral lobes [†] | $68.1 \pm 4.7 \ (57.0 - 79.0)^{a}$ | $74.4 \pm 5.8 \; (64.0 - 84.0)^{\rm b}$ | $74.0 \pm 5.6 \; (64.0 - 88.0)^{\rm b}$ | $72.5 \pm 7.7 \ (52.0 - 85.0)^{ab}$ |
| 21- Angle between lateral | $159.9\pm6.7\;(140.0{-}174.0)$ | $165.9\pm10.1\;(140.0{-}180.0)$ | $164.1 \pm 8.5 \ (141.0 - 176.0)$ | $161.8\pm15.0\ (108.0{-}180.0)$ |
| and find follows 22 - Apex angle of the mid lobe ^{\dagger} | $74.8 \pm 12.9 \; (55.0 - 104.0)^{a}$ | $93.1\pm16.7\;(64.0{-}122.0)^{\rm b}$ | $90.4 \pm 17.8 \; (60.0 - 130.0)^{\rm b}$ | $85.5\pm24.0\;(45.0{-}144.0)^{\rm ab}$ |

| Column | | | | |
|---------------------------|-------------------------------|--------------------------|------------------------------|-----------------------------|
| 23- Length | $2.2 \pm 0.3 \; (1.9 - 3.2)$ | 2.2 ± 0.1 (2.0–2.4) | $2.1 \pm 0.1 \ (1.9 - 2.3)$ | $2.1 \pm 0.1 \ (2.0 - 2.3)$ |
| 24- Width | $0.7\pm0.3~(0.5{-}1.8)$ | $0.6\pm0.1\;(0.5{-}0.7)$ | $0.6\pm0.1\;(0.5{-}0.7)$ | $0.6\pm0.1\;(0.5{-}0.7)$ |
| 25- Thickness | $0.5\pm0.0~(0.4{-}0.6)$ | $0.5\pm0.1~(0.4{-}0.6)$ | $0.5\pm0.1~(0.5{-}0.7)$ | $0.5\pm0.0~(0.4{-}0.6)$ |
| 26- Cuniculus length | $1.2\pm0.3~(0.3{-}1.5)$ | $1.2\pm0.2~(0.7{-}1.9)$ | $1.1 \pm 0.2 \; (0.9 - 1.4)$ | $1.2 \pm 0.3 \ (0.3 - 1.6)$ |
| * Width of the sepals and | d petal at one- and two-third | ts along their length | | |

^{\dagger} Different letters in the same line indicate statistically different means in the Tukey's test (p < 0.05)



Fig. 2. Outline of flower parts indicating the morphological characters used in the morphometric analysis of four populations of *Sophronitis sincorana* occurring in the Chapada Diamantina, Brazil. See Table 1 for character codes

detail. In the largest areas (Serra da Tesoura 1, Beco and Serra da Bacia) plant density was approximately 0.5 plants/m². The projected total number of plants was approximately 50,000 individuals.

Fruit production by *S. sincorana* was noted in four of the five areas surveyed. Fruit production was greater than 1% in only one of the areas, with total production for all five areas being 0.66%. Recruitment (production of seedlings) in populations of *S. sincorana* was observed in 4 of the 5 areas surveyed. The

| | Bacia | Beco | Guiné | Tesoura 1 | Tesoura 2 | Total |
|-------------------------|--------|--------|--------|-----------|-----------|--------|
| Area (m ²) | 57,600 | 21,669 | | 137,500 | 464 | |
| TDP ^a | 0.43 | 0.46 | | 0.1 | 0.53 | |
| D-seedling ^b | 0.002 | 0.003 | | 0.001 | 0 | |
| D-immature ^b | 0.14 | 0.15 | | 0.03 | 0.13 | |
| D-mature ^b | 0.29 | 0.31 | | 0.08 | 0.4 | |
| D-fruiting ^c | 0.002 | 0.002 | | 0.001 | 0 | |
| TNP ^a | 24,960 | 9,970 | 505 | 14,171 | 246 | 49,852 |
| N-seedling ^b | 103 | 66 | 2 | 187 | 0 | 358 |
| N-immature ^b | 8,297 | 3,191 | 270 | 3,601 | 60 | 15,419 |
| N-mature ^b | 16,560 | 6,713 | 231 | 10,383 | 186 | 34,073 |
| N-fruiting ^c | 103 | 33 | 6 | 187 | 0 | 329 |
| % seedling | 0.41 | 0.67 | 0.40 | 1.3 | 0 | 0.72 |
| % fruiting indiv. | 0.41 | 0.3 | 1.19 | 1.3 | 0 | 0.66 |
| Immature:mature | 1:2.0 | 1:2.1 | 1:0.85 | 1:2.9 | 1:3.1 | 1:2.2 |

Table 2. Summary of census data of five populations of Sophronitis sincorana occurring in the Chapada Diamantina, Brazil

^a Total density (TDP) and total number (TNP) of plants (plants/m²)

^b Density (D) and number (N) of seedlings, immature and mature individuals

^c Density (D) and number (N) of individuals bearing fruits in the season

percentage of seedlings was greater than 1% in only a single area, with total production for all five areas being 0.72%. However, due to the small size of the seedlings and the difficulty of locating these small plants on the host plant or the ground, these percentages represent only a minimum estimate. The proportion of immature to mature plants varied from 1:2 to 1:3.1 in the region near Mucugê, and was 1:0.85 in the northern area (the Guiné, in the same municipality).

Variability. Six loci with good resolution in five enzymatic systems were obtained. All loci were polymorphic in all populations studied. MDH-2 was the most polymorphic locus, with five alleles (Table 3). No allele was exclusive to single populations. All populations presented the same most abundant allele in all loci. The populations from Guiné and Serra da Tesoura 2 displayed almost all alleles present in all five populations, except for allele 2 of MDH-2 in Guiné (rare in the other three populations), and the allele 3 of EST in Serra da Tesoura 2 (rare in two populations and frequent in Guiné). The mean number of alleles per locus varied between 3.0 and 3.5, and mean heterozygosity

 (H_e) ranged from 0.33 to 0.48 (Table 4). The Serra da Tesoura 1 population demonstrated the lowest genetic variability (H_e) , while the remaining populations displayed similar values between themselves. All populations showed significant deviations from the expected values of HW equilibrium in at least three loci. All loci were not in HW equilibrium in at least one population, and two of them were not in HW equilibrium in all of the populations (MDH-1 and MDH-2). The reason for the disequilibrium was the deficit of heterozygotes at all loci. The high positive values for $F_{\rm IS}$ (Table 5) reflect this deficit of heterozygotes in the populations.

All populations showed similar values of variability in both morphological analyses (discriminant analysis and MRPP) (Table 4); there was no statistically significant differences in the Kruskal-Wallis test between the D2_m values (H=0.1427, p=0.9863). Spearman rank correlation analysis between morphological and genetic variability did not reveal any statistically significant correlation between H_e and D2_m (r=-0.6000, p=0.4000), nor between H_e and ED (r=0.4000, p=0.6000).

| locus/allele | Population | | | | |
|--------------|------------|-------|-------|-----------|-----------|
| | Bacia | Beco | Guiné | Tesoura 1 | Tesoura 2 |
| DIA | | | | | |
| 1 | 0.321 | 0.346 | 0.133 | 0.043 | 0.278 |
| 2 | 0.554 | 0.481 | 0.667 | 0.848 | 0.611 |
| 3 | 0.125 | 0.173 | 0.200 | 0.109 | 0.111 |
| (N) | 28 | 26 | 30 | 23 | 27 |
| EST | | | | | |
| 1 | 0.086 | 0.205 | 0.034 | 0.174 | 0.087 |
| 2 | 0.845 | 0.773 | 0.655 | 0.826 | 0.913 |
| 3 | 0.069 | 0.023 | 0.310 | - | - |
| (N) | 29 | 22 | 29 | 23 | 23 |
| MDH-1 | | | | | |
| 1 | - | - | 0.017 | 0.040 | 0.074 |
| 2 | 0.919 | 0.904 | 0.783 | 0.860 | 0.704 |
| 3 | - | - | 0.133 | 0.100 | 0.111 |
| 4 | 0.081 | 0.096 | 0.067 | - | 0.111 |
| (N) | 31 | 26 | 30 | 25 | 27 |
| MDH-2 | | | | | |
| 1 | - | 0.038 | 0.095 | - | 0.074 |
| 2 | 0.032 | 0.096 | - | - | 0.037 |
| 3 | 0.613 | 0.615 | 0.643 | 0.840 | 0.593 |
| 4 | 0.097 | 0.058 | 0.214 | 0.040 | 0.037 |
| 5 | 0.258 | 0.192 | 0.048 | 0.120 | 0.259 |
| (N) | 31 | 26 | 21 | 25 | 27 |
| PGI | | | | | |
| 1 | 0.240 | 0.250 | 0.120 | 0.167 | 0.042 |
| 2 | 0.680 | 0.550 | 0.600 | 0.786 | 0.896 |
| 3 | 0.080 | 0.200 | 0.280 | 0.048 | 0.063 |
| (N) | 25 | 20 | 25 | 21 | 24 |
| PGM | | | | | |
| 1 | 0.089 | - | 0.024 | 0.100 | 0.208 |
| 2 | 0.589 | 0.792 | 0.762 | 0.667 | 0.667 |
| 3 | 0.321 | 0.188 | 0.095 | 0.133 | 0.083 |
| 4 | - | 0.021 | 0.119 | 0.100 | 0.042 |
| (N) | 28 | 24 | 21 | 15 | 24 |

Table 3. Allele frequencies at six allozymic loci in five populations of *Sophronitis sincorana* occurring in the Chapada Diamantina, Brazil

N = sample size

Sophronitis sincorana displayed low average values of F_{ST} (0.053). The highest FST was found in the locus EST, mainly due to the high frequency of the allele 3 in Guiné. Low levels of morphological structuring was also found in this species ($A_{MRPP} = 0.018$) using the MRPP analysis. These values were correlated to the low genetic structuring values (F_{ST}) found in this species (Table 5).

Phenetic relationships. Genetic identities between the populations ranged from 0.945 to 0.995 (mean = 0.966). The UPGMA dendrogram obtained from the Nei cluster analysis (1978) unbiased genetic distances (Fig. 3A) reveals that the population from Guiné was the most differentiated population. This differentiation of Guiné occurred mainly due to the high frequency of the allele 3 of

Table 4. Genetic variability at six allozymic loci in five populations and morphological variability based on the morphometric analysis of 26 morphological characters in four populations of *Sophronitis sincorana* occurring in the Chapada Diamantina, Brazil

| Pop. | Ν | A | Р | Ho | H _e | D2 _m | ED |
|-----------|------------|-----------|-----|-------------|----------------|-----------------|------|
| Bacia | 28.7 (0.9) | 3.0 (0.3) | 100 | 0.21 (0.05) | 0.43 (0.07) | - | - |
| Beco | 24.0 (1.0) | 3.2 (0.4) | 100 | 0.30 (0.07) | 0.45 (0.08) | 21.93 | 6.92 |
| Guiné | 26.0 (1.8) | 3.5 (0.2) | 100 | 0.18 (0.05) | 0.48 (0.03) | 22.23 | 6.61 |
| Tesoura 1 | 22.0 (1.5) | 3.0 (0.3) | 100 | 0.23 (0.07) | 0.33 (0.04) | 22.77 | 6.42 |
| Tesoura 2 | 25.3 (0.8) | 3.5 (0.4) | 100 | 0.14 (0.07) | 0.42 (0.08) | 25.11 | 6.70 |

N = mean sample size per locus; A = mean number of alleles per locus; P = percentage of polymorphic loci; H_o = observed and H_e = expected mean heterozygosity per locus (Nei 1978; unbiased estimate); $D2_m$ = median of the Mahalanobis generalized distance of the individuals to the centroid of the population; ED = mean of the Euclidean distance between individuals of the population. Standard deviations in parentheses. A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95

Table 5. F statistics (Wright 1978) at six allozymicloci of five populations of Sophronitis sincoranaoccurring in the Chapada Diamantina, Brazil

| Locus | $F_{\rm IS}$ | F_{IT} | $F_{\rm ST}$ |
|-------|--------------|-------------------|--------------|
| PGM | 0.237 | 0.269 | 0.042 |
| PGI | 0.362 | 0.403 | 0.065 |
| EST | 0.321 | 0.372 | 0.075 |
| MDH-1 | 0.898 | 0.902 | 0.041 |
| MDH-2 | 0.770 | 0.780 | 0.042 |
| DIA | 0.431 | 0.463 | 0.057 |
| Mean | 0.491 | 0.518 | 0.053 |

EST, which is either rare or absent in the other populations. The remaining populations form two groups, one composed of the two populations of Serra da Tesoura, and the other of the populations from Serra da Bacia and Beco.

The UPGMA dendrogram obtained from the cluster analysis of morphological distances showed that the Beco population displayed the greatest differentiation (Fig. 3B). Table 6 demonstrates the classification matrix of the individuals analyzed. The percentage of correct classifications ranged from 73 to 86%. The highest frequency of incorrect classifications occurred between the populations of Guiné and Serra da Tesoura 1 (the populations displaying the smallest linkage distances in the cluster analysis).

The scatter plots of the individual scores on the first three CVA canonical roots are shown

in Fig. 4. The first, second, and third canonical roots explained 58.94%, 24.77% and 16.28%, respectively, of the morphological variation. There is no separation of the populations from Guiné and Serra da Tesoura 1 on the three axes. On the first axis there is a slight separation between the Beco population and the remaining populations, due mainly to the higher values of character 15 (petal apex angle) and lower values of characters 20 and 22 (angle between lip base and apices of lateral lobes, and apex angle of the lip mid lobe) observed in the Beco population (Table 1).

Only four characters presented statistically different mean values among the populations (#3, 4, 20, 22; Table 1). There was no statistical difference in the mean values between the populations from Guiné and Serra da Tesoura 1 in either character. The majority of the statistically different means was observed between the Beco and the remaining populations, especially in the characters #20 and 22. The Beco population may be characterized by the smallest angle of lateral lobes (character #20), smallest angle of the incision of the mid lobe (#22) and by its oblong dorsal sepal (same values for characters # 2, 3 and 4) (Table 1, Fig. 2).

The Mantel tests did not produce statistically significant results for pairwise correlations between genetic and morphological distances (r = 0.261806, p = 0.317), nor between



Fig. 3. Dendrogram showing the phenetic relationships among populations of *Sophronitis sincorana* occurring in the Chapada Diamantina, Brazil. Constructed using the matrix of genetic distances (Nei 1978; unbiased estimate) based on six allozymic loci (**A**) and using the matrix Mahalanobis generalized distance based on 26 morphological characters (**B**) with UPGMA as clustering algorithm

Table 6. Matrix of classification of the individuals in the discriminant analysis of 26 morphological characters in four populations of *Sophronitis sincorana* occurring in the Chapada Diamantina, Brazil

| Population | Percent correct | Beco | Guiné | Tesoura 1 | Tesoura 2 |
|------------|-----------------|-----------|-----------|-----------|-----------|
| Beco | 85.71 | - | 24 | 2 | 2 |
| Guiné | 85.00 | 17 | 2 | - | 1 |
| Tesoura 1 | 73.91 | 1 | 3 | 17 | 2 |
| Tesoura 2 | 72.73 | 2 | 2 | 2 | 16 |
| Total | 79.57 | 20 | 31 | 21 | 21 |
| | | p = 0.215 | p = 0.301 | p = 0.247 | p = 0.237 |



Fig. 4. Representation of the scores on the three first canonical axes of the CVA using 26 morphological characters in four populations of *Sophronitis sincorana* occurring in the Chapada Diamantina, Brazil. A Canonical axes 1 and 2. **B** Canonical axes 1 and 3

genetic and geographical distances (r = 0.530547, p = 0.130) of conspecific populations, nor between the morphological and geographical distances (r = 0.145716, p = 0.417).

Discussion

Under no circumstances should information concerning the location of the populations of S. sincorana be disseminated. The majority of these populations are small and are restricted to an area of just a few square kilometers, making this species very vulnerable to extinction. As this data deals with a species that is sought after by collectors and commercial agents of orchids, the exact location data must remain under absolute secrecy. The presence of S. sincorana in the region around the municipality of Ibicoara represents a significant increase in the distribution range of this species, although the total number of individuals involved is very small. Fire and the predatory collection of these orchids constitute the major threats to the species. Occasionally utility vehicles have been seen loaded with these orchids, even in areas of the National Park. More data are needed about the population dynamics of this species in order to better evaluate the apparently very low levels of recruitment in the surveyed populations.

Although Sophronitis sincorana inhabits almost the same areas as the straw-flower Sygonanthus mucugensis Giul. (Eriocaulaceae) (which is the principal species collected in this area for commercial purposes), S. sincorana is more sensitive to collecting due to a number of factors: 1) the populations and areas of distribution of S. sincorana are smaller than those of S. mucugensis; 2) it would appear that the lifecvcle (time needed to reach maturity/flowering) is longer for S. sincorana; 3) it would appear that because of its habitat, habit, and anatomy S. mucugensis is more resistant to the effects of wild fires that are common in the region; 4) the collection of S. mucugensis for commercial purposes does not require the removal of the entire plant (only the inflorescence), as is the case with S. sincorana.

A very high degree of genetic variability (mainly H_e , but also P and A) was encountered in the populations of S. sincorana surveyed. These values are higher or similar than those seen in other species of orchids (Borba et al. 2001a; Azevedo et al. 2006, 2007; Ribeiro 2006) and much higher than seen in other groups of plants within the campo rupestre vegetation (Jesus et al. 2001; Machado 2005; Conceição 2006; Lambert et al. 2006a, b; Pereira et al. 2007). Moreover, these values are very near the maximum values established for the majority of previously studied plants (Hamrick and Godt 1990, Hamrick et al. 1991), and larger than any normally encountered in the Orchidaceae (e.g. Scacchi and De Angelis 1989, Schlegel et al. 1989, Scacchi et al. 1990, Corrias et al. 1991, Klier et al. 1991, Case 1994, Case et al. 1998, Ehlers and Pedersen 2000, Wallace 2002, Trapnell et al. 2004, Tremblay et al. 2005; but see Sun 1996). These values are also higher than the average reported for monocots, herbs, long-lived perennials, or plants with restricted geographical distribution, wind-dispersed seeds, and sexual reproduction (Hamrick and Godt 1990, Hamrick et al. 1991).

It is likely that these high levels of genetic variability are being maintained, at least at some extent, by the partial self-incompatibility encountered in this species, as well as other characteristics of its reproductive biology, such as pollination-by-deceit, which favors crosspollination (Silva-Pereira et al. unpublished). In spite of self-incompatibility to be common in plants, it is uncommon in Orchidaceae. Hamrick and Godt (1990) and Hamrick et al. (1991) have stressed that breeding system is one of the major traits determining genetic diversity at both population and species level, and the amount of variability may vary between species in a group with contrasting mating systems. Self-incompatibility is especially uncommon in the Laeliinae, mainly in species pollinated by bees (Matias et al. 1996, Borba and Braga 2003, Smidt et al. 2006). It is most commonly observed in other groups in which the behavior of the pollinator itself favors self-pollination, and possibly as a result some of these species present very high levels of genetic variability (Borba et al. 2001b; Azevedo et al. 2007).

However, we must notice that the variability measured by the mean observed heterozygosity (H_0) is much lower than that by the expected heterozygosity (H_e) , in spite of H_o to be considered also high, leading to a high inbreeding coefficient (F_{IS}) . This elevated inbreeding coefficient encountered in populations of S. sincorana, with a large deficit of heterozygotes, indicates the occurrence of structuring within the populations, meaning that the variability of the population is not randomly distributed. This probably reflects a low dispersal of the genotypes, forming groups of related individuals, resulting in crossings among closely related individuals. The behavior of the pollinators of the species can be the cause of this genetic structuring. The species is pollinated by bumblebees (Bombus) that have relatively small foraging ranges (Silva-Pereira et al. unpublished data, Smidt et al. 2006). The behavior of these bees may promote gene flow only among close plants, what would contribute for the genetic sub-structuring of the species. Because S. sincorana is partially selfincompatible, endogamy may occur mainly by biparental endogamy (crosses between related individuals), an important factor for reducing the heterozygosity in small populations, raising their probability of extinction by decreasing the viability and fecundity of the individuals (Handel 1983, Heywood 1991, Bijlsma et al. 2000). Aggregation of all the pollen of a flower in the pollinarium, which is removed and deposited entirely in one pollination event, leads to formation of fruits containing hundreds of thousands seeds pollinated by a single pollen donor, representing full-sib progenies. This overall process is reinforced by the common leptokurtic fashioned seed dispersal of angiosperms, which leads to a patchy distribution of genotypes. High F_{IS} due to geitonogamy or bi-parental endogamy has also been found in other orchid species (e.g.

Wallace 2002), and other incompatible plant species of campo rupestre vegetation (Lambert et al. 2006a).

As could be expected, the highest divergences between mean observed heterozygosity and expected heterozygosity occurred in the two small populations, Guiné and Serra da Tesoura 2, as these two populations are more subjected to genetic drift due to very small number of individuals. Genetic drift probably also led to genetic differentiation of the population of Guiné, expressed mainly by the high frequency of an allele either rare or absent in the other populations. Conversely, these two populations presented the highest number of alleles (21 in the six loci) while the other populations presented either 18 or 19 alleles. This figure would be unexpected for populations more probably subjected to bottlenecks or severe genetic drift. However, inverse correlation between population size and number of private alleles has been found for some orchid species (Sun 1996). Low differentiation and absence of fixation of alternative alleles in different populations is not consistent with founder events by a small number of individuals and variable source populations. Probably gene flow is still extensive enough to be a cohesive force among these populations avoiding inter-population differentiation. The lack of correlation between morphological variation and genetic variation in this species indicates that none of the markers examined should be used separately for either in situ or ex situ conservation purposes.

In spite of the fact that almost all of the populations identified are located within the Chapada Diamantina National Park, they are constantly being affected by anthropic factors, such as: collection and removal; the presence of domestic grazing animals that trample and eat the plants; and fire (either intentional in the traditional management of the areas, or accidental). In this way, small populations such as Serra da Tesoura 2 (less than 250 individuals in 400 m^2) and Guiné run the risk of rapid extinction, for fire burning the vegetation surrounding the rock outcrops where they occur can reach essentially all of the plants.

In the same way, grazing animals or collectors could remove all the plants in a given population. On the other hand, larger populations occurring on extensive rock outcrops would be less susceptible to these effects: the edge of the outcrop itself would serve as a protective zone against fire or grazing, and collections would tend to be made on the external areas, leaving the central zone more protected.

Due to the low genetic and morphological differences between populations, with almost all of the species' variability being located within the populations (and all of them showing high levels of variability), and a low percentage of it being accounted for by population differences, in situ conservation proposals should put priority on protecting populations with the highest number of individuals. Based on our results, a large number of populations is not needed to be protected in order to maintain a large amount of the genetic diversity of the species, instead is preferable to protect entirely a few large populations. However, due to the sub-structure of these populations, with the probable aggregation of groups of closely related individuals, these populations do not consist of panmictic unities. Conversely, local differentiation occurs in these populations, and most likely particularly at the edges of the outcrops. Such local differentiation in the periphery of populations is reinforced by genetic drift, and it may play an important role in the evolution of the species (Grant 1981, Levin 2000), mainly conferring adaptation to the above mentioned pressure which these individuals are suffering. As such, a strong investment in total protection for the rock outcrops, as well and their borders, is imperative. Due to the morphological differentiation of the population of Beco. we suggest that it should be chosen as one of those populations to be included in any conservation planning for the species. Moreover, it must be stressed that special attention must be paid to populations that present any morphological differentiation, because they are the preferred target for collection by growers. Nevertheless, special attention must also be paid to the population of Guiné, which presented the highest genetic variability and differentiation, and is the most isolated and one of the smallest populations of the species. In spite of it being located in a National Park, that population is the most likely to be come extinct, because of the long-term collection of individuals which it has suffered.

We thank two anonymous reviewers for their helpful suggestions. This study was supported by grants conceded by the Fundo Nacional do Meio Ambiente (FNMA #75/2001) and by the Fundação de Amparo à Pesquisa do Estado da Bahia (FA-PESB). E. L. Borba is supported by a grant from CNPq (PQ2).

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