PHENOLOGY, REPRODUCTIVE AND MORPHOLOGY FEATURES AS A TOOL TO CLARIFY *EUTERPE* SPECIES BOUNDARIES

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INTRODUCTION

The primary tool for circumscribing most plant species is the traditional morphological species concept, despite the apparent limitations this concept remains (Stuessy, 1989). If morphological variability is high, it is difficult to delimit species based only on morphology (Listabarth, 1999). Sympatric populations of related species may share some morphological and ecological features. If the biological species concept is used to define a taxon, it presumes reproductive isolation. However, hybridization is a common event in nature, especially among related species that share pollinators (Wendt *et al.*, 001). Hybrid development does not mean that two taxa are only one species. Many cases of hybridization between different species are observed, including in palms (Listabarth, 1999).

The boundaries between putative palms are often obscured by a lack of identified fixed morphological differences, by paucity of informative collection and potential hybridization (Henderson, 2006). Disagreements over whether local forms should be classified as species, infraspecific taxa or natural intraspecific variation have led to varying estimates of species numbers for the palm family (Henderson and Martins, 2002). One example of a taxonomic controversy involves the *Euterpe* genus, which contains seven species distributed in South and Central America. Five species are found in Brazil (Henderson and Galeano, 1996) and some of them produce the palm - heart, locally called *palmito*, which is an exotic gourmet food of high monetary value, but requires the tree destruction when it is collected. *Euterpe edulis* was for many years the most important species for the extraction of palm - heart. Even today, the extraction frequently comes from wild plants, what can affect the population of this species (Reis *et al.*, 000).

Boudet - Fernandes (1989) described *Euterpe espiritosantensis* H.Q.B. Fern., which occurs sympatrically with *E. edulis* in the Santa Teresa municipality, Espírito Santo (ES) State, southeast Brazil. In the most recent taxonomic treatment of this genus, *E. espiritosantensis* was considered as a synonym of *E. edulis* (Henderson and Galeano, 1996). Although these two palms are valid synonymies, they continued to be treated as two distinct species in subsequent publications (e.g. Martins *et al.*, 007), and by the local people in Espírito Santo.

OBJECTIVES

A critical revaluation of species boundary is necessary by the fact that both are currently listed as endangered species (Kollmann *et al.*, 007). The goal of our study was to investigate whether *E. edulis* and *E. espiritosantesis* could be confidently recognized morphologically in narrow sympatric occurrence in Santa Teresa, and if this morphological distinction could reflect reproductive isolation. Thus, we compared their morphology and reproductive biology, and also conducted artificial treatments to investigate potential hybridization between them.

MATERIAL AND METHODS

2.1 - Study area and species
Fieldwork was carried out in the Reserva Biológica Augusto Ruschi, ES, southeast of Brazil. The reserve is covered predominantly by primary Atlantic Forest. For climatic variations and a detailed description of the study area see Mendes and Padovan (2000).

The palms reach 5–12 m, and are characterised by a slender, single unbranched stem. They are monoecious, with conspicuous branched inflorescences. Flowers are numerous and inconspicuous. We selected a site in which *Euterpe edulis* and *E. espiritosantensis* grow in narrow sympatry. All palm trees with exposed stems were tagged in 1 ha divided into 25 plots of 20 x 20 m each. All individuals with vestiges of reproductive phases, such as scars of old inflorescences or peduncular bracts, were classified as adults. The individuals without these reproductive features were considered immature.

2.2 - Phenology and flowering features

Observations were conducted during one flowering season from April 2004 to December 2005 for 1212 tagged mature individuals of *E. edulis* and *E. espiritosantensis* using binoculars. Using a scaffold, the following reproductive features were measured: length of peduncular bract, duration of peduncular bract (days), number of inflorescences per stem, number of rachillae per inflorescence, number of staminate and pistillate flowers per inflorescence, and duration of male and female inflorescence phases (days).

2.3 - Floral visitors

Floral visitors to the palms were observed in August and September of 2004. Fifteen plants were observed (total of 60h) on sunny days, between 0600 h and 1800 h. Visits were observed directly or with binoculars, and scaffolds were used to allow visual proximity to the inflorescences. Insect visitors were collected for identification.

2.4 - Reproductive biology and seed germination

Five to six individuals of each palm morph were used for the following reproductive experiments: natural pollination, self - pollination, cross - pollination, agamospermy and interspecific cross - pollination. Percentage of fruit set was determined for each pollination treatment.

We compared germination rates between treatments for both palm morphs. Recently collected fruits (15 days) were planted in large pots (5 L) in a greenhouse. The substrate used was a mix of vermiculite, sand and natural organic fertiliser (3:2:1). Five replicates were performed for each pollination treatment with 30 seeds each. Percent germination was estimated by counting the seedlings over a period of three months (September to November 2005).

2.5 - Statistical analyses

The measurements of reproductive features were compared using a t - test. Data expressed as percentages were transformed (x2 = arcsine Vx) prior to statistical analysis and compared by analysis of variance (Anova). The Tukey test was used to compare means when F was significant at p 0.05.

RESULTS AND DISCUSSION

Individuals of *Euterpe edulis* and *E. espiritosantensis* were mixed in the area, whithout showing distinct habitat preferences. Their populations varied in density: *Euterpe edulis* had 910 individuals per hectare, whereas *E. espiritosantensis* had 302, all growing in narrow sympatry. Almost half, i.e., 462 of *E. edulis* and 123 of *E. espiritosantensis*, were adults, but only a few of them flowered during our observation season (*E. edulis* 142; *E. espiritosantensis* 22), indicating that mature individuals do not necessarily flower each year.

The reproductive features of the two palms are similar. Inflorescence buds are born axillary and are intrafoliar at anthesis. The inflorescence opens via an abaxial split in the peduncular bract. The length of the peduncular bract ($t = -5.43$, d.f. = 86; $P < 0.001$) and the time that the bracts enclose the inflorescence ($t = 3.31$, d.f. = 242; $P < 0.01$) were statistically different for *E. edulis* (X = 99.5+11.9 cm, N = 58; and X = 16.5+8.5 days, N = 203) and *E. espiritosantesis* (X = 115+14.2 cm, N = 30; and X = 11.8+6.9 days, N = 41). Other phenological features studied did not differ ($P > 0.05$) between the palms. Inflorescences have a short peduncle and are branched into rachillae of first order. The rachillae carry staminate and pistillate flowers in triads, except for the very tips that bear paired or single staminate flowers. Both palms sometimes produce inflorescences with only staminate flowers. The rachis, rachillae and flowers are cream - coloured in *E. edulis* and reddish in *E. espiritosantensis*. The immature fruits are green in *E. edulis* and vinaceous in *E. espiritosantensis*, but in both palms become dark purple when maturing.

The flowering period of *E. edulis* lasted from April 2004 to November 2005 with a peak in July. The flowering of *E. espiritosantensis* overlapped a small period of *E. edulis*, occurring from March to December, but it showed a distinct peak in September. There were synchronies between male and female phases in each palm morph. The 142 reproductive stems of *E. edulis* produced 223 inflorescences, of which 44 (19.7%) had only staminate flowers. *Euterpe espiritosantensis* produced 22 reproductive stems with 39 inflorescences, 18 (46.1%) of them being staminate only. Few stems produced staminate and pistillate inflorescences simultaneously (13 [5.8%] stems for *E. edulis*, and only two [5.1%] for *E. espiritosantensis*). Staminate and pistillate flowers started opening in the morning at about 0700 h, emitting a pleasant scent, which is more intense in the staminate phase. Pistillate flowers remained open for three days, while most staminate flowers fell off in the first day. Pollen was exposed as temperature increased and nectar was produced by the flowers in both palms. The first mature fruits were observed two to three months after flowering and the last five to seven months after flowering had ended.

Throughout the flowering period, floral visitors were attracted by abundant pollen and nectar production in staminate flowers. Visits usually began at 0800 h, increasing throughout the morning, and peaked between 1000 h and 1400 h, after which activity stopped. The guild of floral visitors of both *Euterpe* was predominantly composed of four species of Apidae (*Trigona* spinipes, *Nanotrigona testaceicornis, Oxitrigona sp. and Apis mellifera*). Other species were also recorded: three species of Vespidae (*Brachygastra lecheguana*, *Polybia paulista* and *Poyibia dimidiata*) and one of Muscidae (*Musca domestica*). *Trigona spinipes* and *Nanotrigona testaceicornis* were the most frequent bee visitors observed. All bee species had a similar behaviour: they arrived on the inflorescence and walked into the staminate flowers, collecting pollen. Bees on pistillate flowers also walked and touched the stigma.

Other sympatric species complex of palm (e.g. Henderson *et al.*, 000; Borchsenius, 1997; Bacon and Bailey, 2006) frequently have the same flower features, sharing the same pollinators, although there is some overlap in the flowering season, their flowering peaks are temporally separated. Sequential flowering guarantees offers of resources for a long period and the permanence of the pollinator in the area, but the differences in the flowering peak could reduce hybridization (Listabarth, 2001). Thus, we can suppose that difference in the flowering peak, as observed for *E. edulis* and

E. espiritosantensis, appears to have greater importance to limiting gene flow between closely related sympatric palms than the shift in flowering and pollination behaviour during the diversification process for the Arecaceae family.

In several inflorescences, flowers did not develop into fruits (42% for *E. edulis* and 24% for *E. espiritosantensis*). Both *Euterpe* palms developed fruits by all reproductive, however with low reproductive efficiency (fruit - set less than 15%), indicating the absence of self - and interspecific - incompatibility. Cross - pollination produced the most fruits in *E. edulis* (13.1%, N = 4271) and *E. espiritosantensis* (10.8%, N = 4355), and no fruit was developed by agamospermy, which was excluded from the statistical analyses. There was no significant difference ($P > 0.05$) among the reproductive experiments or between the palms. In *Euterpe espiritosantensis*, self - pollination produced the fewest fruits (6.2%, N = 3601), and 10.5% (N = 5202) of the fruits were developed by interspecific - crosses. On the other hand, for *E. edulis*, interspecific - crosses produced the fewest fruits (8.4%, N = 4354), and self - pollination was the second most important reproductive method (10.1%, N=3246). Our results on the reproductive biology of both *Euterpe* are in harmony with several other studies related to palm species (e.g. Henderson *et al.*, 000; Listabarth, 1999, 2001). No fruits were developed from agamospermy, as recorded for *E. espiritosantensis* (Bovi *et al.*, 994) and other palms (Borchsenius, 1997; Listabarth, 1999). Both developed fruits following self - pollination, with percentages similar to cross - pollination. Self - compatibility was previously reported for Arecaceae (Bovi *et al.*, 994; Borchsenius, 1997), but there is strong evidence for selection for outcrosses. The arrangement of the flowers in triads and the pollen/ovule ratio confers a characteristic of a xenogamous system. Geitonogamy could occasionally occur in individuals that produce more than one inflorescence with synchrony of male and female phases. Differences in inflorescences signals, as the distinct colours observed for *Euterpe edulis* and *E. espiritosantensis*, may contribute to reproductive isolation mechanisms as observed for others palms species.

In both *Euterpe* palms all seeds produced in the reproductive experiments germinated significantly more in *Euterpe edulis* (control = 65.3%; cross - = 60.7%; interespecific = 67.3%; N = 30 to each treatment) than *E. espiritosantensis* (control = 29.3%; cross - = 35.3%; interespecific = 34%; N = 30 to each treatment), except in self - pollination (55.3% and 34.2%, respectively). Other palms also show the potential for artificial hybridization (Bovi *et al.*, 994; Listabarth, 1999), and hybrid zones between palm species may be common. Interspecific crosses can occur between related species, indicating recent divergence with weak genetic differentiation.

Several palm species are separated mainly on the basis of variation in vegetative and reproductive morphology, with little or no variation in floral characteristics (Borchsenius, 1997; Bacon and Bailey, 2006). In this context, estimates of the number of species of palms vary greatly, and some of the past debate over the recognition of palm species has been caused by the paucity and poor quality of available herbarium specimens (Henderson, 1999). Henderson and Galeano (1996) addressed the status of *E. espiritosantensis* based on observations from available herbarium specimens, and in their opinion *E. espiritosantensis* and *E. edulis* have similar characteristics. Even Henderson (2006), recognized that current knowledge of morphological variation in palms is superficial, and there may be more than double the currently accepted number of species. Given the large similarity in palms congener species it is interesting to note that our investigated putative entities differ at the study location. *Euterpe edulis* has the basis of the colour of their leaf - sheaths green to yellow greenish and whitish inflorescences, while *E. espiritosantensis* has the basis orange to reddish brown and reddish inflorescence. Additionally, *E. espiritosantensis* has immature fruits vinaceous, and *E. edulis* green coloured. Furthermore, Martins *et al.*, (2007) identified different enzymatic systems that could be used to distinguish seeds of both palms. The peduncular bract is longer in *E. espiritosantensis* than in *E. edulis* and both *Euterpe* palms have distinct flowering peaks, different investment in male flower production and different percentages of germination in fruits from all reproductive treatments. These characters show that *E. edulis* and *E. espiritosantensis* have important morphological and reproductive particularities, and are potentially reproductive isolated.

Our data does not deny hybridization events between these two morphs. Nevertheless, even distinct species could be characterized by substantial but not necessarily complete reproductive isolation. Sympatric distribution can arise from sympatric speciation or secondary sympatry of allopatrically derived species (Jiggins, 2006). Our knowledge about geographic distribution of *E. espiritosantensis* is limited, but it is probable that this palm always occur in sympatry with *E. edulis*. A large - scale study involving population genetic structure of both species occurrence will be important for answering questions about speciation process and genetic species boundaries.

CONCLUSION

This taxonomic controversy involves endangered species, and can have a negative impact on conservation assessments. The establishment of justified recommendations in conservation biology requires critical evaluation of species limits (Bacon and Bailey, 2006). Our data strongly indicates that *E. espiritosantensis* deserves status of species, which is an essential step to safeguard individuals of this endangered taxonomic entity. We can suppose that *E. espiritosantensis* is suffering from the same predatory harvesting of *E. edulis*. Based on its low population density and low fruit set and seed germination, we can conclude that it is under significant threat, and deserves special attention for future conservation action. (We thank the staff of the Reserva Biológica Augusto Ruschi; the staff of the Entomology Laboratory of Universidade Federal de Viçosa for insect identification; CNPq/ Programa Mata Atlântica 690149/01 - 8 for funding; FAPERJ for the post - doctoral fellowship of D.D. Cruz; and CNPq for a productivity grant to T. Wendt).

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