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A Morphometric Analysis of a Putative Hybrid between *Pitcairnia albiflos* and *P. staminea* (Bromeliaceae)

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Abstract. Interspecific hybridization between *Pitcairnia albiflos* and *P. staminea* (Bromeliaceae) was investigated using morphometric analysis. Nine morphological traits representing inflorescence size and flower shape were recorded for different plants in a mixed population at Pão de Açúcar, Rio de Janeiro, Brazil in 1996. Examination of the morphometric variables using Analysis of Variance (ANOVA) and Discriminant Function Analysis supported a hypothesis for the existence of naturally occurring hybrids that are intermediate and distinct from the parental species.

Key words: Bromeliaceae, *Pitcairnia,* natural hybridization, Brazil, rare species

Introduction

The genus *Pitcairnia* L'Héritier is the largest genus in the subfamily Pitcairnioideae (Bromeliaceae). Smith and Downs (1974) placed 260 species in two subgenera: *Pitcairnia* (220 spp.) and *Pepinia* (40 spp.). Varadarajan and Gilmartin (1988) elevated the subgenus *Pepinia* (Brongn. ex André) Mez to generic rank, *Pepinia* (42 spp.), based on geographic distribution, vegetative characteristics, and flower and seed morphology. In Brazil, *Pitcairnia* (38 spp.) form three distinct geographic groups: 20 species occur in the Amazon region, eight in the central Brazilian state of Goiás, and ten in the Atlantic Forest (Wendt & Chamas 1997). The Atlantic Forest group, which is dominated by rare and threatened species, contains the two closely related species involved in this study: *Pitcairnia albiflos* Herb. and *P. staminea* Lodd. These saxicolous herbs found on rocky outcrops near the ocean are limited to a few isolated populations in Rio de Janeiro State; they are frequently allopatric (Wendt 1994). *Pitcairnia staminea* and *P. albiflos* are easily differentiated by the color of their flowers, red and white, respectively. In a mixed population on Pão de Açúcar, we observed plants showing a variety of pink floral colors. The genus *Pitcairnia* has flowers that are usually red, rarely white or yellowish, and very rarely blue or green (Smith & Downs 1974). Pink flowers were not previously reported, not even in the last taxonomic revision involving the *Pitcairnia* species of Rio de Janeiro State (Wendt 1994).

The role of natural hybridization in plants, a widespread phenomenon, is frequently discussed by monographers and evolutionary biologists (e.g., Rieseberg 1997, Arnold 1997, Eckenwalder 1998). Although bromeliad species easily hybridize by hand manipulation (McWilliams 1974), little is known about natural hybridization within Bromeliaceae. Misinterpretation or the failure to recognize natural bromeliad hybrids largely reflect lack of taxonomic knowledge and small herbarium collections. Many species of *Tillandsia* are easily crossed artificially (Read 1984), and some of the best known natural hybrids involve *Tillandsia* species (Gardner 1984, Luther 1985). Few records of natural hybrids are available for genera such as *Vriesea* (Read 1984) and *Pitcairnia* (Luther 1984). Interspecific hybridization in plants has been the focus of studies conducted during the past several decades (Arnold 1992). Numerous techniques have been used to investigate putative hybrids, and methods involving morphometric analyses frequently have been applied (DePamphilis & Wyatt 1989, Mackay & Morrison 1989, Freeman et al. 1991, Wilson 1992, Valverde et al. 1996, McDade 1997, Dibble et al. 1998).

The aim of this work was to use statistical analysis of certain morphometric traits to test the hypothesis that hybrids between *Pitcairnia albiflos* and *P. staminea* exist in a mixed population of the putative parental taxa at Pão de Açúcar, Rio de Janeiro.

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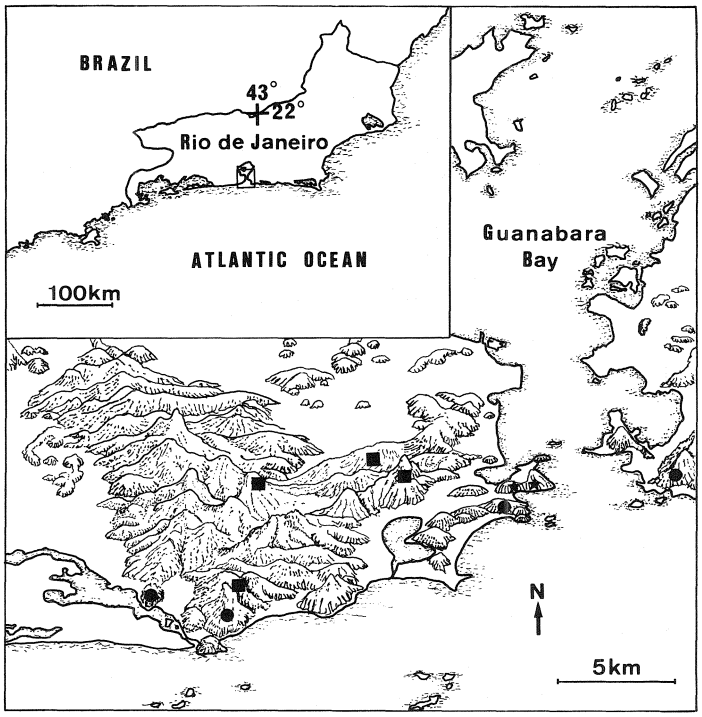

FIGURE 1. Distribution of *Pitcairnia albiflos* and *P. staminea* obtained from herbarium data (adapted from Wendt 1994). ■ = *P. albiflos*; ● = *P. staminea*; ★ = *P. albiflos* and *P. staminea* co-occurring at Pão de Açúcar.

MATERIALS AND METHODS

The study site is located on Pão de Açúcar, a bell-shaped gneiss-granitic outcrop, near Guanabara Bay in the city of Rio de Janeiro, Brazil (22°57′S, 43°59′W). It is among the most famous and attractive rock outcrops of countless ones along the southeastern Brazilian coast. Although within the domain of the Atlantic rain forest broadly defined, its vegetation differs markedly from that of the surroundings because of edaphic and microclimatic conditions (Carauta & Oliveira 1984, Porembski et al. 1998). The vegetation on Pão de Açúcar is rich in endemic species, and bromeliads are major physiognomic elements (Meirelles et al. 1999). The outcrop appears as a bald mountain with vegetation usually installed on a thin soil layer, forming elliptical soil-islands with various sizes related to substrate inclination. *Pitcairnia albiflos* and *P. staminea* are known from a few, frequently distinct neighboring outcrops surrounded by urban development (FIGURE 1). At Pão de Açúcar, the species are broadly sympatric along the rocky slope, but their populations are separated by habitat preference: *Pitcairnia albiflos* occurs in small, sun-exposed patches; and *P. staminea* occurs in large patches, often shaded by scrubs and trees. The observed intermediate forms, recognized by their pink flowers, frequently occur on disturbed sites invaded by weeds. The distance from a given putative hybrid to the nearest *P. albiflos* and *P. staminea* varied from a few (~5) to no more than 1000 m.

A total of 64 individuals were randomly selected from different patches at the study site. Of these, 16 belonged to *Pitcairnia albiflos,* 19 to *P. staminea,* and 29 to the putative hybrids (TABLE 1). The nine morphometric variables chosen were corolla length, corolla width, pedicel length, floral bract length, pistil length, vertical distance of stigma to anthers, scape length, number of flowers per inflorescence, and number of opened flowers per day. The corolla width was measured at the widest point of the distal end, where the petals are most divergent. The distance from stigma to anthers indicated how much the stigma exceeded the anthers. These measurements were taken in the field in May 1996, when plants of the two species and the putative hybrid were blooming together and could be recognized by the color of their flowers.

TABLE 1. Morphometric variables used to characterize *Pitcairnia* species and one putative hybrid in a study at Pão de Açúcar, Rio de Janeiro, Brazil, 1996. Values are means and standard deviations; means with the same letter are not significantly different (Unequal N HSD test, $P < 0.05$) and F values of one-way ANOVA are given (*** $P < 0.001$).

Plant character	*Pitcairnia albiflos* $n = 16$	*P. albiflos-staminea* intermediates $n = 29$	*Pitcairnia staminea* $n = 19$	One-way ANOVA F values
Corolla length (cm)	4.72 ± 0.49 a	3.96 ± 0.54 b	3.90 ± 0.25 b	17.23***
Corolla width (cm)	2.84 ± 0.26 a	2.01 ± 0.48 b	1.69 ± 0.17 c	45.02***
Pedicel length (cm)	1.78 ± 0.54 a	1.93 ± 0.33 a	2.75 ± 0.38 b	31.27***
Bract length (cm)	1.08 ± 0.18 a	1.18 ± 0.47 a	2.87 ± 0.41 b	120.77***
Pistil length (cm)	5.76 ± 0.77 a	6.63 ± 0.47 b	8.10 ± 0.2 c	92.89***
Stigma-anther distance (cm)	0.56 ± 0.14 a	0.30 ± 0.19 b	0.32 ± 0.24 b	9.99***
Scape length (cm)	62.06 ± 10.83 a	101.41 ± 15.95 b	120.26 ± 20.49 c	56.32***
No. flowers/inflorescence	17.25 ± 3.17 a	42.51 ± 12.06 b	51.26 ± 13.84 c	42.85***
No. opened flowers/day	2.93 ± 0.92 a	2.65 ± 0.93 a	3.31 ± 2.05 a	1.34

Analysis of variance (ANOVA) was performed for each trait in each taxon. When ANOVA results were significant at $P < 0.05$, a multiple comparison test (Unequal N HSD) was undertaken to detect differences between the taxa. Stepwise discriminant analyses also were performed to examine multivariate morphometric differences among the taxa and the putative hybrids. All statistical tests were conducted using Statistica 4.2 procedures of Statsoft, Inc. (1993).

RESULTS

Mean values differed significantly between parental taxa and putative hybrids for eight of the nine measured morphological attributes (TABLE 1). For seven of the nine traits, the mean values of hybrids were intermediate between means of both parental species. Corolla width, pistil length, scape length, and number of flowers of the hybrids differed significantly from parental species (TABLE 1). Hybrids did not differ significantly from *Pitcairnia staminea* in corolla length and stigma-anther distance, nor did they differ from *P. albiflos* in pedicel length or bract length.

TABLE 2. Standardized Coefficients for Canonical Variables on the first two Roots, derived from discriminant function analysis.

Plant character	Root 1	Root 2
Corolla length (cm)	−0.792	−0.428
Corolla width (cm)	−0.174	0.825
Pedicel length (cm)	−0.286	0.366
Bract length (cm)	0.593	0.746
Pistil length (cm)	1.031	−0.119
Stigma-anther distance (cm)	−0.212	0.572
Scape length (cm)	0.338	−0.183
No. flowers/inflorescence	0.165	−0.322
No. opened flowers/day	−0.037	0.398
Eigenvalue	14.529	2.068
Cumulative proportion	0.875	1.000

The three groups were neatly separated in the bidimensional discriminant space, with the putative hybrids in an intermediate position between parental species (FIGURE 2). Root 1, accounting for 87.5% of the variance, summarized the correlation among pistil length, corolla length, and bract length (TABLE 2). The placement of plants in FIGURE 2 can be explained in simple morphometric terms, pointing out the most significant ratios. Thus along root 1 (left to right: *Pitcairnia albiflos,* hybrids, and *P. staminea*), flowers range from those with a relatively shorter pistil to those with a longer one, from flowers with a relatively longer corolla to those with a shorter one, and from flowers with a relatively shorter bract to those with a longer one. Root 2, accounting for 12.5% of total variance, summarized the correlation of corolla width, bract length, and stigma-anther distance.

The squared Mahalanobis distances (in parentheses) showed that the distance between *Pitcairnia staminea* and *P. albiflos* (106) is larger than the distances that both species showed to their hybrids (38 and 32, respectively). Finally, the percentage of grouped cases correctly classified was 100%.

DISCUSSION

Several morphometric methods are used to show intermediacy as evidence for hybridization. These methods are criticized, because they do not allow a distinction between hybridization and primary divergence (Wilson 1992). Rieseberg (1995) argues that hybrids are not always morphologically intermediate to their parents, because the expression of parental vs. interme-

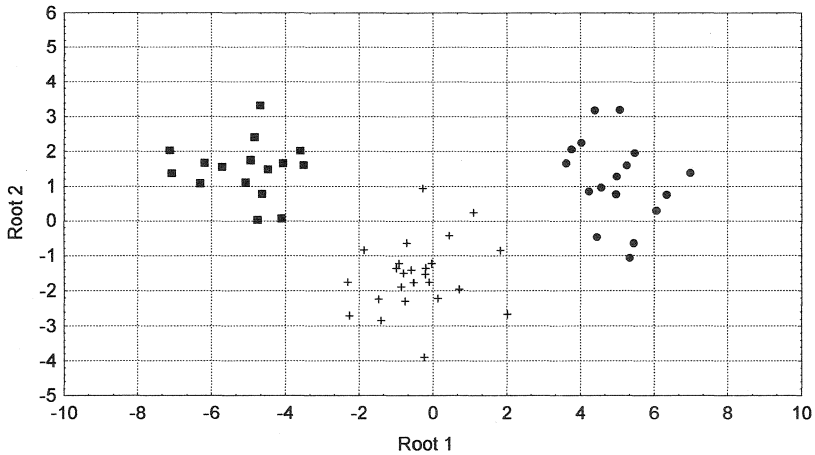

FIGURE 2. Scatter plot of scores derived from discriminant functions Root 1 vs. Root 2 produced by Stepwise Discriminant Analysis applied to nine morphometric characters for mixed populations of *Pitcairnia* in Pão de Açúcar: *P. albiflos* (■), *P. staminea* (●), and hybrids between *P. albiflos* and *P. staminea* (+).

diate character states in hybrids depends on the nature of the genetic control of a particular character, as well as on interactions with the environment. Despite these problems, systematics has a long record of successfully identifying hybrids as such, using morphological intermediacy as a primary criterion (e.g., Gardner 1984, Valverde et al. 1996). Even when assumptions are less than precise, a preponderance of intermediate character states remains as evidence against divergence in favor of hybridization (Wilson 1992). Our morphometric analyses of *Pitcairnia albiflos, P. staminea,* and the putative hybrids support our hypothesis regarding hybridization, because the putative hybrid plants were intermediate to their parental species in most characters measured.

Pitcairnia albiflos (Herbert 1826) and *P. staminea* (Loddiges 1823) are known from the last century. Treated in many classic Bromeliaceae monographs (Baker 1889, Mez 1894, 1896, 1935, Smith & Downs 1974), the species were always identified taxonomically without ambiguity. Herbaria material has indicated their co-occurrence at Pão de Açúcar since 1957 (Fontoura et al. 1991), but the possibility of hybridization was not raised prior to our study, not even in the most recent revision on these species (Wendt 1994). Grant (1981) argues that natural hybridization and gene flow often occurs between sympatric species that are disjunct and non-interbreeding over a wide area but that hybridize locally in one or only a few sites. Hybridization may not affect the distinctiveness of the species involved; but interspecific hybridization is a potential threat to the integrity of the parental species if introgression follows. Introgression, in turn, has the greatest impact on populations of rare species. The morphological distinctiveness among hybrids and both parental species, in turn seemed to imply absence of introgression, because apparently pure parental plants are represented in the investigated mixed population. Unfortunately, genuine pure parental populations from other localities (in allopatry) were not obtained for comparison in this study. Conversely, the high abundance of hybrid individuals in the field could be explained by a high frequency of fortuitous interbreeding events between the parental species; thus backcrossing presumably would be possible (Heiser 1973). *Pitcairnia albiflos* and *P. staminea* flower simultaneously (Wendt 1994). Despite their distinct microhabitat preference and different pollination syndromes (white and scented flowers in *P. albiflos,* red and non-scented flowers in *P. staminea*), we observed trigonid bees visiting both species, as well as the putative hybrids, during our field measurements.

Additional studies of reproductive interactions between parental and hybrid genotypes and of the distribution and fitness of parental and hybrid genotypes in the field will help explore the implication of this hybridization on future evolution and conservation of these rare species.

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