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“Inbreeding depression in *Vriesea gigantea*, a perennial bromeliad from Southern Brazil”

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Inbreeding depression in *Vriesea gigantea*, a perennial bromeliad from Southern Brazil

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Running title: Inbreeding depression in *Vriesea gigantea*

Key words: Brazilian Atlantic Rainforest, Bromeliaceae, fitness, mating system

Abstract

Vriesea gigantea is a bromeliad distributed in the Atlantic Rainforest, Southeastern Brazil. It is characterized by the presence of tanks which are used as an important source of resources by populations of associated biota. Habitat destruction, fragmentation and predatory colection have decreased natural populations of *V. gigantea*. Inbreeding depression is a reduction in fitness in the progeny of closely related individuals. In plants species the effect of inbreeding depression is assigned to selfing or biparental inbreeding. We aim to describe the occurrence of inbreeding depression (δ) in three natural populations of *V. gigantea*, and to correlate this phenomenon with fertility and mating system of the species. A total of 54 mother-plants were sampled and 108 flowers used for pollination treatments (selfing, outcrossing and control). In the mother-plants we analyzed plant and inflorescence height and number of flowers. In the progenies, the parameters seed set, germination and survival rate and final weight were evaluated. The results indicated no differences between treatments, but a low to moderate level of inbreeding depression was detected in *V. gigantea* (δ = 0.02 to 0.39), according to molecular data of previous study. Based on the findings, we may infer that *V. gigantea* populations have a mixed mating system that tolerate some degree of inbreeding, with evolutive potential to selfing as main reproductive system.

Introduction

Vriesea gigantea Gaudich is an epiphytic, saxicolous, or terrestrial bromeliad, with geographic distribution in Southeastern Brazil, specifically in the Atlantic Rainforest biome (Figure 1). It is found in an altitudinal track of 5 to 500m (Smith and Downs, 1977; Reitz, 1983). As many other bromeliad species, *V. gigantea* is very appreciated as ornamental plant and its wild populations have been destroyed or restricted to small size due to habitat destruction, fragmentation and predatory gathering (Bered *et al.*, 2008). Like the vast majority of bromeliads, *V. gigantea* is characterized by the presence of tanks which are used as an important source of resources by populations of associated biota. The bromeliad tank may be considered an essential component in natural ecosystems, since it expands the supply of water, nutrients, microhabitat, shelter, feeding and breeding site for many plant and animal species (Benzing, 1995; Rocha *et al*., 1997; Rocha *et al*., 2004; Cascante-Marín, 2006). Recent studies showed that *Vriesea gigantea* populations from Southern Brazil are fertile considering flower, fruit, and seed production (Paggi *et al.*, 2007). The species is self-compatible and showed pollinator limitation in Itapuã State Park population, which was considered a consequence of habitat fragmentation, and specifically, a disruption of the bat pollination mutualism (Paggi *et al.*, 2007).

The genetic structure of plant populations reflects the interactions of different processes, including a long-term evolutionary and short-term ecological history of the species, such as the effects of habitat fragmentation, shifts in distribution and population isolation (Gonzáles-Astorga *et al.*, 2004). The patterns of genetic diversity of nuclear and plastids molecular markers of *V. gigantea*, including populations from the total geographical distribution, indicated latitudinal trend of decreasing diversity from North to South away from the equator, consistent with historical forest expansion from the Northern half of the present distribution range (Palma-Silva *et al.*, 2009). Further species expansion appears to be impeded by lack of gene flow at the current range edges (Palma-Silva *et al.*, 2009). Moreover, Paggi (2009) studying the mating system of *V. gigantea*, using molecular markers, showed that populations from Southern Brazil have moderate outcrossing rate with high pollen gene pool structure, also indicating low gene flow via pollen.

According with some theoretical models, the evolution of different mating systems is determined by several factors including inbreeding depression, genetic control of the sex types and optimal allocation of resources to female and male function (Charlesworth, 2006). Inbreeding depression, defined as the reduction in fitness in the progeny of closely related individuals, may be caused either by the homozygous expression of deleterious alleles after inbreeding (partial dominance or mutation-selection balance) or a decrease in heterozygotes that exhibit a fitness advantage over homozygotes (Culley *et al.*, 1999). In plant species this is often associated with increased seed abortion, low germination rates, high seedling mortality and poor growth and flowering of offspring (Dudash and Fenster, 2000; Oostermeijer *et al.*, 2003; DeVere *et al.*, 2008), therefore inbreeding depression occurs in any stage of plants life.

Depending on the value of inbreeding depression (δ), either complete selfing (δ < 0.5) or complete outcrossing (δ > 0.5) have been predicted to evolve as the reproductive mode of a species (Lande and Schemske, 1985; Cheptou, 2002). In this view, when inbreeding depression is above 50%, selfing alleles are lost from the population because the fitness of plants possessing these alleles is lower than the plants possessing alleles for outcrossing (Hull-Sanders *et al*., 2005). The inbreeding depression can be estimated in a direct or indirect way, through measures of phenotypic traits, such as biomass, reproductive potential, and reproductive success; or through molecular approaches which quantify genotypic frequencies that generate an inbreeding coefficient (*F*) of adult plants relative to the expected *F* of progeny based on the selfing rate (s) (Goodwillie *et al.*, 2005).

This study aim to analyze the effects of different mating types – selfing and outcrossing - on characteristics that reflect fitness in the progenies in three populations of *V. gigantea* in Southern Brazil, estimating the ocurrence of inbreeding depression; and to aggregate informations for development of strategies to species conservation.

Material and Methods

Study sites

The study was conducted in three populations of *V. gigantea* from Southern Brazil. The Maquiné population (29°48' S, 50°16' O) is located in the coastal north region of the Rio Grande do Sul State (Figure 1). The climate is subtropical with average annual temperature of 21.5℃. Maquiné has a total area of 622km², which houses the Natural Reserve of the Serra Geral, with an average altitude of 38 m. The sampled *V. gigantea* subpopulations are located outside the Reserve area. The Itapuã State Park (ISP) population (51°05' O, 30°27' S), has a total area of aproximately 5566 ha (Figure 1), being used for research, environmental education and leisure time. The local climate is classified as subtropical humid, with average temperature of 17.5℃, and no dry season. The Ecological Station of Taim (EST) population (31°56' S, 52°25' W), is located in the narrow strip of land between the Atlantic Ocean and Mirim Lake, in the South of Rio Grande do Sul state (Figure 1). The station is distinguished mainly by the diversity of migratory birds, and the richness of its swamps, having an average temperature of 18℃.

Pollination treatments

The field work was performed in the reproductive season of *V. gigantea*, in January and February/2008. Hand-pollination treatments (outcross and self pollination) were performed in two flowers per plant, randomly sampled, one for each treatment, totaling 108 flowers from 54 individuals, considering all populations. The flowers were bagged until opening and manipulated according each treatment. The flowers from outcrossing pollination treatment were previously emasculated to avoid its self pollination, and received pollen from another plant; in the self pollination treatment, two anthers from the same flower were used to pollinate it; and in the control, no pollination treatment was made, one flower was randomly chosen of each mother-plant. After, the flowers were bagged and marked with wire of different colors.

Analyzed parameters

A total of 54 individuals (mother-plants) of *V. gigantea* were analyzed considering the following parameters: plant and inflorescence height and number of flowers per plant. These parameters indicate the plants reproductive potential and vitality that could be affected by an inbreeding depression phenotype. Also to determine whether there was any evidence of inbreeding depression in the early stage life of this species, we compared the fitness of outcrossed and selfed progenies considering the following aspects: a) number of seeds per fruit, by an indirect countering, comparing the mass of 20 seeds and the mass of all seeds of each fruit; b) % germination, thirty seeds sampled per progeny; c) survival rate, by periodic countering the seedlings remaining; d) final weight of the seedlings after 11 months in culture, in each treatment and control, using an analitical balance for weigh the total mass of all the seedlings of each treatment divided by number of seedlings in each progeny.

Germination

Fruits originated from each treatment and control, were collected in June 2008. The seeds germination was conducted *in vitro*; 30 seeds were disinfected by washing in alcohol of 70 followed by bleach, and placed in petri dishes with vermiculite previously sterilized. The dishes were incubated in an acclimatized room, with relative humidity near 100%, and a photoperiod of 16 hours in the presence of light at 25℃, and eight hours in the dark at 22℃. The germination was monitored daily.

Inbreeding depression

The inbreeding depression (δ) measure was performed using the following formula (Husband and Schemske, 1995):

$$\delta = 1 - (Ws / Wo)$$

Where *Ws* is the mean *fitness* of self pollination treatment; and *Wo* is the mean *fitness* of outcross pollination. The inbreeding depression was estimated for each parameter and analyzed separately.

Results

The parameters evaluated in mother-plants of *V. gigantea* are presented in Table 1. Taim population showed higher value for mean plant height than Itapuã; and Maquiné population showed higher value for mean number of flowers per inflorescence than Taim.

In agreement with the total means presented in Table 2, there were no significant differences among the treatments considering each population. The total means obtained from the parameters evaluated in the progeny from each pollination treatment are presented in Table 2. It was not found statistic differences among treatments for none of the parameters evaluated (Table 2). Despite, the progeny originated from self pollination treatment showed, generally, lower values of number of seeds per fruit and germination rate than the progeny from outcross pollination (Table 2). The parameters analyzed in the progenies per population are showed in Figure 2. Considering the self pollination treatment, Maquiné population presented the lowest number of seeds (~315.64 seeds per fruit), and germination was lower in Taim (86% germination); Itapuã population showed the highest number of seeds and germination rate in the outcross pollination treatment (~686.05 seeds per fruit; 93% germination; Figure 2a and 2b). The weight reached over 11 months of growing ranging from 0.015g to 0.050g per seedling (Figure 2c). The survival rate was high, reaching until 95%, with exception of control population of Taim that showed 49% of lost seedlings during growing time (Figure 2d). In agreement with the total means presented in Table 2, there were no significant differences among the treatments considering each population.

The inbreeding depression (δ) estimated from each analyzed parameter per sampled populations, was considerate low to moderate, varying from 0.02 (germination rate), to 0.39 (seed per fruit; Figure 3). The final weight parameter showed no inbreeding depression ($\delta = 0$).

Discussion

The results obtained from the mother-plants of *V. gigantea* indicate few differences considering the reproductive and plant vitality features (plant and inflorescence height and number of flowers per plant). The differences observed among populations may be assigned to envirnment conditions of each population with differences in habitat quality. In this way, we may sugest that the high humidity of Taim contribute for a greater size plant; whereas formation of the primary Forest in Maquiné, an ancient forest with higher epiphytic arrangement of bromeliads, contribute for a strong investment of plant in flowers that ensure its reproduction.

Althought the differences found between populations, the mother-plants analysis could be used as a control over the current situation of inbreeding depression in the studied populations, since it was not detected small fitness in the sampled plants. The timing of inbreeding depression in a life cycle may have substantial fitness consequences. Early stage inbreeding depression may reduce the fitness of the progeny to zero, whereas with late stage inbreeding depression, the proportional reproductive contribution to the next generation is > zero (Charlesworth, 1980; Husband and Schemske, 1996; Hull-Sanders *et al.*, 2005). In addition, the identification of the most affected life history stage by inbreeding depression can aid in the interpretation of a population's demographic patterns (Kittelson *et al.*, 2000). Selfed genotypes that experience inbreeding depression could influence population dynamics through differential changes in seed bank input, recruitment, fecundity, or tolerance to herbivores (Hamilton and Mitchell-Olds, 1994). We considered, based on the mother-plants analysis, that none substantial inbreeding depression effect was detected in the sampled plants.

Results from progenies analysis showed high seed set, which is an indicative of the high fertility of *V. gigantea* populations (Table 2). Despite the absence of significant differences between the pollination treatments, the results from self pollination treatment revealed a low seed production, more markedly in Maquiné (Figure 2). From seed set, the estimated inbreeding depression showed greater value (0.39, Maquiné population; Figure 3), which is similar with results based on nuclear molecular markers (Paggi, 2009; δ = 0.509), in the Maquiné population. Also, as *V. gigantea* is a self-compatible species (Paggi *et al.*, 2007), exhibiting mixed mating system (Paggi, 2009), it is likely that express inbreeding depression at early stages of reproduction, like seed set, particularly in small populations trapped in habitat fragments (Aizen *et al.*, 2002). The highest germination rate was observed in the progeny from outcrossing of Itapuã population (93%; Figure 2c). The inbreeding depression for germination rate was low in all populations, ranging from 0.02 to 0.05 (Figure 3), indicating that the germination potential of *V. gigantea* is not a characteristic greatly affected by selfing. Partially, the seed set and germination parameters agree with the expected for many plant species, where inbreeding depression occurs more often during growth and seed set in angiosperms (Husband and Schemske, 1996).

After eleven months of growing, the final weight for all progenies were considerably low (comparing with *V. gigantea* growing in culture with MS medium in other study, Paggi *et al*., 2007), considering seedlings derived of control, selfing and outcrossing (Table 2). These results can be attributed to some factors such as: mineral substrate (vermiculite) used during culture, which is very poor; the difficulty manipulation of seedlings to avoid fungi contamination and adhesion of vermiculite particles in the leaves, and the conditions of light and water provided in growth environment. In the Maquiné population was observed a higher weight in the selfed than in the outcrossed progeny (Figure 2c), contrasting with the previous data from seed set and germination rate. We believe that these factors interfered minimizing the potential growth of *V. gigantea* seedlings and leading to inaccuracy in weighing procedure. For these reasons we could not estimate the inbreeding depression for the final weight parameter.

The survival rate was 85% of seedlings from outcrossing pollinated treatment (Table 2), which remained until the complete period of growth evaluated (11 months). In the Taim population the self pollinated progeny showed higher survival (90%) than outcrossing progenies, presenting no inbreeding depression (values negative or zero). Only in the Itapuã population a very low inbreeding depression for survival was detected (0.12;Figure 3). However, usually survival of seedlings in natural conditions does not follow these rates, because it is one of the most vulnerable stages in the life cycle of plants, with high seedling mortality (Harper, 1997; Winkler *et al.*, 2005).

The inbreeding depression per population showed the highest values in Maquiné and Taim for seeds per fruit parameter (Figure 3). Maquiné population can be considered a primarily outcrossing population according to Paggi (2009). Concordantly, it was the population with higher inbreeding depression values (δ) for seed set and germination rate. The Itapuã population showed a low inbreeding depression for all traits evaluated, which can be associated to its relevant history of land use. The Park was used for stone exploration during many years, which reduced plants and animals species populations, including *V. gigantea* populations. Therefore, populations that may have had very small effective population sizes (Ne

< 500) for a long time, or those that have recovered from population bottlenecks, should be less sensitive to inbreeding depression due to the purging of deleterious recessive alleles (Brook *et al.*, 2002)

Many models of mating system evolution predict that inbreeding depression should decrease from generation to generation as the selfing rate in a population increases (Charlesworth *et al.*, 1990; Uyenoyama and Waller, 1991; Hull-Sanders *et al.*, 2005). Selfing should evolve in a population because often has a large genetic advantage, unless some strong selective force acts against inbreeding. That is, inbreeding depression is likely often to be the most important disadvantage (Charlesworth, 2006). The results found in this study corroborated with those obtained by Paggi (2009) and allow us to suggest that Southern populations of *V. gigantea* can tolerate some degree of inbreeding and could be adapting to selfing as the main reproductive system.

Even, the quantification of inbreeding depression, breeding effective population size, minimum viable population size and levels of genetic variation and gene flow in natural populations provides specific and comparable measurements of processes that affect endangered populations (DeSalle and Amato, 2004). In this study, we attempted to estimate the inbreeding depression in Southern populations of *V. gigantea* and to provide new insights to our previous researches which include population demographic patterns, genetic diversity and mating system of this species (Paggi *et al.*, 2007; Palma-Silva *et al.*, 2008; Paggi, 2009; Palma-Silva *et al.*, 2009). These approaches will contribute to better understand the evolutionary process occurring in the *V. gigantea* margin range populations and will contribute to plan efficient conservation strategies.

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Legend of figures

Figure 1. Study sites in Rio Grande do Sul state, Brazil. A) Maquiné; B) Itapuã State Park (ISP); C) Ecological Station of Taim (EST). The gray shadow is *Vriesea gigantea* geographic distribution.

Figure 2. Means of the analyzed parameters in progenies per population and per treatment. (a) Nº seeds/fruit; (b) Germination rate; (c) Final weight; (d) Survival rate.

Figure 3. Inbreeding depression (δ) estimated for each parameter, and per population analyzed. Final weight did not show δ for any parameter.

Tables

Table 1. Parameters evaluated in mother-plants of *V. gigantea* in each population.

Parameters	ITAPUÃ	MAQUINÉ	TAIM	TOTAL
Plant height (m) [1]	0.63 [b]	0.70 [ab]	0.80 [a]	0.71
Inflorescense (m) [2]	1.59 [ns]	1.40 [ns]	1.42 [ns]	1.47
Nº flowers [3]	155.17 [ab]	194.08 [a]	144.66 [b]	164.63
Nº obs	24	12	18	54

[1] $F = 6.47$, $P = 0.0032$; [2] $F = 1.81$, $P = 0.1742$; [3] $F = 3.79$, $P = 0.0292$. * Means followed by the same letter, in the line, did not showed difference by Tukey test (5%). n.s. = non significant by Tukey test.

Table 2. Parameters analyzed in progenies of *V. gigantea* for each treatment.

Parameters	CONTROL	SELFING	OUTCROSSING
Nº seeds/fruit [1]	609.55 [ns]	499.81 [ns]	646.48 [ns]
Germination rate [2]	0.86 [ns]	0.89 [ns]	0.91 [ns]
Final weight (g/plant) [3]	0.04 [ns]	0.04 [ns]	0.03 [ns]
Survival rate [4]	0.73 [ns]	0.80 [ns]	0.85 [ns]

[1] $F = 2.86$, $P = 0.0642$; [2] $F = 0.87$, $P = 0.4232$; [3] $F = 1.48$, $P = 0.2351$; [4] $F = 1.43$, $P = 0.2470$. n.s. = non significant by Tukey test.

Fig. 1

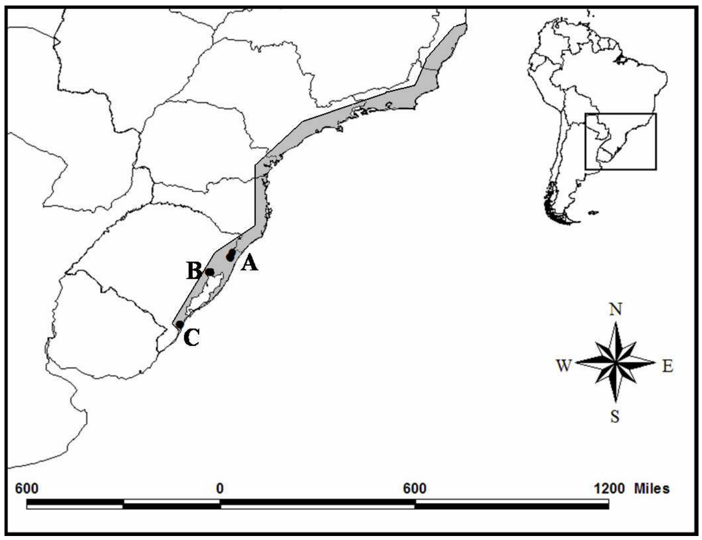
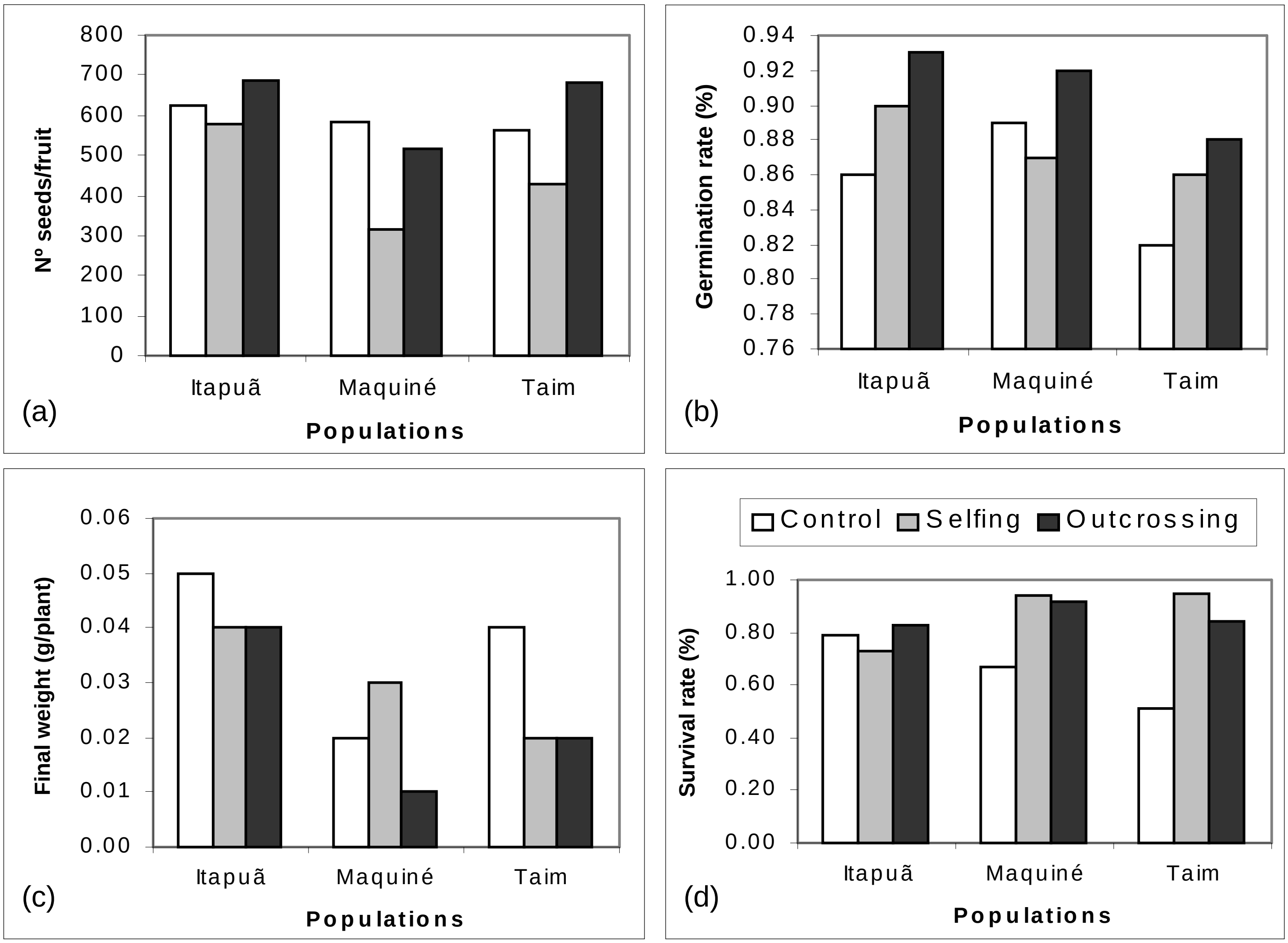
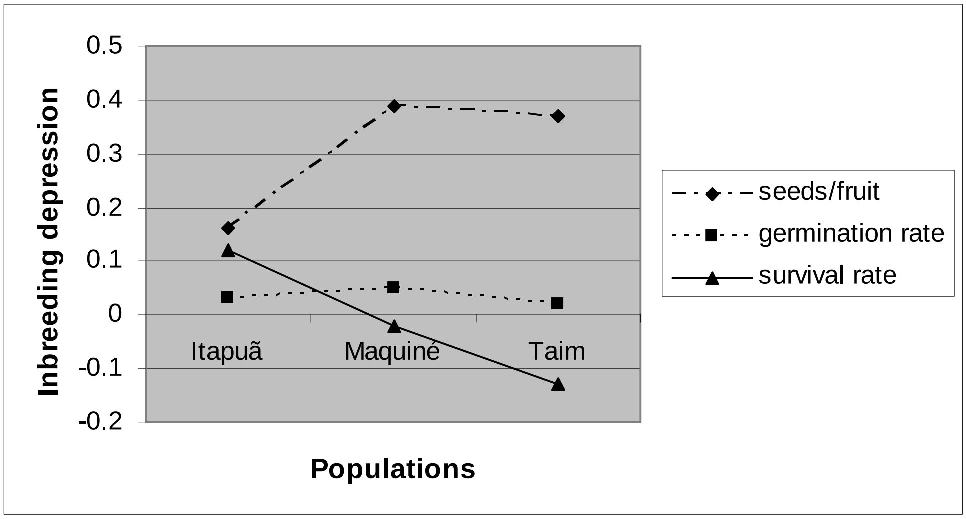


Fig.2



(a)
Nº seeds/fruit
800
700
600
500
400
300
200
100
0
Itapuã
Maquiné
Taim
Populations
(b)
Germination rate (%)
0.94
0.92
0.90
0.88
0.86
0.84
0.82
0.80
0.78
0.76
Itapuã
Maquiné
Taim
Populations
(c)
Final weight (g/plant)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Itapuã
Maquiné
Taim
Populations
(d)
Control
Selfing
Outcrossing
Survival rate (%)
1.00
0.80
0.60
0.40
0.20
0.00
Itapuã
Maquiné
Taim
Populations

Fig. 3


0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
Inbreeding depression
Itapuã
Maquiné
Taim
Populations
seeds/fruit
germination rate
survival rate