



## Risk of genetic vulnerability and aspects of the reproductive biology of *Psychotria ipecacuanha* (Rubiaceae), a threatened medicinal plant species of Brazilian forests

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### ABSTRACT

*Psychotria ipecacuanha*, commonly known as Ipeca, is a medicinal plant of pharmacological and economic value. The species is distylous; it has populations with two floral morphs, one with long and one with short styles. Apart from the presence of two floral morphs in a balanced ratio (1:1), reciprocal herkogamy of the reproductive organs between alternative morphs is desirable to maintain cross-pollination. The risk factors for genetic erosion and conditions for sexual reproduction in natural populations of *P. ipecacuanha* were investigated. The main risks for genetic erosion in four populations studied were: habitat change in the forest fragment where they occurred and in the surrounding area over the last 20 years; proximity to agricultural areas; frequency of drought affecting the forest fragment; and the area occupied by the species within the forest fragment. All evaluated populations were isoplethic with the reciprocity of reproductive organs varying across populations. Anthropogenic factors, associated with morphological and reproductive characteristics (e.g., low reciprocity between anther and stigma and low pollen production), indicate risks for the maintenance and reproduction of Ipeca in the population of the municipality of Denise. Habitat loss, small clusters, and the low number of reproductive plants jeopardize the survival of the studied populations.

**Keywords:** distyly, floral morphs, genetic erosion, Ipeca, reciprocal herkogamy

## Introduction

Genetic erosion can be defined as a permanent reduction in the richness or regularity of local alleles, or the loss of combinations of these alleles in a given area, over the course of time, resulting in a loss of evolutionary potential and a reduction in the direct use of plant and population genetic resources (Maxted *et al.* 2002).

The rate of species loss far exceeds the origin of new species, and unlike the mass extinctions of species in the

past, those occurring currently are mainly results of human activities (Kideghesho *et al.* 2005). The reproductive system, life cycle, and ploidy level of a species affect the movement of genes and genetic diversity. The issue of genetic erosion is addressed within the main context of the biology of individual species.

Ecogeographic studies collect and synthesize ecological, geographic, and taxonomic information as a way of establishing predictions underlying the formulation of conservation strategies and collection priorities (Crane 2003).

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*Psychotria ipecacuanha* (Ipeca), a synonym of *Carapichea ipecacuanha*, see (Andersson 2002), is a distylous shrub found exclusively in shaded forest understories (Oliveira & Martins 2002). *Psychotria ipecacuanha* occurs in Central America (e.g., Panama, Nicaragua, and Costa Rica), South America (Colombia), part of the Brazilian Amazon (states of Rondônia and Mato Grosso), and the Atlantic Forest (mainly in the states of Bahia, Espírito Santo, Rio de Janeiro, and Minas Gerais) (Skorupa & Assis 1998; Assis & Giulietti 1999). In their natural environment, Ipecas are rarely found as isolated plants, and tend to grow in circular or elliptical clusters.

At the first meeting of the committee of Conservation of Genetic Resources of Medicinal and Aromatic Plants, *P. ipecacuanha* was recognized as a priority for conservation studies (Vieira *et al.* 2002). In 2013, *P. ipecacuanha* was considered vulnerable to extinction due to the effects of predatory exploitation, trade, and export, the fragmentation and deforestation of its forest habitat, and due to the requirement for cross-pollination, increasing the rareness of subpopulations and making them prone to deleterious stochastic, genetic, population, and environmental effects (Zappi *et al.* 2013). The medicinal and economical value of *P. ipecacuanha* is based on the expectorant, amebicidal, and vomitive substances contained in the isoquinolinic alkaloids emetine and cephalin found in the roots (Giraldo *et al.* 2015).

Distylous species exist in populations with two floral morphs. The pin morph has long pistils and short stamens (called long-styled morphs), while the thrum morph presents short pistils and long stamens (called short-styled morphs) (Ferrero *et al.* 2009). Apart from the presence of floral morphs in a balanced ratio (1:1), reciprocal herkogamy of the reproductive organs between the alternative morphs (long-styled and short-styled) is desirable for cross pollination. This floral polymorphism is usually accompanied by an intra-morphic auto-incompatibility system and a series of accessory floral features, that differ between floral morphs (size of flowers, lobe stigma, number and size of pollen grains; Ganders 1979). Due to the intra-morphological self-incompatibility system, only legitimate pollen from the opposite morph is able to fertilize the ovules and produce viable seeds (Barrett 2002). The reproduction of distylous species also depends on factors related to pollen viability (Silva & Vieira 2015) and changes in floral morphology, which can affect the position of the reproductive organs (Hodgins & Barrett 2008).

Based on ecological conditions and habitat requirements for species conservation, our objective was to test the hypothesis that the intensity of anthropogenic disturbance can influence the isopleth, floral morphometry, pollen viability, and population size of four natural Ipeca populations in the southwest region of Mato Grosso, Brazil.

## Materials and methods

Collection expeditions were carried out from October 2015 to October 2016 to seven municipalities in the southeastern region of Mato Grosso - Brazil (Fig. 1) where Ipeca has previously been reported in populations of forest fragments. In only four of these municipalities (Barra do Bugres - BAB; 14°52'S 57°53'W, 263 m above sea level a.s.l., Denise - DEN; 14°39'S 57°05'W, 191 m a.s.l., Nova Olímpia - NOL; 14°47'S 57°15'W, 181 m a.s.l., and Tangará da Serra - TGA; 14°38'S 57°19'W, 457 m a.s.l.) Ipeca populations were found. On a local spatial scale, the distances between the nearest clusters within a given population ranged from 40 to 100 m. Populations of Ipeca with groupings of more than three plants (rametes) in the reproductive phase were evaluated. Voucher specimens (Fig. 2) were deposited in the Herbarium of the State University of Mato Grosso, Brazil (TANG 3290, 3291, 3292, and 3293).

The climate of the region is characterized by two well-defined seasons: rainfall, between October and April, and drought, between June and August. The vegetation is considered a transition from the Amazon Forest to the Cerrado biomes and is currently fragmented due to the implantation of monocultures. Data on forest fragments were obtained by direct observations during expeditions, from conversations with local residents, or surveys exploiting information from the internet, road maps, and institutions.

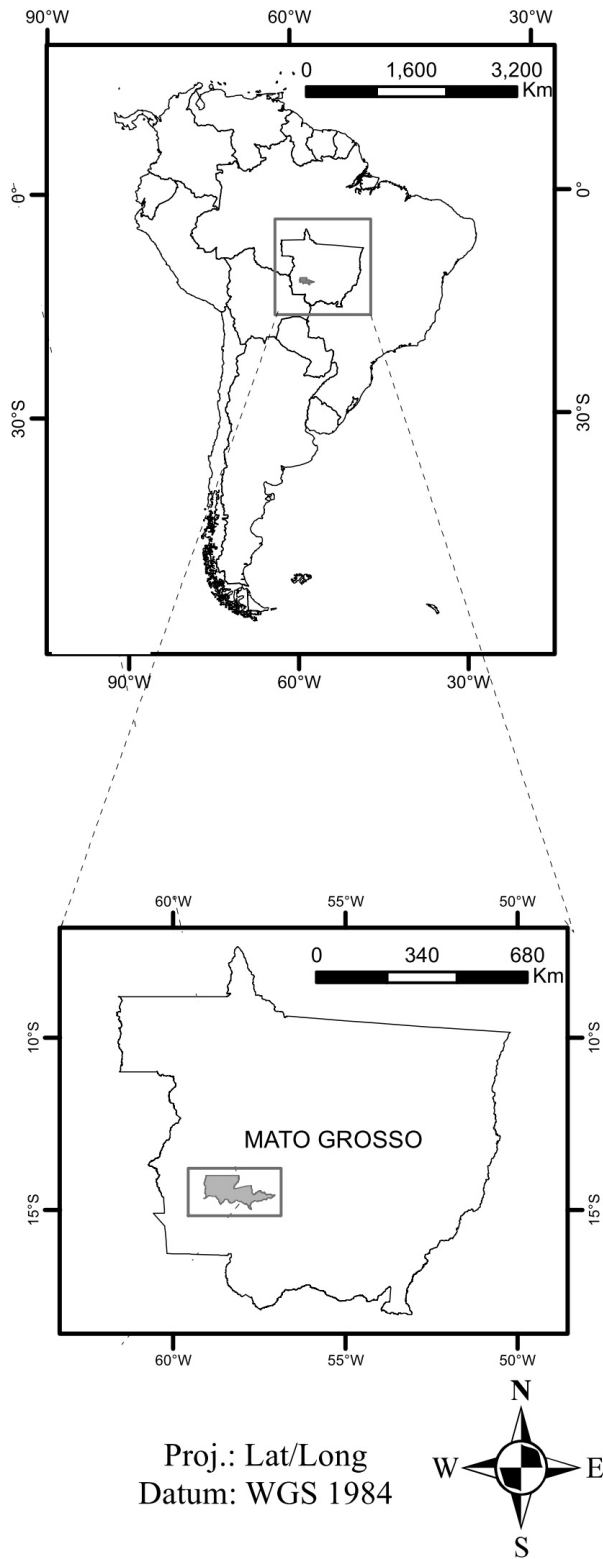
Five soil samples per population were collected and soil fertility parameters were analyzed. The geographic coordinates and altitude were determined with a GPS device. Rainfall and temperature data were provided by the meteorological institute of Brazil - INMET.

The risk of genetic erosion in *P. ipecacuanha* was evaluated by a methodology adapted from Oliveira & Martins (2002). For each forest fragment, the risk of genetic erosion was determined by adding the scores attributed to 20 factors (Tab. 1). A score between 0 and 10 was assigned to each factor. The relative risk was determined by the sum of each risk score of the factor, and is calculated as the percentage in relation to the maximum sum of all factor scores (200 or 100%) (Tab. 1). The term "agricultural" used for factors F3 and F4 indicates areas used for agriculture and/or livestock husbandry. The term "population center" used for factor F5 (Tab. 1) indicates the city closest to the forest fragment. "Main road" (Factor F6) indicates the nearest state highway (with or without asphalt paving) (Tab. 1). The sum of the scores represents the threat of genetic erosion to which *P. ipecacuanha* is exposed in the four municipalities analyzed.

To evaluate the ratio between short- and long-styled morphs, flowering plants of both floral morphs were counted and identified in the four studied forest fragments. In each population, the numbers of clusters, area occupied by the



cluster, total number of plants per population, and number of plants in the reproductive stage per floral morph were observed.



**Figure 1.** Map showing the study location of *Psychotria ipecacuanha*, Mato Grosso-Brazil.

The morphometry of short- and long-styled morphs was analyzed. For each morph, five flowers were collected from four plants per population, making a total of 20 flowers per morph/population. The flowers were stored in 70 % alcohol. In the laboratory, using millimeter graph paper and a stereoscope microscope, we measured floral structures were measured using millimeter graph paper and a stereoscope microscope as follows: corolla length (mm); stigma lobe length (mm); anther size (mm); style height (mm); and stamen height (mm) (Fig. 3).

The reciprocal placement of anthers and stigma was further studied using the reciprocity index developed by Sánchez *et al.* (2008; 2013) by investigating the influence of corolla tube length on the herkogamy of the two floral morphs (Faivre & McDade 2001). The reciprocity index compares the relative position of the sexual organs (anthers and stigmas) of each flower with the reciprocal organ in the flowers of the alternative morph. The index varies between 0 and 1 (the closer to 1, the higher the reciprocity). Calculations can be processed with RECIPROCITY, a simple macro for Excel (available at <http://webs.uvigo.es/plantecology/software.es.html>).

The viability of pollen grains was evaluated in five pre-anthesis buds of four plants per morph, in all four populations. The buds were stored in 70 % alcohol and all anthers per bud were subsequently mounted on a slide in a drop of acetic carmine. Up to 200 grains per slide were analyzed (Koch *et al.* 2010).

Pollen production per floral morph of five flowers per morph per population was calculated. One ground anther per flower was mounted on a slide in a drop of acetic carmine and the number of pollen grains was multiplied by the number of anthers per flower (Silva & Segura 2015).

Stigma receptivity was tested during anthesis in five flowers per morph per population, using hydrogen peroxide (3%) (Kearns & Inouye 1993).

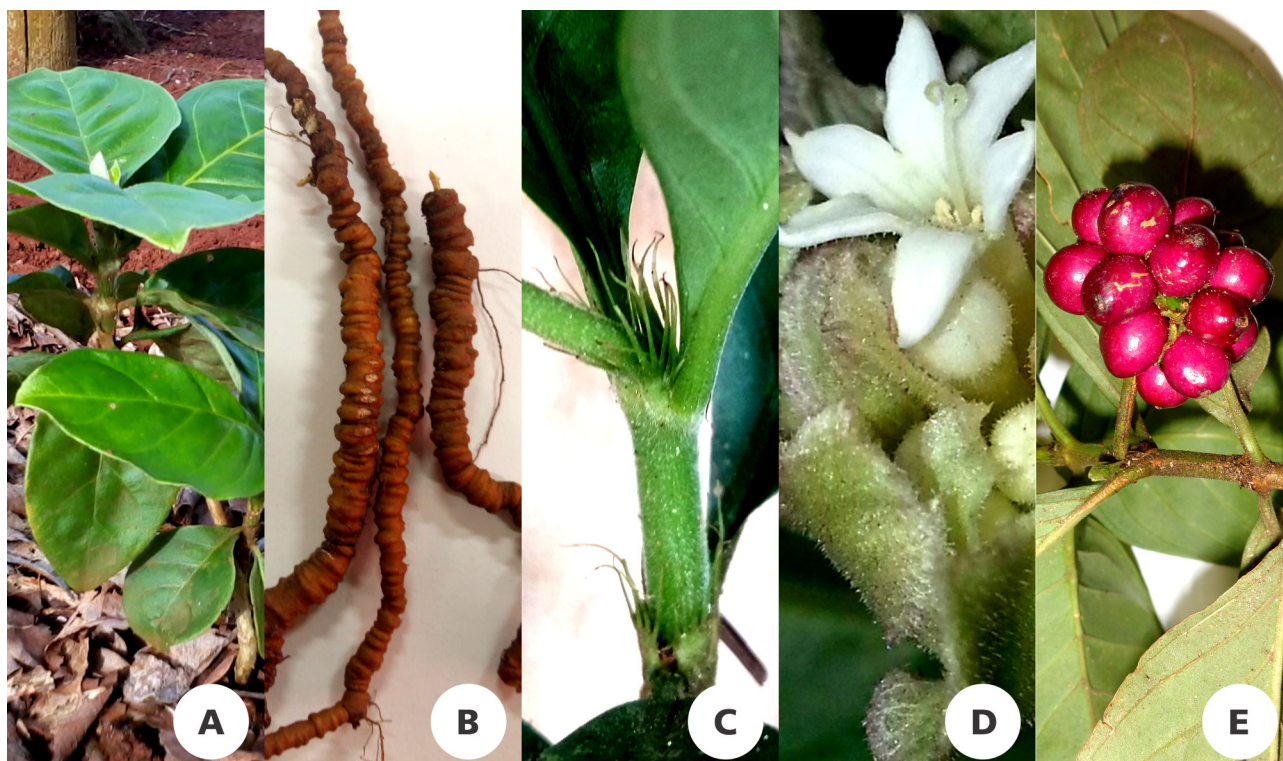
Differences in the frequency of floral morphs and pollen production among populations were evaluated by the Chi-square test. To analyze differences in floral structures, the Shapiro Wilk test was performed to verify the normality of errors and the Kruskal-Wallis (non-parametric) test was used to evaluate floral morphometry. In case of significantly different floral structures, the Scott-Knott test was performed. Program R, version 3.3.1 was used to perform the Shapiro-Wilk, Kruskal-Wallis, and Scott-Knott tests (R Development Core Team 2014).

## Results

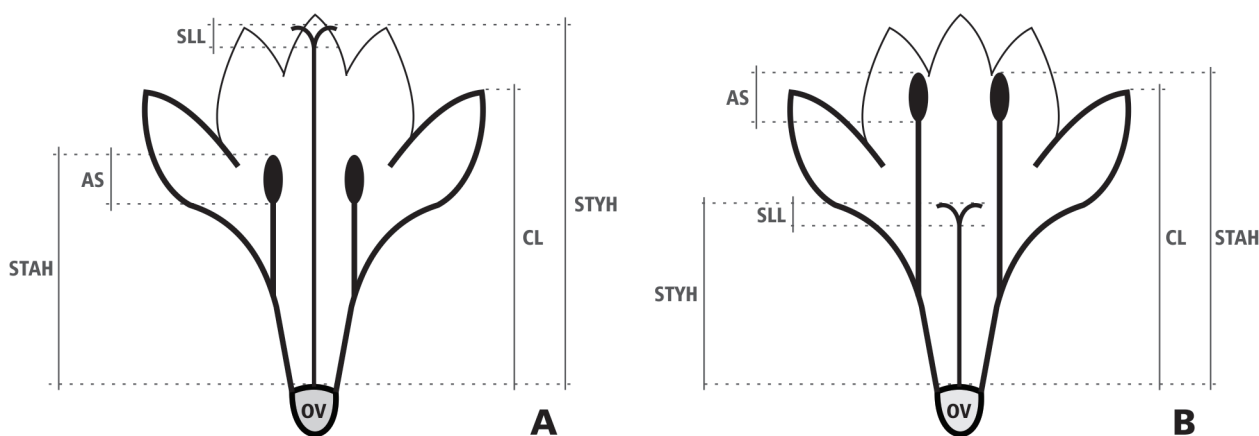
Annual rainfall varied from 1578 to 1750 mm, and the mean temperature was 22 °C. All populations were found in shaded areas within the forest fragments, on acid, clayey soils rich in organic matter.

The number of *P. ipecacuanha* clusters per population ranged from 4 to 10. The cluster diameter varied from 0.80

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**Figure 2.** *Psychotria ipecacuanha* **A.** Habit **B.** Rooted roots of ipecac **C.** Interpetiolar stipules **D.** Inflorescence **E.** Ripe Fruit



**Figure 3.** Morphometry (mm) of long styled (**A**) and short styled (**B**) flowers of *Psychotria ipecacuanha*. Corolla Length (CL), Style Height (STYH), Stigmatic Lobe Length (SLL), Stamen Height (STAH) and Anther Size (AS)

$\pm 0.41 \text{ m}^2$  (Nova Olímpia) to  $3.88 \pm 5.33 \text{ m}^2$  (Tangará da Serra) (Tab. 2).

The population from Barra do Bugres had the lowest risk of genetic erosion (43.33%) (Tab. 3). This population dwells in a preserved forest fragment, which is part of a private property.

The highest risk of genetic erosion was recorded in the population of Denise (Tab. 3). The proximity of the species to the fragment border and the population center, the advance of the agricultural frontier, the occurrence of forest fires, the species' susceptibility to cattle trampling, and fragment size are factors affecting the survival of the *P. ipecacuanha* population in Denise (Tab. 3).

The main risks of genetic erosion in all four populations studied involved habitat change in the forest fragment and surrounding areas in the past 20 years, proximity to agricultural areas, and the frequency of drought affecting the forest fragment and the area occupied by species within a forest fragment (data not shown). Together, these factors contributed 43% of the genetic erosion threat in the study populations.

Both floral morphs of *P. ipecacuanha* were found in the studied forest fragments, in a balanced ratio (Tab. 2). A rather low number of reproductive plants was recorded; 90 plants were found in the forest fragment in Denise, 30

**Table 1.** Risk factors of genetic erosion of *Psychotria ipeacacuanha* populations assessed in in four forest fragments in the state of Mato Grosso, Brazil. SI = Source of information (O = direct observation in the study area during collection expeditions, I = Interview with local inhabitants, C = Consultation of available databases).

Code	SI	Factors contributing to genetic erosion	Score
F1	O	1. Exploitation level of the wild habitat within the forest fragment	
		Industrial exploitation	10.00
		Exploitation by the surrounding populations (e.g., wood collection by neighboring communities)	6.66
		Hunting and collection by small local communities	3.33
		Fully protected	0.00
F2	O/I	2. Changes in the habitat type in the forest fragment and surrounding areas over the last 20 years	
		Increasing level of disturbance	10.00
		No changes	5.00
		Decreasing level of disturbance	0.00
F3	C	3. Availability of agricultural land in surrounding areas	
		> 70 ha/km <sup>2</sup> cultivated area	10.00
		30-70 ha/km <sup>2</sup> cultivated area	5.00
		< 30 ha/km <sup>2</sup> cultivated area	0.00
F4	O/C	4. Pressure of surrounding farmland on the forest fragment	
		Large-scale cultivation in the surroundings	10.00
		Fields of family agriculture in the surroundings	7.50
		Soil suited for cultivation, agricultural fields at a distance of 3 km from the forest fragment borders.	5.00
		Soil suited for cultivation, agricultural fields at a distance of 3-10 km from the forest fragment borders.	2.50
		Soil inadequate for cultivation	0.00
F5	O/I	5. Distance of the forest fragment from the largest population center	
		< 20 km	10.00
		20-50 km	5.00
		> 50 km	0.00
F6	C	6. Distance of forest fragment from the main road	
		< 5 km	10.00
		5-10 km	5.00
		> 10 km	0.00
F7	O/C	7. Distance of forest fragment from new development projects (irrigation system, touristic complex, mining site, hydroelectric power system, and areas destined for agrarian reform)	
		< 5 km	10.00
		5-30 km	5.00
		> 30 km	0.00
F8	O/I	8. Frequency of drought affecting the forest fragment	
		Known to have occurred in two or more subsequent years	10.00
		Mean occurrence once or more in 10 years, but not in consecutive years	5.00
		Mean occurrence less than once in 10 years	0.00
F9	I	9. Accidental fire in the forest fragment	
		Small forest fragment, known as fire-prone	10.00
		Large forest fragment, known as fire-prone	5.00
		Area not known as fire-prone	0.00
F10	C	10. Estimated area of the forest fragment where the species was found	
		< 5 ha	10.00
		5-100 ha	5.00
		> 100 ha	0.00
F11	O	11. Distribution of the target species	
		Limited and rare distribution within forest fragment	10.00
		Limited but common distribution within the forest fragment	6.66
		Generalized, but rare at the site of occurrence	3.33
		Abundant and widely distributed	0.00
F12	O	12. Extension of the wild habitat of the target species within the forest fragment	
		Very limited (5%)	10.00
		Limited (5-15%)	6.66
		15-50%	3.33
		Wide (0.50%)	0.00



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**Table 1.** Cont.

Code	SI	Factors contributing to genetic erosion	Score
F13	O	13. Distance of the site where the target species was found from the border of the forest fragment	
		< 10 m	10.00
		10-50m	5.00
		> 50 m	0.00
F14	O	14. Susceptibility of the target species to animal grazing	
		High, including signs of herbivoria	10.00
		High, but no sign of herbivory found	6.66
		Low, signs of herbivory found	3.33
		Low, no sign of herbivory found	0.00
F15	O/I	15. Value of the target species in traditional medicine	
		Surrounding population does not know about or believe in the medicinal value nowadays	10.00
		Known only by a very limited number of people (e.g., elderly, midwives. etc.)	5.00
		Widely known by the surrounding population	0.00
F16	O/I	16. Level of exploitation of the target species	
		Industrial exploitation	10.00
		Exploitation by the surrounding population	6.66
		Local exploitation	3.33
		Protected or unexploited	0.00
F17	O	17. Distance from the site where the target species was found and the nearest path in the forest fragment	
		< 2 m	10.00
		2-5 m	5.00
		> 5 m	0.00
F18	O/I	18. Conservation status in situ of the target species	
		Germplasm is not known nor found within any nearby protected area	10.00
		Germplasm is known and occurs within a protected area, but with insufficient or unknown conservation status	5.00
		Germplasm known and found within a protected area, with good conservation status.	0.00
F19	O/I	19. Conservation status ex situ of the target species	
		Germplasm is not known to be represented in any genebank	10.00
		Germplasm represented in a genebank, but the information available is poor, irregular or incomplete	5.00
		Germplasm represented in a genebank, with a satisfactory level of information available.	0.00
F20	O	20. Cluster area	
		< 1m <sup>2</sup>	10.00
		1 – 5 m <sup>2</sup>	5.00
		>5 m <sup>2</sup>	0.00
		TOTAL SUM	200=100 %

**Table 2.** Sample size and ratio between floral morphs of four natural populations of *Psychotria ipecacuanha* in the state of Mato Grosso, Brazil. TAG: Tangará da Serra; BAB: Barra do Bugres; NOL: Nova Olímpia; DEN: Denise. (S) Short-styled; (L) Long-styled morphs.

Populations/ Code	Number of clusters	Total number of plants per populations	Number of flowering plants		Ratio between morphs
			L	S	
TAG	04	49	05	07	$\chi^2 = 0.33$ p=0.56
BAB	05	52	16	14	$\chi^2 = 0.13$ p=0.72
NOL	05	22	10	07	$\chi^2 = 0.3$ p=0.47
DEN	10	90	19	11	$\chi^2 = 2.13$ p=0.14

**Table 3.** Risk factors of genetic erosion observed in natural *P. ipecacuanha* in populations of the state of Mato Grosso, Brazil. TAG: Tangará da Serra; BAB: Barra do Bugres; NOL: Nova Olímpia; DEN: Denise.

Code	Risk factors																				Total sum	%
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20		
TAG	6.66	10	10	10	5	10	0	10	0	5	6.66	10	5	10	5	5	10	5	5	5	133.32	66.66
BAB	3.33	10	10	10	0	0	0	10	0	5	0	10	0	3.33	5	5	0	5	5	5	86.66	43.33
NOL	3.33	10	10	10	10	10	0	10	0	5	0	10	10	10	5	0	10	5	5	10	133.33	66.67
DEN	3.33	10	10	10	10	0	0	10	10	10	0	10	10	10	5	6.66	10	5	5	5	139.99	70.00



of which were in the reproductive phase. In the population of Nova Olímpia, occupying a 41.6 % smaller cluster area than the Denise population, 17 of the 22 plants found were in the reproductive phase (Tab. 2).

Significant differences in stamen and stigma height between short- and long-styled morphs *P. ipecacuanha* were recorded in all studied populations, reinforcing the distilia of the species (Tab. 4). Although the degree of reciprocity was not completely accurate between the stigma height and anther of alternative morphs among populations (Fig. 4 A-B), the reciprocity index proposed by Sánchez *et al.* (2013) was higher in populations where the corolla length was not significantly different among the floral morphs (Tab. 4), *e.g.*, in Barra do Bugres and Nova Olímpia, with values of 0.91 and 0.85 respectively.

The reciprocity index of the Tangará da Serra population was 0.81. In this population, the lowest degree of reciprocity was observed in stigma height of the short-styled morph in relation to stamen height of the long-styled morph (Tab. 4, Fig. 4). The reciprocity index of the population in Denise was 0.69, which was 25 % lower than that of the population in Barra do Bugres.

In all evaluated populations, the stigma lobes of the short-styled morphs were longer, and these differences were statistically significant (Denise:  $\chi^2= 11.20$ ;  $p=0.0008$ ; Barra

do Bugres:  $\chi^2=18.85$ ;  $p=0.00001$ ; Tangará da Serra:  $\chi^2= 4.54$ ;  $p=0.03$ ; and Nova Olímpia:  $\chi^2= 7.70$ ;  $p=0.005$ ) (Tab. 4).

Our results suggest there is no direct relationship between large anther size and pollen production. In populations where the anther size was statistically different among floral morphs, *e.g.*, those of Tangará da Serra and Barra do Bugres (Tab. 4), the pollen production was not high (Tab. 5). The pollen viability was high (> 79 %) in all studied populations (Tab. 5). The stigma receptivity was 100% for both floral morphs in all studied *P. ipecacuanha* populations.

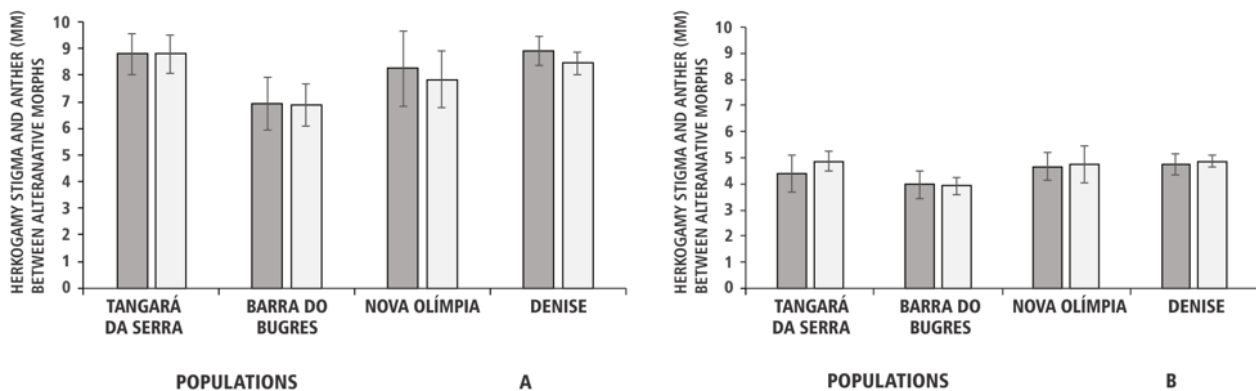
## Discussion

The *P. ipecacuanha* populations studied in the state of Mato Grosso are distributed in transition areas between the Amazon, Cerrado, and Pantanal biomes (Nascimento & Ribeiro 2017). These three biomes host numerous plant and animal species threatened by extinction, and are affected by damage caused by anthropogenic action. As the pressure to open new areas is increasing, in order to increase meat and grain production for export, the natural resources of these regions are progressively depleted, in addition to the predatory exploitation of the region-specific plant germplasm (Skorupa & Assis 1998).

**Table 4.** Flower morphology of short- and long-styled morphs in four natural populations of *Psychotria ipecacuanha* in Mato Grosso, Brazil. CL: Corolla length (mm); StiLL: Stigma lobe length (mm); AS: Anther size (mm); StyL: Style length (mm); StaH: Stamen height (mm). (S) Short-styled; (L) Long-styled morphs. N= 20.

Populations/ Code	CL $\pm$ SD	StiLL $\pm$ SD	AS $\pm$ SD	StiH $\pm$ SD	StaH $\pm$ SD
TAG	S=9.57 $\pm$ 0.54 a	S=1.30 $\pm$ 0.37 a	S=1.97 $\pm$ 0.11 a	S=4.40 $\pm$ 0.70 b	S=8.80 $\pm$ 0.71 a
	L=8.70 $\pm$ 0.47 b	L=1.07 $\pm$ 0.18 b	L=1.47 $\pm$ 0.11 b	L=8.80 $\pm$ 0.77 a	L=4.87 $\pm$ 0.36 b
BAB	S=7.27 $\pm$ 0.52 a	S=1.42 $\pm$ 0.29 a	S=1.70 $\pm$ 0.25 a	S=3.97 $\pm$ 0.54 b	S=6.87 $\pm$ 0.79 a
	L=6.95 $\pm$ 0.62 a	L=1.00 $\pm$ 0.16 b	L=1.47 $\pm$ 0.11 b	L=6.94 $\pm$ 0.98 a	L=3.92 $\pm$ 0.33 b
NOL	S=8.82 $\pm$ 1.01 a	S=1.32 $\pm$ 0.40 a	S=1.65 $\pm$ 0.23 a	S=4.67 $\pm$ 0.54 b	S=7.85 $\pm$ 1.07 a
	L=8.30 $\pm$ 1.48 a	L=1.00 $\pm$ 0.22 b	L=1.55 $\pm$ 0.15 a	L=8.25 $\pm$ 1.40 a	L=4.75 $\pm$ 0.69 b
DEN	S=9.52 $\pm$ 0.47 a	S=1.27 $\pm$ 0.34 a	S=1.85 $\pm$ 0.23 a	S=4.75 $\pm$ 0.41 b	S=8.45 $\pm$ 0.42 a
	L=8.62 $\pm$ 0.35 b	L=1.00 $\pm$ 0.00 b	L=1.85 $\pm$ 0.23 a	L=8.90 $\pm$ 0.55 a	L=4.87 $\pm$ 0.22 b

Note: Different letters in a same populations indicate statistically significant differences ( $P < 0.05$  by the Scott-Knott test).



**Figure 4.** Hercogamy (mean values) between heights of stigma (dark bar) long styled vs anther (light bar) short styled (A); stigma (dark bar) short styled vs stamen (light bar) long styled (B) in populations of *Psychotria ipecacuanha* from four municipalities of Mato Grosso. The error bars refer to the standard deviation of the mean.

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**Table 5.** Pollen production and viability in flowers (%) of the short-styled (S) and long-styled (L) morphs of *Psychotria ipecacuanha* in the state of Mato Grosso, Brazil.

Populations / Morphs	Tangará da Serra		Barra do Bugres		Nova Olímpia		Denise	
	L $\bar{X} \pm SD$	S $\bar{X} \pm SD$	L $\bar{X} \pm SD$	S $\bar{X} \pm SD$	L $\bar{X} \pm SD$	S $\bar{X} \pm SD$	L $\bar{X} \pm SD$	S $\bar{X} \pm SD$
Pollen production	*3.856±1.432	1.961±367.5	2.532±910.7	2.866±580.9	4.165±709.7	2.821±1.278	1.673±933.1	1.420±463.8
Pollen viability	88.7±7.9	90.5±5.3	88.3±10.3	94.8±2.2	79.50±12.8	83.3±6.8	89.6±3.7	86.2±7.6

Note: \*Morphs differ significantly ( $p < 0.05$ ) by the  $\chi^2$  test.

The clay soil texture of the forest fragments sampled from the state of Mato Grosso has also recorded in other studies, although *P. ipecacuanha* occurs mainly in sandy soils (Veloso 1947). The topography and slope of the land, the degree of acidity of the surface horizons, the quality of the drainage, the physical nature of the soil, and the of the vegetation cover influence the occurrence of the species (Veloso 1947).

Factors such as distance from the fragment to the largest population center and *in situ* and *ex situ* conservation status were major risk factors for the *P. ipecacuanha* populations in the Atlantic Forest (Oliveira & Martins 2002). Habitat loss was observed inside and around the forest fragments studied, which was aggravated by the proximity of large agricultural areas, traces of logging and hunting, openings of clearings, and the presence of animals as cattle (personal observation). Habitat loss was one of the reasons for the low genetic diversity observed in natural populations of *Psychotria hastisepala* in the state of Minas Gerais (Silva & Vieira 2013). In addition, habitat loss diminishes the areas of clusters, and can cause an imbalance in the ratio between morphs (anisoplethy), as well as reducing the number of physiologically mature plants for reproduction (Silva & Segura 2015).

The isoplethy (Tab. 3) and reciprocity of reproductive intermorphic organs (Fig. 4) are important, and were confirmed in the study populations; however, they are not the only factor able to promote reproduction among the morphs of the species. Reproduction also depends on mechanisms associated with heteromorphic incompatibility, a system that prevents self-pollination between plants of the same morph (Barrett 2000), the effective action of pollinators (Silva *et al.* 2010), morphological variation among floral morphs (Silva & Vieira 2015), and maintains the environmental characteristics required for species occurrence or persistence.

Isoplethy in *P. ipecacuanha* has been recorded (Rossi *et al.* 2005), in natural populations of the Atlantic Forest in Minas Gerais and Rio de Janeiro and is consistent with the results of the present study. For the genus *Psychotria*, isoplethy was also recorded in other distylous species such as *P. tenuinervis* (Virillo *et al.* 2007), *P. brachypoda* (Fonseca *et al.* 2008), *P. carthagenensis* (Koch *et al.* 2010; Faria *et al.* 2012); *P. hastisepala* (Silva & Vieira 2015), *P. elata* (Silva & Segura 2015), *P. cephalophora* (Watanabe *et al.* 2015), and *P. deflexa* (Matias *et al.* 2016).

Anther fixation to the corolla tube indicated, independent of the floral morph, a close relationship between corolla

length and anther position. Given that reciprocal herkogamy promotes intermorph cross-pollination, morph differences in reproductive success may occur in populations where reciprocity of anthers and stigmas differs by the direction of pollen flow (Faivre & McDade 2001; Armbruster *et al.* 2009; Valois-Cuesta *et al.* 2012) Based on morphometric, pollen production, and herkogamy characteristics, the populations of Barra do Bugres and Denise presented the best reproductive safety results in the present study.

In addition, we found that herkogamy was higher for the short-styled than the long-styled morphs in all populations, as previously reported for other *Psychotria* species (Sá *et al.* 2016) and *Palicourea demissa* (Valois-Cuesta *et al.* 2011). The absence of herkogamy between reproductive organs of short- and long-styled flowers generates the probability of self-pollination and asymmetric pollen flow (Perez-Barrales *et al.* 2014).

Asymmetric pollen flow could be promoted through the foraging activity of pollinators, which results in divergence between potential and realized functional genders, a fundamental process that may lead to functional dioecy (Valois-Cuesta *et al.* 2012). However, gender specialization requires confirmation, also considering several populations, because pollinators and other floral characteristics of the plants can vary among populations.

Longer stigma lobes in the short-styled morph may receive higher amounts of pollen than long-styled stigma in lobes (Barrett 2000). The different lengths of stigma lobes of the floral morphs of *P. ipecacuanha* confirms observations in other populations of the same species of the Atlantic Forest (Rossi *et al.* 2005), and other species such *P. carthagenensis* (Koch *et al.* 2010) and *P. cephalophora* (Watanabe *et al.* 2015).

Significant differences in the size of the short-styled anther, recorded in two populations of this study, may be related to the size of the pollen grains, which are generally larger in this morph (Virillo *et al.* 2007; Koch *et al.* 2010; Faria *et al.* 2012). Conversely, the long-styled morph, despite a smaller anther size, produced a significantly higher amount of pollen in at least one population. This greater pollen grain production, usually in long-flowered anthers, may compensate for asymmetric pollen flow between the floral forms, providing the stigmas of the less exposed short-styled flowers with a greater pollen load (Ganders 1979).

The >80 % viability of pollen grains, a common characteristic of heterostylous species, has also been recorded in *P. poeppigiana* (Coelho & Barbosa 2004), *P. barbiflora* (Teixeira & Machado 2004), *P. carthagenensis* (Koch *et al.* 2010), *P. conjungens*, *P. sessilis*, *P. hastisepala* (Silva & Vieira





2013), and *P. elata* (Silva & Segura 2015). This may assist in the reproductive success of the species (Valois-Cuesta *et al.* 2012).

Our results indicate a relatively low risk of genetic erosion in three of the four populations analyzed. Anthropogenic factors such as a reduced cluster size, low number of reproductive plants, habitat loss, and advance of the agricultural frontier jeopardize the maintenance of natural *P. ipecacuanha* populations in all analyzed populations.

The intensity of anthropogenic disturbances mainly influenced the population size of *P. ipecacuanha*. Three of the four populations evaluated presented a cluster area of less than 5 m<sup>2</sup>. Demographic stochasticity is an important factor leading to the local extinction of very small populations (Byers & Meagher 1992), and Ipeca clusters are frequently isomorphic; that is, a given cluster exhibits either short- or long-styled flowers (Rossi *et al.* 2005).

Our studies corroborate the results presented by Oliveira *et al.* (2010), in 10 Ipeca populations occurring in the biomes of Atlantic Forest and Amazon. According to those authors, the Ipeca populations consisted of a handful of clusters and few populations contained more than six clusters.

The anthropogenic factors associated with morphological and reproductive characteristics, *e.g.*, the low reciprocity between anther and stigma, and low pollen production, support risks for the maintenance and reproduction of Ipeca in the population of Denise-MT. Further studies addressing the frequency of pollinators and dispersers in the study areas are required.

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