

Tibouchina pulchra (Melastomataceae): reproductive biology of a tree species at two sites of an elevational gradient in the Atlantic rainforest in Brazil

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Received: 7 November 2011 / Accepted: 31 March 2012 / Published online: 21 April 2012
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Abstract Low-temperature environments interfere with plant reproduction by reducing the frequency of pollinators, and this may favour reproductive strategies such as self-pollination and apomixis. *Tibouchina pulchra* is a common tree species that occurs at high and low sites of the Brazilian Atlantic rainforest. This study focussed on the pollination biology and breeding system of this species, describing the pollinators and the reproductive success at the two sites of an elevational gradient. Observations were made to determine extent of flowering and fruiting, to identify the richness and abundance of pollinators, and to record data on the floral and reproductive biology at these two sites. Despite more dense flowering at the high site, five visits of bees (two species) were recorded during the observation time (60 h), whereas at the low site there were 948 visits (seven species) during the same period. In contrast with the low site, the flowers of the high site released and received few pollen grains on the stigma. At the high site less fruit was set with fewer seeds as a result of open pollination than at the low site; at that site, however, more seeds were obtained from cross-pollination than at the low site. *Tibouchina pulchra* is self-compatible; however it is not apomictic and needs pollinators for seed set at both sites. Life-history traits other than the breeding system, for example more dense flowering, advantage of greater

fertility in cross-pollination, and multiple reproductive events during the lifetime of the tree may reduce inbreeding depression, increase the hybrid vigour, and balance the lack of pollinators at the high site.

Keywords *Bombus morio* · Buzz pollination · High and low sites · Reproductive assurance · Southeastern Brazil

Introduction

Plant–pollinator interactions are hugely variable, because of diverse degrees of specialization and spatially and temporally unstable conditions, which make predictability difficult (Johnson and Steiner 2000; Waser et al. 1996). This variability gives rise to spatial mosaics of interactions and, consequently, plants pollinated by animals are subject to high reproductive uncertainty (Harder and Thomson 1989; Wilson and Thomson 1991). This interaction system thus provides ecology and evolutionary biologists with powerful tools for testing hypotheses (Gugerli 1998; Malo and Baonza 2002; Totland 2001).

Environments with low temperatures, and, therefore, reduced and more unpredictable growing seasons, for example those occurring at high elevations, interfere with plant reproduction (Bell and Bliss 1980). For instance, the reproductive phenology of plants pollinated by insects is constrained by the low activity of their pollinators, because of low temperatures at high elevations (Freitas and Sazima 2006). Moreover in high Andean and Alpine sites, studies suggest that such environmental conditions coupled with intense winds reduce the number of pollinators and, consequently, transfer of pollen grains to the stigma of conspecific flowers (pollen limitation), reducing the chances of cross-pollination (Arroyo et al. 1985; Totland 1993). Thus,

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it is believed these are important forces which drive the evolution of apomixis and self-pollination in species at high altitudes (Molau 1993; Sandvik et al. 1999). However, such pollination studies on elevational gradients have rarely been conducted, because of spatial, taxonomic, and sampling difficulties (Devoto et al. 2005). Empirical investigation of the effect of high elevation on the pollination and breeding systems of plant species are very scarce (Gugerli 1998), especially those targeting tree species in humid forests.

The transition from cross-pollination to apomixis and/or spontaneous self-pollination often occurs at sites where cross-pollination is chronically low or temporarily disabled, caused either by the absence of conspecific individuals or by the lack of pollinators (Charlesworth and Charlesworth 1987). Therefore, apomixis and/or spontaneous self-pollination can be seen as a form of reproductive assurance under conditions in which visits by insects are rare or even nonexistent, for example at high altitudes (Lloyd and Schoen 1992; Sandvik et al. 1999). Moreover, this pollinator-independent reproduction is a “selective automatic advantage”, because the seeds from these breeding systems will have two copies of the maternal genes, whereas in cross-pollination systems there would be one only (Jain 1976).

Approximately 20,000 species of flowering plants offer only pollen as floral reward and are adapted to pollination by bees that depend on pollen to feed their larvae (Buchmann 1983; Michener 2007). Some of these species are grouped in the family Melastomataceae that comprises 4,200–4,500 species and is well represented in tropical and subtropical ecosystems of the Americas, where there are approximately 3,000 species (Renner 1993). Bees collect pollen from the tubular and poricidal anthers of species of this family, by vibratory movements of their wing muscles, and consequently the pollen is expelled, adhering to the ventral part of the visitor, a place that often makes contact with the stigma; these are the characteristics of buzz pollination (Buchmann 1983). Some Melastomataceae species, for example those of the genus *Tibouchina*, have heterantherous flowers with high herkogamy, promoting cross-pollination and reducing the possibility of spontaneous self-pollination (Renner 1989). For these species, the floral morphology of which suggests dependence on pollinators for pollen transfer, it is supposed there are differences between reproductive phenology, amount of pollen reaching the stigmas, and breeding systems between sites of low and high elevation, because of variation of pollinator abundance and activity.

In this study the pollination biology and the breeding system of *Tibouchina pulchra* Cogn. (Melastomataceae) was evaluated at two sites of an elevational gradient in the Atlantic rainforest in southeastern Brazil, to compare reproductive phenology, visitors/pollinators, and breeding system. This was done to address four major questions:

1. Are there differences between reproductive phenology?
2. Is pollinator availability different between the two sites and if so how does it affect fruit set?
3. Does a smaller amount of pollen reach the stigma, thus reducing fruit set at higher elevation?
4. Does pollinator-independent fruit set occur at higher elevation to ensure reproduction?

Materials and methods

Study species and sites

Tibouchina pulchra Cogn. is a common canopy tree that grows in disturbed sites and secondary forests in the Atlantic rainforest of Brazil (Leitão-Filho et al. 1993). The flowers are large, white on the first day, changing their color to pink/purple during anthesis (Fig. 1a, b; details of flower colour changes are given by Pereira et al. 2011), and produce a pleasant and weak perfume. Further, they are hermaphrodite, heterantherous, and herkogamous. Flowers of this species interact with large bees able to buzz the poricidal anthers and transfer the pollen to the stigmas of conspecific flowers. By successful pollination and fertilization each fruit may produce more than 2,000 seeds (VLG Brito, Unicamp, Brazil; unpublished results). The seeds are dispersed by gravity and often colonize recently fragmented sites (Zaia and Takaki 1998).

The study was conducted at two extreme sites of an elevational range: Núcleo Santa Virgínia (NSV) and Núcleo Pinguaba (NP), both located at Serra do Mar State Park on the northern coast of São Paulo state. The NSV (23°19'S, 45°05'W) is located in the municipality of São Luis do Paraitinga-SP, at elevations ranging from 870 to 1,100 m a.s.l. The regional climate is temperate tropical without a dry season (Tabarelli and Mantovani 1999). The NP (23°20'S, 44°50'W) is located in the municipality of Ubatuba-SP, on the coastal plain (0–10 m a.s.l.), stretching from the seashore to the foot of the mountains that form the Serra do Mar mountain range. At this site, the climate is tropical rainy, with a super-humid season from October to April (Morellato et al. 2000). The mean monthly temperature was 16.1 and 21.3 °C, monthly precipitation was 172.5 and 146.5 mm and the mean monthly wind speed was 4.7 and 4.1 ms⁻¹ during the study period (2008–2009) at the NSV and NP sites, respectively (CPTEC 2010).

Phenology

The study sites were visited monthly from February 2008 to March 2009 to define the phenological pattern of flowering and fruiting. For this purpose 45 individuals were



Fig. 1 Flowers of *Tibouchina pulchra* showing: **a** first day flower, white coloured, with *Bombus morio* landed on short anthers and on filaments of the long anthers, and contacting the stigma with the end

of its abdomen; **b** first day flower changing its colour (end of the day), note that borders of the petals have turned purple. Scale bars 2 cm

tagged and monitored at each site. The phenophase intensity could be defined by the phenology index that consists of an ordinal scale with values from zero to four, which generates subjective categories (posts or ranks) of phenological intensity. On this scale, the values zero, one, two, three and four, respectively, correspond to the absence of phenological activity, and 1–25, 26–50, 51–75, and 76–100 % of phenological intensity. The phenological index of each population at each site was calculated by averaging the sum of the values of each individual during a given month (Bencke and Morellato 2002). In the event of repetition of the phenological observation in the same months from different years we used the average of the two values. The activity peak was defined as the period with the highest phenological index assigned to each phenophase. Vouchers were deposited at the herbarium of Universidade Estadual de Campinas (UEC). The identity of the voucher plants was confirmed by A. B. Martins from the Universidade Estadual de Campinas.

Flower visitors and pollinators

The observations were made during the flowering peak, covering the period of highest daily visitor activity: from 0500 to 1500 hours. Observations were made on 6–12 flowers each day, over six non-consecutive sunny days spread during the same flowering season, totalling 60 h at each site. Floral visitors which contacted the stigma and the anthers were regarded as pollinators. The visit frequencies of all bees were recorded during the given period. The importance of each visitor as pollinator was determined by the relative frequency values of visits and contact with the stigma. Bees were captured with entomological nets, labelled and identified with the help of specialists or by comparison with reference collections of the Museu de

Zoologia, Universidade Estadual de Campinas (ZUEC), where the specimens were deposited.

Pollen dynamics

The availability of pollen was assessed during the first day of anthesis in unbagged flowers of both sites. Flowers were collected every 2 h from 0500 to 1500 hours and preserved in 70 % FAA. The pollen was carefully removed from the anthers and spread in a haemocytometer to estimate the total number of available grains from a large and a small anther of each flower ($n = 15$ flowers on 15 individuals, each time at both sites). The stigmas were removed, cleaned with sodium hypochlorite solution, and stained with aniline blue for observation of pollen grains and pollen tubes with a fluorescence microscope (adapted from Martin 1959). The pollen load on the stigma surface of each flower ($n = 15$ flowers on 15 individuals, each time at both sites) was estimated according to the pollen load index that considers categories from 0 to 4 with 0, 1–10, 11–25, 26–50, and 51–100 %, respectively, of the stigma surface being covered with pollen grains (Valdivia and González-Gómez 2006). Each time an average value of the index was estimated for both sites.

Breeding system, seeds and germinated seeds

The breeding system was assessed by means of controlled pollination experiments at both sites, in accordance with those described by Radford et al. (1974): apomixis, autonomous pollination, hand self-pollination, hand cross-pollination, and open pollination. For each experiment, 30 flowers were used (from ca 10 individuals), except for open pollination, for which 100 flowers were used (from ca. 20 individuals) at each site. Approximately one month after

pollination, the percentage of fruits from each treatment was evaluated. The percentage of seeds in fruits from hand self-pollination, hand cross-pollination, and open pollination at both sites was estimated by counting seeds in 15 fruits of each treatment, by use of graph paper. Thirty seeds from each fruit produced by these flowers were spread on wet paper. After 17–21 days seeds that germinated were counted and the percentage of germinated seeds was estimated.

Statistical analysis

The phenological index of the flowering and fruiting peak was compared between sites by use of the two-sample t test. Amounts of pollen in flowers, percentage of seeds, and percentage of germinated seeds were compared by use of two-way ANOVA (time and place for the first test and place and treatment for the others) ranking the data as proposed by Zar (1996). The Shapiro–Wilk test revealed that all these data deviated significantly from normal distribution (pollen amount in flowers: $SW = 0.904$, $p < 0.001$; percentage of seeds: $SW = 0.935$, $p < 0.001$; percentage of germinated seeds $SW = 0.882$, $p < 0.001$). The Bonferroni post-hoc test with ranked data was used for multiple comparisons when necessary. Numbers of fruits produced in open pollination at the two sites were compared by use of the chi-squared test. For all the tests, we assumed a confidence interval of 95 %. Systat11 software (Systat Software, Point Richmond, CA, USA) was used for statistical analysis.

Results

Phenology

Reproductive phenological asynchrony of *T. pulchra* occurred between the two sites (Fig. 2). The flowering phenology at NSV (high site) occurs later (January–June) than at NP (low site) (December–April), with flowering peaks in March and February, respectively. However, the flowering peak density is higher at NSV (phenological index = 2.27 ± 0.17) than at NP (phenological index = 1.71 ± 0.13) ($t = -2.826$, $p < 0.05$). Moreover, flowering at NSV lasted five months, whereas at NP it lasted four months and from the fifth month there were few flowers only. The fruiting peak was lower at NSV, with a phenology index of 0.46 ± 0.07 ; at NP this index was 3.04 ± 0.17 ($t = 15.215$, $p < 0.001$). The fruiting time was also lower at NSV, where fruits developed during the seven months from February to September; at NP fruits developed during the 11 months from January to November.

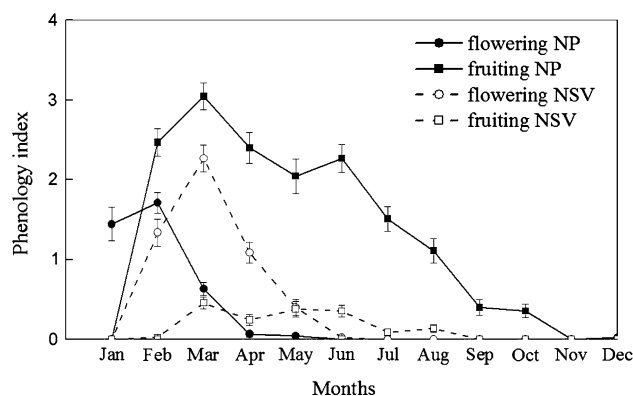


Fig. 2 Reproductive phenology of *Tibouchina pulchra* at high (NSV) and low (NP) sites. Each point indicates the phenology index (mean \pm SE) for each month during a year

Flower visitors and pollinators

The frequency of visits by bees was very low at NSV, with only five visits during the whole observation period, whereas at NP 948 visits were recorded during the same period (average 19.2 ± 6.9 visits/flower/day). The visit peaks occurred between 1000 and 1100 hours at both sites. The number of species of floral visitors was also lower at NSV where two species of bees were recorded, *Bombus morio* and a small unidentified species; only *Bombus morio* acted as pollinator. At NP seven species of bees were recorded, among which *Bombus morio*, *Xylocopa brasili-anorum*, *Xylocopa frontalis*, *Epicharis flava*, and *Eufriesea* sp. acted as pollinators. *Bombus morio* is the main visitor of *T. pulchra* (Fig. 1b), accounting for 89 % of visits, followed by *Epicharis flava* (6.2 %), *Xylocopa brasili-anorum* (3%), *Xylocopa frontalis* (1.5 %), and *Eufriesea* sp. (0.2 %). In general, the pollinators visited first-day white flowers; second-day pink flowers were rarely visited.

Pollen dynamics

Throughout the first day of anthesis of the flowers the amount of pollen grains in the anthers differed between sampling times ($F_{(5,168)} = 17.296$, $p < 0.001$) and between sites ($F_{(1,168)} = 78.981$, $p < 0.001$), and there was an interaction between these two factors ($F_{(5,168)} = 12.226$, $p < 0.001$), indicating that the pattern of variation is different between the two sites over time (Fig. 3a). At the beginning of the day, the amount of pollen in the flowers at NSV is less than at NP ($t = 2.659$; $p < 0.05$). But this pattern does not continue—the amount of pollen in the flowers remains almost unchanged throughout the day at NSV whereas at NP the amount of pollen decreased substantially until the end of the observation period (Fig. 3a). Moreover, the deposition dynamics of pollen on stigmas indicates that at NSV the flowers receive almost no pollen

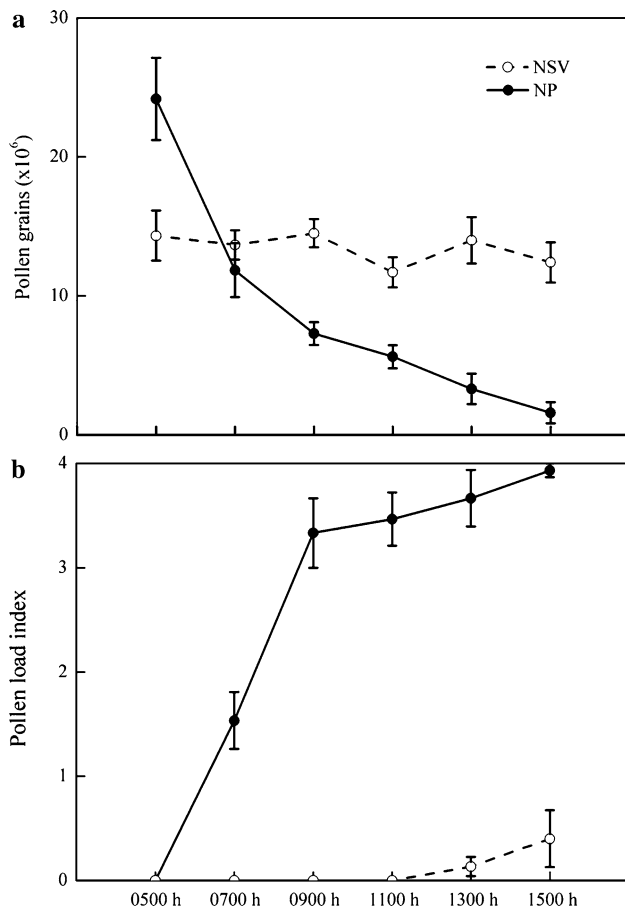


Fig. 3 Pollen dynamics of *Tibouchina pulchra* at high (NSV) and low (NP) sites. Each point indicates **a** the mean (\pm SE) number of pollen grains per flower and **b** the mean (\pm SE) pollen load index on the stigma during the first day of anthesis

grains throughout the day whereas at NP the pollen load increases, almost achieving the saturation of the stigma surface at the end of the bee’s activity (Fig. 3b).

Breeding system, seeds, and germinated seeds

The individuals at NSV produced no fruits by apomixis, whereas autonomous pollinated flowers developed 16 % of fruits, although these fruits were seedless. Fruit formation occurred in 93 and 90 % of the hand self-pollination and hand cross-pollination treatments, respectively (Table 1). On the other hand, individuals at NP developed one fruit by apomixis, which was aborted after a few weeks, but no fruits developed after autonomous pollination treatments. The fruiting of hand self-pollination and hand cross-pollination treatments was also high: 84 and 86 %, respectively (Table 1). However, at NSV only 10 % of fruit formation occurred under natural conditions whereas at NP 79 % of fruits were formed ($\chi^2 = 95.864$; g.l. = 1; $p < 0,001$) (Table 1).

Table 1 Fruit set from emasculated, autonomous self-pollinated, manual self-pollinated and cross-pollinated, and open-pollinated *Tibouchina pulchra* flowers at the high (NSV) and low (NP) sites

Treatment	Fruit set (% NSV)	Fruit set (% NP)
Emasculation (apomixis)	0 (0/28)	3,3 (1/30)
Autonomous self-pollination	16 (5/31)	0 (0/32)
Hand self-pollination	93 (28/30)	84 (26/31)
Hand cross-pollination	90 (26/29)	86 (25/29)
Open pollination	10 (10/102) ^b	79 (75/95) ^a

Different letters indicate significant differences by chi-squared test. Figures in parentheses are number of fruits/number of flowers

The percentage of seeds per fruit was not statistically different at the sites ($F_{(1,84)} = 0.021$; $p = 0.885$), but was different between treatments ($F_{(2,84)} = 4.191$; $p < 0.05$) and there was interaction between sites and treatment factors ($F_{(2,84)} = 6.967$; $p < 0.01$) (Fig. 4a). The percentage of seeds per fruit in hand self-pollinated treatment was equal at both sites (NSV 0.29 ± 0.11 , NP 0.28 ± 0.13 , $t = 0.252$, $p > 0.05$); however, the percentage of seeds was significantly different between the sites for hand cross-pollinated (NSV 0.52 ± 0.22 , NP 0.32 ± 0.16 , $t = 2.635$, $p < 0.05$) and open-pollinated flowers (NSV 0.31 ± 0.18 , NP 0.53 ± 0.27 , $t = 2.635$, $p < 0.05$) (Fig. 4a).

The percentage of germinated seeds was not statistically different between sites ($F_{(1,84)} = 0.219$, $p > 0.05$) and there was no interaction between sites and treatments ($F_{(2,84)} = 2.051$, $p > 0.05$) (Fig. 4b). However the percentage of germinated seeds was different between treatments ($F_{(2,84)} = 13.944$, $p < 0.01$), being higher for hand cross-pollinated treatments than for hand self-pollinated ($t = 5.190$, $p < 0.05$) and in open-pollinated flowers ($t = 4.810$, $p < 0.05$). The percentage of seeds germinated in hand self-pollinated and open-pollinated flowers was not different ($t = 0.380$, $p > 0.05$) (Fig. 4b).

Discussion

Differences in the density and extent of flowering, such as occurred in *T. pulchra* between the NSV and the NP sites, affect the number and activity of pollinators (Buzato et al. 2000), i.e., the greater the number of flowers and the longer the flowering period, the greater is the availability of resources for pollinators as the greater the chances that pollination occurs. However, despite the higher density and extent of flowering in the NSV, plants set few fruits and had a short fruiting period. Fruit formation under natural conditions coupled with the results of pollen dynamics indicates that the flowers of NSV suffer major limitations in relation to the amount of pollen that reaches stigmas

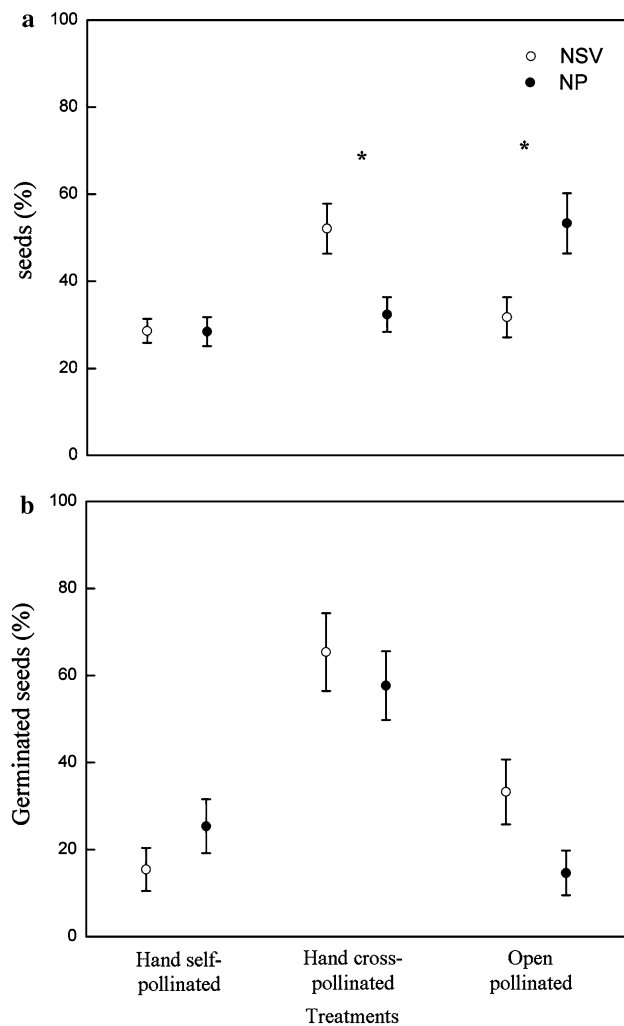


Fig. 4 Percentage (mean \pm SE) of **a** seeds and **b** germinated seeds of *Tibouchina pulchra* after different pollination treatments at high (NSV) and low (NP) sites. Asterisks show significant differences by Bonferroni post-hoc ranked test between areas

(pollen limitation) explaining the low fruiting at this site. The hypothesis of increased pollen limitation with increasing altitude was also recorded for Andean and Alpine environments (Arroyo et al. 1985; Totland 1993), but these data were never tested for tree species in tropical forests. According to these authors, the increased pollen limitation could be related to the reduced activity and density of pollinators at higher elevations, primarily pollination by bees. The low visit frequency and the subsequent pollen limitation may be one of the factors that affect the genetic diversity of plant populations along altitudinal gradients (Semagn et al. 2000; Yan et al. 2009). However, this remains unstudied for Brazilian native species, and *T. pulchra*, being abundant, is a good model for this type of study.

This study indicates that pollinator richness and abundance are lower for *T. pulchra* at the high site. Lower bee

diversity at higher elevation than at lower elevation has also been found in other environments, for example the Tasmanian forest (Hingston 1998), Chilean Andes (Arroyo et al. 1982, 1985), and European Alps (Müller 1880; Totland 1993). The small number of pollinators is the main consequence of extreme abiotic conditions, for example low temperatures, high winds, and short growing seasons (Bliss 1971; Gugerli 1998), which occur to a greater extent at the NSV site than the NP site. Pollinator activity at both sites can be related to the results obtained from the experiments on pollen dynamics. At the NSV site the flowers are rarely visited by bees, resulting in low pollen removal from the anthers and low deposition on the stigmas. At the NP site pollinator activity, and removal and deposition of pollen are reversed: there are frequent visits of a variety of pollinator species, thus the amount of pollen removed and the amount deposited on the stigmas are high. Other studies also show that pollination is low in higher environments and this may result in low fruit and seed set (Duan et al. 2007; Law et al. 2010). In this species, pollinators were large bees and they preferred new white flowers to pink old ones, as reported by Pereira et al. (2011).

At the NP site the greater frequency of bee visits to *T. pulchra* flowers coincides with relative low humidity and mild temperatures during most of the day, as mentioned for other *Tibouchina* species namely, *T. cerastifolia*, *T. clinopodifolia*, and *T. gracilis* (Franco et al. 2011). These abiotic factors are favourable for pollen extraction during most of the day, being only hampered in the early mornings when humidity is high and temperature is low. The decrease in the number of visits to *T. pulchra* flowers after the period of high activity coincides with the small amount of pollen in the anthers. In fact, when visiting flowers with poricidal anthers, bumblebees prefer unvisited flowers and avoid those with few or no pollen grains (Larson and Barrett 1999). That *Bombus morio* is the main pollinator of *T. pulchra* at both sites supports the importance of this bee species in the pollination systems at both low and high elevations within the Atlantic forest, as has been reported by several authors (Brito et al. 2010; Freitas and Sazima 2006; Pinheiro and Sazima 2007).

The reproductive biology experiments indicate that *T. pulchra* is self-compatible at both sites; however this species is not apomictic, because the single fruit produced on emasculation treatment aborted. This result is in agreement with those reported after other studies conducted in the Atlantic rainforest on *T. pulchra* or the species *T. selowiana*, *T. cerastifolia*, *T. clinopodifolia*, *T. gracilis*, and *T. semidecandra* (Franco et al. 2011; Goldenberg and Varassin 2001; Pereira et al. 2011). On the other hand, *T. stenocarpa*, besides self-compatibility, performs autonomous pollination (Goldenberg and Shepherd 1998),

although the viability of the seeds produced in these treatments was not tested. The development of fruits of *T. pulchra* after autonomous pollination at NSV may be because of vigorous movements of flowers under windy and rainy conditions, which may lead to pollen release, as recorded for some Melastomataceae species even under conditions of pronounced herkogamy (Renner 1989). In addition, water droplets carrying small amounts of pollen may also be a form of autonomous pollination. When the pollen grains reach the stigma, they release growth hormones and stimulate the development of the ovary, even if the pollen load is not sufficient for seed maturation (Stephenson 1981). This may explain the formation of seedless fruits after autonomous pollination at NSV. Thus, *T. pulchra* is pollinator-dependent for reproduction at both sites despite its self-compatibility.

The smaller amount of seeds produced after open pollination at NSV than at NP is likely to be related to low pollinator frequency. It was demonstrated for *Cambessedia hilariana* (Melastomataceae) that an increase in the number of visits enhanced the number of seeds produced (Fracasso and Sazima 2004). Although percentages of germinated seeds were quite similar at both sites for all treatments, hand cross-pollination is more successful for *T. pulchra*, because more seeds germinated after this treatment than after open pollination and hand self-pollination, which could be attributed to different selectivity of gametes (Mishra and Drolsom 1973). Nevertheless, *T. pulchra* is a pioneer species and produces thousands of flowers like others in the genus (Franco et al. 2011; Silva 2006; Zaia and Takaki 1998). Hence, the amount of viable seeds produced by only one tree is probably high enough to guarantee the reproductive success of this species, even with low percentage germination. Moreover, the longevity of the seeds of *T. pulchra* may be another aspect of reproductive success—its seeds germinate after one year (Zaia and Takaki 1998) and even after two years (VLG Brito, Unicamp, Brazil unpublished results).

At high elevations, where pollinators are scarce, *T. pulchra* does not ensure reproductive success via autonomous pollination or apomixis, as would be expected in these circumstances and is provided by the hypothesis of reproductive assurance (Lloyd and Schoen 1992). On the other hand, other mechanisms may balance pollen limitation at NSV: for example more pronounced flowering than at the NP site. In fact, the production of more flowers and the subsequent high density, and the length of the flowering period, may increase the attractiveness to pollinators, and abundance and pollinator activity (Buzato et al. 2000; Mitchell et al. 2004). Moreover, plants with an oversupplied quantity of flowers and/or ovules, as for *T. pulchra*, may be favoured in environments where visits from heavily laden pollinators that could pollinate many flowers are

occasional (Ashman et al. 2004; Burd 1995). Another hypothesis that should be tested is if the flowers of *T. pulchra* are bigger at NSV than those at NP, and if pollinators tend to visit species with bigger flowers instead of those with smaller ones (Geber 1985), greater flower size should be favoured where pollinators are scarce. Moreover, more seeds in hand cross-pollinated flowers of *T. pulchra* at the high site indicates that this population may favour cross pollination and this could reduce inbreeding depression and increase hybrid vigour (Lloyd 1992).

Life history factors may also reduce the selective pressures on species lacking reproductive assurance (Pannell and Barret 1998). For *T. pulchra* they are:

- the condition of perennial species with multiple reproductive events during lifetime (iteroparity) which ensures that a species continues reproducing after an unsuccessful year; and
- the ability to produce a viable seed bank for many years, assuming that seeds which did not germinate at the beginning of a growing season can survive to germinate in subsequent seasons.

This seems to be true for *T. pulchra* populations at NSV, an annual flowering species that produces many viable seeds capable of dormancy and guarantees its reproductive success even when pollinators are scarce.

Acknowledgments We thank the Instituto Florestal (Parque Estadual da Serra do Mar, Núcleo Santa Virginia and Núcleo Picinguaba) for permits to study pollination biology in protected public land, I. Bressan for technical help in the laboratory, A. L. Lima, D. P. Maia, M. L. Pereira, and A. E. Ferreras for valuable help in the field, and L. Galetto and S. Buzato for helpful comments on previous versions of the manuscript. We also thank an anonymous reviewer for significant contributions. This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (grant numbers 131969/2008-0 to V.L.G.B. and 302452/2008-7 to M.S.) and by the State of São Paulo Research Foundation as part of the Thematic Project Functional Gradient (process number 03/12595-7), within the BIOTA/FAPESP Program—The Biodiversity Virtual Institute (<http://www.biota.org.br>). COTEC/IF 41.065/2005 and IBAMA/CGEN 093/2005 permits.

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