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Establishment, Reproduction and Genetics of Epiphytic Bromeliad Communities during Premontane Forest Succession in Costa Rica

Alfredo Mario Cascante Marín
Establishment, Reproduction and Genetics of Epiphytic Bromeliad Communities during Premontane Forest Succession in Costa Rica

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CHAPTER 1

General introduction

The diversity of the epiphytic life-form

Epiphytes – organisms living on a plant or in the dead outer tissues of a plant without drawing water or food from its living tissues (Barkman 1958) - display an enormous variety of forms and structures that account for most of the extraordinary complexity of many tropical wet forest ecosystems. Vascular plant epiphytes, which include ferns, orchids, bromeliads, and aroids, represent a life-form restricted to the tropical areas of the World, with only a few scarce representatives in temperate zones. The non-vascular epiphytic plants, among them the mosses, liverworts and lichens, exhibit a more widespread distribution around the globe. Figures of the diversity of vascular epiphytes estimate the richness of the group amounting to about 30 000 species, representing 896 genera and 83 plant families (Madison 1977, Gentry & Dodson 1987 a, b). These richness values certainly increase when non-vascular species are considered. Among vascular epiphytes, the monocots greatly contribute to the diversity of the group with orchids being overly dominant (ca. 20 000 spp.). The Neotropics contain a higher diversity of vascular epiphytes than the Paleotropical areas, owing to the great evolutionary radiation of some exclusive groups such as bromeliads (ca. 1140 spp.), cacti (ca. 130 spp.), and ericaceous plants (ca. 300 spp.), as well as in several aroid and gesneriad genera (ca. 1030 and 360 spp., respectively). Several orchid genera that have many species of epiphytes are, moreover, exclusively found in tropical America, such as Pleurothallis (1500 spp. of epiphytes), Epidendrum (720 spp.), Maxillaria (570 spp.), Stelis (540 spp.) and Masdevallia (400 spp.) (Madison 1977, Gentry & Dodson 1987 a, b). In the Neotropics, vascular epiphyte communities (including hemi-epiphytes) attain their greatest diversity at mid-elevation mountain forests with prevalent humid conditions and they account for most of the differences in plant species diversity between floras of sites with contrasting regimes of humidity, constituting up to 35 percent of the overall plant diversity and 63 percent of all the individuals (Gentry & Dodson 1987b and reviewed by Wolf & Flamenco-S. 2003).
How are epiphytes adapted to their specific habitat?

Epiphytes are remarkable in taking advantage of an ecological niche that is not usually associated with the general perception of a plant habitat. There is not a specific set of characteristics (growth form, water/carbon balance regime, source of nutrient ions, pollination mode, and seed type) that uniquely identifies the epiphytic life form in plants (Benzing 1987, 1990). Whatever these mechanisms are, they must fulfill the critical requirements for the epiphytic life style, which are imposed by the condition of living within the tree crowns. Epiphytes lack connection with the ground and grow on tree bark surfaces with limited capacity to retain humidity, thus the ability to absorb and store water and nutrients represents a crucial pre-adaptation. In this respect, bromeliads (members of the pineapple family) represent a good example of novel adaptations to deal with the water-stressing and nutrient-limited conditions of the forest canopy. They have developed specialized trichomes to collect humidity from the air, morphological arrangements to create a reservoir among the rosette’s leaves for collecting rain water, and metabolic mechanisms (Crassulacean acid metabolism, CAM) to avoid water loss without affecting the regular photosynthetic processes (Benzing 2000). These water reservoirs or ‘tanks’ also accumulate dead organic material from other plants and organisms (mainly insects) that decompose and constitute a source of nutrients that are later absorbed through specialized cells at the leaf bases (Benzing 1990).

The non-homogenous spatial distribution of the host-trees within the forest canopy demands an efficient dispersal mechanism for the colonization of empty microsites. This requires the adoption of r-selection features, such as a high shoot-root ratio and the production of many small and easily-dispersed propagules. The latter is exemplified by the tiny-dust-like seeds or spores produced by the thousands in orchids and ferns, often with a sculptured outer layer to aid in air flotation, and the seeds of bromeliads, which are winged or plumed to increase air buoyancy (Dressler 1981, Benzing 1990, 2000). Alongside with effective dispersal, the attachment to the host-tree or phorophyte constitutes an important factor during establishment. The hairy appendage of many bromeliad seeds is also functional in this respect. The formation of adventitious roots with a primary function of attachment to the phorophyte bark is common among epiphytes and promotes clonal growth as plants may form different independently rooted nodules (Madison 1977, Benzing 1990).

Reproductively, epiphytes share several features with weedy terrestrial plants, associated with strategies emphasizing the production of many seeds and the chance of establishment, yet most of them display high specialization in terms of pollination mechanisms, particularly in mature forest species (Gentry & Dodson 1987a). Well-documented are the interactions
between orchids and Euglossine bees (Dodson et al. 1969, Dressler 1981) and the floral adaptation of many Tillandsioid bromeliads for hummingbird (Gardner 1986, Martinelli 1994, Benzing 2000) and bat pollination (Vogel 1969, Benzing 2000).

**Epiphyte communities in secondary forest habitats**

Epiphytes are known to play an important role within old-growth forest ecosystems (Benzing 1990, 2000), but the ecological processes influencing the composition of epiphyte communities during secondary succession have been little explored. The neotropical montane habitat of many epiphyte species is under permanent threat due to conversion of forest into farming lands (Sánchez-Azofeifa et al. 2001, Hamilton 2001) which are subsequently abandoned to natural processes of forest regeneration. From a conservationist point of view, information on the mechanisms of colonization of secondary forests by epiphytes is important for the understanding of forest regeneration.

The study of epiphyte communities in secondary forests is a topic of research in its early stages. Most of the recently published papers have basically compared the epiphyte diversity and community composition between secondary and adjacent primary forests (Dunn 2000, Barthlott et al. 2001, Krömer & Gradstein 2003) and the spatial distribution of epiphytes and host-tree characteristics (van Dunné 2001, Merwin et al. 2003). The documented general reduction in epiphytic species diversity of secondary forests as compared to mature forests is ascribed to differences in the physical characteristics among forest habitats, such as lower diversity and density of phorophyte (host-tree) structures and microclimatic conditions (Barthlott et al. 2001, Krömer & Gradstein 2003).

Along with the changes in species richness and population structure in secondary forests, it is expected that forest regeneration affects the levels and distribution of genetic variation among epiphyte populations in successional habitats. Similarly to terrestrial plants, it is expected that the magnitude of the changes in the population genetics of epiphyte species will depend on the interplay between life-history traits associated with reproduction and dispersal (Loveless & Hamrick 1984, Hamrick & Godt 1996). In colonizing species the repeated colonization episodes, genetic bottlenecks, founder effects, and drift play a more significant role in influencing the genetic variation and population differentiation compared to plants with other life history strategies (Barrett & Shore 1989, Whitlock & McCauley 1990). The genetics of vascular epiphytes in general is a still largely virginal field of research.
Current perspectives about plant community assembly

The development of plant communities has been traditionally described as a scenario in which the presence or absence of the species is determined by competitive interactions and where specialization results in competitive displacement of the less fitted species for the particular habitat or niche conditions (Levin 1970). This notion was recently termed the “niche-assembly perspective” (Hubbell 2001). Accordingly, plant species have been customary classified under categories aimed to reflect their ability to establish in particular successional stages, such as pioneer, early and old-secondary and climax species (Budowski 1965) or in wider categories, such as nomads and stationary species (van Steenis 1958). Species may occupy either side of the spectrum of vegetation succession, as exemplified by shade intolerant or pioneer species exclusively adapted to the high irradiance of early stages of succession and by species adapted to climax condition of the forest (shade-tolerant or climax species). However, a great majority of species are likely to represent ecologically near-equivalent species located in the middle of that spectrum.

An alternative model proposes that plant community assemblages are non-equilibrium situations ruled by chance, history, and random dispersal (“dispersal assembly perspective”, Hubbell 2001). Early observations suggested that community assembly does not conform to a rigid sequence; instead, the composition of species at different stages of succession seemed to vary from place to place at a local scale, indicating that chance and opportunity are involved in the process (van Steenis 1958). This indicates that species establishing in a specific area or successional forest stage are near-equivalent competitors and the degree of their success is influenced by factors related to seed supply and stochastic processes affecting seed dispersal. In the tropical rain forest of Barro Colorado Island (Panama), the great variability and unpredictability of plant species richness (even for pioneer species) in forest gaps was attributed to limited recruitment or the inability of the species to reach all favorable sites (Hubbell et al. 1999). A review of studies of experimental seed-sowing in terrestrial plants, mostly herbaceous species of temperate zones and a few tropical representatives, found that in almost half of the studies examined there was evidence of populations being seed-limited (Turnbull et al. 2000). However, Levine & Murrell (2003) have suggested prudence in interpreting correlative studies of spatial distribution of individuals and experiments of seed addition as direct evidence of seed limitation in plant communities as long as alternative hypotheses were not excluded. Nevertheless, both views of community assembly (niche versus dispersal) are not mutually exclusive, rather they operate in any community (Hubbell 2001), and their relative importance is probably determined by history and species-specific characteristics of the community.

Pittendrigh (1948) described the communities of vascular epiphytes on single host-trees in terms of light-preferences (i.e., from the niche-assembly perspective), with species tolerant to high irradiation located near the top of the tree and the more shade-tolerant species towards the inner parts of the tree crown and on the trunk. For cryptogamic species – lichens, mosses and liverworts - it has been suggested that dispersability and initial establishment play an important role in defining the communities’ identity (Barkman 1958). In his extensive work of the bryophyte communities along an altitudinal gradient in the Colombian Andes, Wolf (1993a) concluded that bryophytes and lichens in a specific habitat are ecologically (near) equivalent species and that the specific composition of the branch-community is determined by the species arriving at early stages of colonization, which would explain the variability among samples of apparently identical habitats. Very recently, the same author (Wolf 2005) suggested that at the landscape level also differences in the composition of vascular epiphyte communities in the area of Chiapas, Mexico, were better understood when differences in seed supply (dispersal assembly perspective, sensu Hubbell 2001) are considered. In one of the few – probably the only published – experimental studies of dispersal limitation in vascular epiphytes, Ackerman et al. (1996) found that the number of successfully established seedlings of the tropical orchid Tolumnia variegata increased in plots where seed availability was artificially enhanced. In apparent agreement with the previous study, juvenile bromeliads have been shown to grow clumped around mother plants (Benzing 1978, Bader et al. 2000). Apart from dispersal-related factors, however, there is a clear need to include life-history characteristics related to seed germination, growth and reproduction of the species into the assembly rules that determine the establishment of epiphytes and the composition of their communities (Wolf 2005).

Aims and content of this thesis

The research project that is the subject of this thesis was conducted in the region of Monteverde, Costa Rica, an area scientifically recognized by the biological importance of the cloud forest that lies on the top parts of the Cordillera (mountain range) of Tilarán on the north-western part of the country (Fig. 1). The region has received a great deal of attention
from the research community during the last decades, which has contributed to the high levels of biological knowledge of the flora and fauna (Nadkarni & Wheelwright 2000), only comparable to few other neotropical sites. Epiphytes are very abundant and diverse in the region, comprising one-third of the vascular plant species (Haber 2000). The areas on the pacific slope have a more fragmented forest cover due to anthropogenic activities related to cattle raising and coffee plantations, constituting at present a heterogeneous mosaic of old-growth and altered forest remnants, intermixed with grazing areas and forest patches in different stages of natural succession. A setting that is suitable for the study of colonization processes in epiphytic plants (Fig. 2).

The main purpose of this thesis was to investigate the population structure and community composition of epiphytic bromeliad communities in relation to their colonization ability, life history strategy and breeding system. In particular, life-history traits related to dispersal, seed germination, growth and reproduction were examined in selected species with seemingly different habitat preferences in secondary and mature forest habitats. For the first time in epiphytes, full attention is paid to the levels and distribution of genetic diversity within and among populations in different successional forest habitats. The results were used to test the hypotheses of community assembly: niche- versus dispersal assembly.

Chapter 2 describes the community composition of populations in secondary and mature forests in the study region. The population structure of the dominant species in each habitat was compared, as well as factors related to the spatial distribution of individuals within their host-trees. In Chapter 3, we carried out a seed-sowing experiment in the field to test whether the occurrence of species is limited by factors that play a role during the early stages of establishment (germination and early seedling growth and survival over a two-year period). Seed dispersal was examined by a series of seed trapping experiments aimed at determining the amount of seeds dispersed into different successional habitats in relation to the species’ local distribution and abundance (Chapters 4 and 5). Additionally, Chapter 5 includes a seedling transplantation experiment which evaluates their survival and growth over a one-year period in different forest habitats and in relation to micro-climatic conditions of temperature and air humidity measured in the canopy of these habitats. We described and compared the reproductive biology of selected species in detail in Chapters 6 and 7, emphasizing reproductive traits that are likely to affect seed production and further seedling recruitment. The levels of outcrossing of two selected species were estimated using microsatellite markers especially designed for the studied species, to genotype mother plants and their offspring (Chapter 6). Full attention to the levels of population genetic variation and its distribution among successional habitats is given in Chapters 8 and 9. Here, for the first time microsatellite markers are used in genetic studies of epiphytic plants.
Figure 1. Location of the region of Monteverde on the Tilarán Mountain Range, Costa Rica. The enlarged area represents part of the pacific slope region.

Figure 2. Aerial picture of study region at the Upper San Luis area (Monteverde, Costa Rica) showing the pattern of forest distribution (1997). Source: Instituto Geográfico Nacional, Ministerio de Obras Públicas y Transporte (MOPT), Costa Rica.
References


General Introduction


CHAPTER 2

Epiphytic Bromeliad Communities in Secondary and Mature Forest in a Tropical Premontane Area


(Accepted in Basic and Applied Ecology)

Summary

We analyzed the differences in species richness, community composition, population structure, and within-tree location of epiphytic bromeliads in contiguous secondary and mature forests in a premontane area in Costa Rica. Diversity in the mature forest was highest, and the communities differed in their composition as well as in the recruitment rates of the dominant species. Guzmania monostachia and Catopsis nutans dominated the secondary forests, whereas Tillandsia fasciculata and T. tricolor were more abundant in the mature forest. The secondary forest species showed high rates of seedling recruitment while the opposite was found for the mature forest species. Species presence and abundance among and within habitats did not correlate with their physiological (i.e. CAM vs. C3 photosynthesis) or morphological attributes. The spatial distribution patterns were similar among habitats; bromeliads tended to aggregate on a few relatively large phorophytes. The species shared a similar vertical stratification within habitats, except for the two dominant species in the early and mid-successional stages, although its ecological implication is not clear. With some exceptions, conspecifics of different ages were located on similar substrate types (i.e. stems, primary-, or secondary-, tertiary branches) within the tree-crowns, which suggests limited within-tree dispersion. Differences in species composition and rates of seedling recruitment among secondary and mature forest may arise from ecophysiological differences among species; however, the combined effect of seed availability and dispersal differences may have a larger influence. Thus, epiphyte community assembly can only be understood when the differences in habitat conditions, the availability of propagules, their dispersal characteristics and requirements for seedling establishment are known.
Introduction

The extraordinary diversity and abundance of vascular epiphytes is a particular characteristic of mature tropical montane forests. The contribution of epiphytes to overall diversity (reviewed in Wolf & Flamenco-S. 2003) and to forest biomass (Nadkarni 1985, Hofstede et al. 1993) has been well documented for some undisturbed forests. Deforestation of tropical forests for agriculture and cattle grazing continues; as soil productivity decreases, lands are abandoned and are subsequently invaded by successional vegetation or secondary forests (FAO 1993). While secondary forest dynamics has received more attention from forest ecologists (Brown & Lugo 1990, Finegan 1996 and references therein), equivalent studies on epiphytes are still rare.

The few studies on epiphytes in secondary forests report a reduction in species richness (Dunn 2000, Barthlott et al. 2001, Krömer & Gradstein 2003). Differences in the physical characteristics of secondary forests, such as lower phorophyte (host-tree) structure diversity and a more homogeneous microclimate, supposedly explains that pattern (Barthlott et al. 2001). The availability and dispersability of propagules has also been suggested as an important determinant of community assembly (Benzing 1981, Yeaton & Gladstone 1982, Wolf 1994, 2005, van Dunné 2001). Comparative studies on epiphyte communities in mature and successional forests provide insights into the process of community assembly during forest recovery. The incorporation of the variables of species richness and composition are important in the identification of trends in floristic diversity, both are useful indicators of vegetation recovery and change independently along the successional stages (Finegan 1996).

Bromeliads (Bromeliaceae) are restricted to the Neotropics, where they contribute considerably to the diversity of epiphytic angiosperm communities, in effect only surpassed by orchids and pteridophytes (Benzing 2000). The few studies of bromeliads in successional habitats have addressed the spatial distribution (Bader et al. 2000, van Dunné 2001, Wolf 2005) and the changes in species richness and composition (Dunn 2000, Barthlott et al. 2001, Wolf 2005). Comparative accounts on the population structure of secondary and mature forest species are still scarce. Particularly, there is a tendency to neglect small plants (juveniles) when surveying epiphytes because of limitations that arise from the observational technique and the difficulty in distinguishing and identifying small juveniles in a complex canopy environment.

We studied the species richness, abundance and community composition of epiphytic bromeliads in two successional forests of different age and one primary forest stand in
a tropical premontane locality in Costa Rica. Additionally, we examined the relationship between population structure, vertical stratification and within-tree location with the forest successional stage. Specifically, we addressed the following questions: (i) Is the diversity of epiphytic bromeliads correlated with the forest successional stage? (ii) Do the population structures of the dominant species differ among habitats? (iii) Is the presence and abundance of epiphytic bromeliads among habitats associated with physiological or morphological traits of the species? and iv) Do the patterns of spatial distribution change with the forest’s successional stage?

Materials and Methods

Study site
This study was carried out at the Ecolodge San Luis and Research Station, a 62-ha private reserve located on the Upper San Luis River Valley on the Pacific slope of the Monteverde area, Province of Puntarenas, Costa Rica (10°17’10” N, 84°47’40” W, 1050 m a.s.l). The area is classified as humid premontane (bmh-P) according to Holdridge’s life-zone system (Haber 2000) and it borders the Monteverde Cloud Forest Preserve and the Children’s Eternal Rain Forest Reserve. Weather records from the station report a mean annual rainfall of 3282 mm (± 489), with about 80% of the rain occurring from May to November; the air temperature ranges between 17–25 °C. The terrain is irregular and steep and the original forest cover has been fragmented into a mosaic of different land-uses as consequence of dairy and coffee activities but some mature forest patches were spared. Recently, several pastures and coffee plantations have been abandoned and subsequently invaded by secondary forest trees which in turn are being colonized by epiphytes. The epiphytic bromeliad flora of this area comprises five genera and some 28 species: *Catopsis* (4), *Guzmania* (6), *Racinaea* (1), *Tillandsia* (11) and *Werauhia* (6) (unpubl. data).

Study plots
The station manages several areas in different successional stages, from active pastures to old growth or mature forest. During 2002, a 300 m² area was studied within each of three habitats that differed in their successional stages, located within 700 m from each other. Each sampling area was further divided into three 100 m² plots randomly distributed to encompass the within-habitat variation. The early succession (ES) represents a c. 3 ha pasture abandoned eight to twelve years ago, the distance between plots ranged from 50 to 230 m. The regenerating forest is patchily distributed, in a matrix of grassy areas that enclose the station’s facilities. This area is surrounded by active pastures, secondary forests of intermediate age, mature and selectively logged forest patches. The mid-succession
Host trees characteristics
The woody vegetation in each successional habitat was described by recording the richness and abundance of tree species with a stem diameter ≥5.0 cm at breast height (1.3 m,dbh) in the study plots. Voucher specimens were deposited at the National Herbarium of Costa Rica (CR). We recorded the number of stems and their diameter (when several stems were present their values were added up), the number of branch bifurcations (forks) thicker than 2.5 cm at the base, and tree height using a graduated pole or a clinometer (Suunto PM-5/360, Finland).

Bromeliad diversity and community composition
We identified and counted all the bromeliads growing on trees ≥5.0 cm dbh within the plots. Their size was measured to the nearest 0.1 cm, as the length of the largest leaves stretched and measured from the base of the rosette. Morphological differences among species and previous knowledge from field experiments were utilized to identify all individuals up to 0.5 cm in size to species level. Ramets from the same genet or plant were recorded separately. Species richness (S), the Shannon (H', log_e) and Simpson (D) indices were used to compare bromeliad diversity among the three sampled habitats (Magurran 1988). Vouchers were deposited at the National Herbarium of Costa Rica.

Population structure
Individuals of the most abundant species in each habitat were classified into one of the size categories described in Table 1. Size class limits are meant to represent the life stages of
the species in the study area and are based on field observations and preliminary data from germination and seedling establishment experiments and growth rate measurements in the same area (see Chapter 3).

Table 1. Size class limits (cm) representing the life-stage categories used in the field to distinguish the previous-year seedlings (PSD), older seedlings (OSD), juveniles (JUV), sub-adults (SAD) and adult plants (ADL) of the most abundant epiphytic bromeliad species in the study site.

<table>
<thead>
<tr>
<th>Category (life stage)</th>
<th>Bromeliad species</th>
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<tr>
<td></td>
<td><em>Catopsis nutans</em></td>
</tr>
<tr>
<td>PSD</td>
<td>≤1.0</td>
</tr>
<tr>
<td>OSD</td>
<td>1.1 – 2.0</td>
</tr>
<tr>
<td>JUV</td>
<td>2.1 – 4.0</td>
</tr>
<tr>
<td>SAD</td>
<td>4.1 – 6.0</td>
</tr>
<tr>
<td>ADL</td>
<td>&gt; 6.0</td>
</tr>
</tbody>
</table>

**Distribution and abundance**

We examined the distribution of bromeliads among trees in each successional habitat by comparing the frequency of occupied and empty trees using the log-likelihood ratio method for contingency tables. Bromeliad abundance and substrate availability per tree (Tree Size) was examined by calculating “Tree Size” as the summation of the standardized values of stem diameter (cm), tree height (m) and number of branch bifurcations (forks) per tree. This correlation was evaluated by means of a Pearson product-moment correlation test.

The vertical stratification of the most abundant species per habitat was determined by measuring the distance from the ground to the bromeliad’s attachment point on the host-tree using a tape ruler. When inaccessible, it was estimated to the nearest decimeter with a graduated plastic pole (3 m) from the highest possible tape ruler measurement. The data
were sorted according to categories in Table 1 and the differences in vertical distribution were separately tested for each habitat with an analysis of covariance. Species and life stage were entered as main factors and tree height as covariate. Data were square-root or log-transformed when necessary to improve normality and remove heteroscedasticity. Computations were carried out using the program STATISTICA for Windows® (StatSoft Inc. 2000).

**Bromeliad location on the host-tree**
We examined the bromeliad position within the tree by recording several variables related to the substrate they occupied. ‘Substrate type’ refers to the location on stems or trunks, or on primary-, secondary- or tertiary branches. Since most of the tertiary branches were located near the periphery of the tree crown, no further subdivision was considered necessary. ‘Position on the substrate’ describes the bromeliad’s point of attachment either (1) superior or on the upper surface, (2) lateral or on the sides or (3) inferior or underneath the branch or stem. ‘Substrate inclination’ refers to the angle formed by the stem or branch with respect to an imaginary horizontal axis in the vicinity of a bromeliad (about 5 cm on both sides) and divided into four categories: (A) horizontal or nearly so, (B) less than 45 degrees, (C) higher than 45 degrees, and (D) vertical or nearly so.

Using contingency table analyses, we separately determined the association between life stage and each variable, after controlling for species and successional stage. The probability distribution to test for general association in the dataset was calculated by following the Mantel-Haenszel test (Stokes et al. 2000), which is based on randomizations of the column levels among the levels of the row variables while keeping the marginal totals fixed. The statistic \(Q_{GMH}\) is approximately distributed as chi-square with \((s-1) (r-1)\) degrees of freedom.

**Results**

**Host tree characteristics in the successional habitats**

The three successional habitats differed in tree diversity and composition (Table 2). Shannon diversity values \(H'\) increased with the age of the successional vegetation while species dominance \(D\) decreased. Tree abundance in the ES was lower than in the MS and MF and the species from the younger vegetation were habitat-specific. In the ES vegetation *Acnistus arborescens* (Solanaceae) represented 78% of the trees. In the MS and MF stages, the dominance of a particular species was less pronounced. *Inga mortoniana* (Fabaceae),
Dendropanax arboreus (Araliaceae) and Beilschmiedia brenesii (Lauraceae) accounted for 52% of all the trees in the MS stage. In the MF, most of the species (74%) were represented by a single tree and the four most common species, Cupania glabra (Sapindaceae), D. arboreus, I. mortoniana, and Lonchocarpus oliganthus (Fabaceae), constituted 38% of all the recorded trees. Structurally, the trees in the ES were lower in stature (< 6 m) and had on average more stems and a higher stem diameter value than trees in the MS (Fig. 1). The MF vegetation was composed of taller trees, with a higher number of branch bifurcations and larger stem diameters than trees in the other two successional stages (Fig. 1).

**Table 2.** Values for the descriptive parameters of diversity and composition of the arborescent vegetation in three forest successional stages at a tropical premontane site, Costa Rica. Data from trees ≥5.0 cm dbh in three 100 m² plots in each habitat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Forest successional stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early succession</td>
</tr>
<tr>
<td>Species richness (S)</td>
<td>6</td>
</tr>
<tr>
<td>Plant families</td>
<td>5</td>
</tr>
<tr>
<td>Shannon index, H’</td>
<td>0.84</td>
</tr>
<tr>
<td>Simpson’s index, D</td>
<td>0.62</td>
</tr>
<tr>
<td>Total tree abundance</td>
<td>36</td>
</tr>
<tr>
<td>(range per plot)</td>
<td>(4—20)</td>
</tr>
</tbody>
</table>

**Bromeliad diversity and composition**

Eleven species (subfamily Tillandsioideae) were identified in the three successional habitats (Table 3). Species richness (S) and Shannon Diversity values (H’) indicate an increase in diversity of the bromeliad community with age of the successional stage. Species dominance values (Simpson Index, D) were similar for the ES and MS but decreased in MF. The identity of the dominant species changed between secondary and mature forest. The frequency of Guzmania monostachia and Catopsis nutans was significantly higher in the ES and MS.
stages, while in the MF the dominant species were *Tillandsia tricolor* and *T. fasciculata* ($G = 2791.5, df = 6, P < 0.001$). The former species together represented 96% and 83% of all the bromeliads in the ES and MS stages, respectively. In the MF, 88% of the plants were *Tillandsia* spp. while the abundance of *G. monostachia* and *C. nutans* was reduced to 5.1%.

**Figure 1.** Descriptive values for the parameters of host-tree physiognomy in three successional habitats in a tropical premontane forest, Costa Rica. Each parameter is represented by a graph with habitat on the x-axis: ES = early succession, MS = mid-succession and MF = mature forest. Symbols represent the median (dark square), 25% - 75% percentiles (box) and minimum and maximum (whiskers). Statistical values from non-parametric tests (Kruskal-Wallis ANOVA) are indicated inside the graphs.

**Bromeliad population structure**

The relative contribution of each life stage (size class) to the local population (i.e. the population structure; Fig. 2) is an indicator of the recruitment rate. Populations with high recruitment rates will have a higher number of individuals in the smallest size-classes (seedlings and juveniles). Conversely, populations with a low recruitment will mainly be composed of individuals in the upper size-classes (sub-adults and adults) (cf. Oostermeijer et al. 1994). The most abundant species in the ES and MS stages, *G. monostachia* and *C. nutans*, showed a population structure characterized by high recruitment (Fig. 2). Contrary, the most common species in MF, *T. fasciculata* and *T. tricolor*, showed different patterns. In the ES stage, the small population of the first species exhibited a high recruitment structure and it presented an intermediate population structure in the MS and MF stages, with similar
proportions of individuals in the smallest and largest size classes. For *T. tricolor*, which did not occur in the ES stage, its population structure was nearly intermediate in the MS and MF stages but with proportionally fewer seedlings than the other *Tillandsia*.

Table 3. Species diversity, composition and abundance (number of ramets) of epiphytic bromeliads in three successional stages of a tropical premontane forest, Costa Rica

<table>
<thead>
<tr>
<th>Bromeliad species</th>
<th>Abundance per habitat (300 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early succession</td>
</tr>
<tr>
<td><em>Catopsis nutans</em> (Sw.) Griseb.</td>
<td>934</td>
</tr>
<tr>
<td><em>C. nitida</em> (Hook.) Griseb.</td>
<td>6</td>
</tr>
<tr>
<td><em>Guzmania monostachia</em> (L.) Rusby ex Mez</td>
<td>1952</td>
</tr>
<tr>
<td><em>G. sanguinea</em> (André) André ex Mez</td>
<td>--</td>
</tr>
<tr>
<td><em>Racinaea spiculosa</em> (Griseb.) M.A. Spencer &amp; L.B. Sm.</td>
<td>2</td>
</tr>
<tr>
<td><em>Tillandsia fasciculata</em> Sw.</td>
<td>59</td>
</tr>
<tr>
<td><em>T. juncea</em> (Ruiz &amp; Pav.) Poir.</td>
<td>--</td>
</tr>
<tr>
<td><em>T. leiboldiana</em> Schltdl.</td>
<td>--</td>
</tr>
<tr>
<td><em>T. multicaulis</em> Steud.</td>
<td>2</td>
</tr>
<tr>
<td><em>T. tricolor</em> Schltdl. &amp; Cham.</td>
<td>--</td>
</tr>
<tr>
<td><em>Werauhia werckleana</em> (Mez) J.R. Grant</td>
<td>--</td>
</tr>
<tr>
<td>Unidentified species</td>
<td>--</td>
</tr>
<tr>
<td>Total abundance</td>
<td>2 955</td>
</tr>
<tr>
<td>Species richness, $S$</td>
<td>6</td>
</tr>
<tr>
<td>Shannon index, $H'$</td>
<td>0.74</td>
</tr>
<tr>
<td>Simpson’s index, $D$</td>
<td>0.54</td>
</tr>
</tbody>
</table>
Bromeliad distribution and abundance

Almost half (45.8%) of the 144 examined trees hosted at least one bromeliad. The proportion of trees supporting bromeliads was higher in the ES (92%) than in the MS and MF (30% and 28%, respectively) \((G = 46.4, \text{df} = 2, P < 0.001, \text{Fig. 3})\). Regardless of the successional stage, most epiphytes aggregated on a few trees (Fig. 3). In the ES, seven out of the 36 examined phorophytes contained almost 60% (1770) of all the recorded ramets, each tree harbouring from 170 to 407 ramets. Similarly, in MS five out of the 46 sampled phorophytes contained 76% (842) of the bromeliads; from 122 to 302 ramets per tree. In MF, four out of the 46 surveyed trees housed 58% (366) of the bromeliads, each tree having from 43 to 123 ramets. No host-tree specificity was detected in the MS or MF stage; however, 70% of the trees hosting bromeliads in ES belonged to *A. arborescens*. The substrate availability was positively correlated with the bromeliad abundance per tree (Fig. 4). High values of substrate availability usually described a relatively tall tree with a thick stem and a wide crown, especially in the MS and MF stages.

**Figure 2.** Population structure of the most abundant bromeliad species in three successional habitats in a tropical premontane forest, Costa Rica. Data are proportions per life stage represented by sections in each bar (bottom to top): previous-year seedlings (hatched bold), older seedlings (hatched light), juveniles (white), sub-adults (dotted) and adults (black). Successional habitats (x-axis) are: ES (early succession), MS (mid-succession) and MF (mature forest). Sample sizes (number of ramets) are shown below the x-axis.
Figure 3. Bromeliad density per host tree in different successional habitats in a tropical premontane forest. Bars represent percentages of trees in each habitat according to bromeliad density categories.

Figure 4. Regression analyses for the standardized parameter of substrate availability (Tree Size) and the number of bromeliads per host tree in three successional habitats in a tropical premontane forest, Costa Rica. In each graph: (A) early succession, (B) mid-succession and (C) mature forest. $R^2$ is the coefficient of determination and its significance after a t-test is indicated by *** $P<0.001$. Dotted lines represent the 95% confidence intervals.
In order to account for differences in tree height, the vertical distribution is graphically expressed as a relative measurement of the bromeliad position with regard to the respective tree height. Tree heights were divided in ten equally distributed bands and the bromeliad distribution was partitioned accordingly (Fig. 5). The vegetation structure greatly determined the bromeliad’s vertical stratification. In the ES stage, the majority of bromeliads (>90%) were at 1.3—3.1 m above the ground with an average host-tree height of 4.4 m. In MS, most bromeliads occurred at 7.3—11.0 m above the ground with an average host-tree height of 12.2 m, and in MF, bromeliads were at 9.7—17.5 m above the ground with an average host-tree height of 19.4 m. *Guzmania monostachia* significantly occupied relatively lower strata of the phorophytes, while *C. nutans* occurred higher in the canopy of the ES and MS stages (Table 4, Fig. 5). *Tillandsia tricolor* and *T. fasciculata* had intermediate positions when present. In the MF stage, *T. fasciculata* occurred at lower positions than *T. tricolor*.

Regarding the life stages, no particular or consistent pattern was detected. In the ES stage, seedlings of *G. monostachia* were on average located significantly higher in the canopy than older plants; seedlings occupied lower positions in the MS and the developmental stages showed no significant differences in distribution in the MF. In contrast, seedlings of *C. nutans* occupied lower positions than juveniles and adults in ES and MF, while no differences were detected in the MS stage. As for the two *Tillandsia* species, only *T. fasciculata* seedlings were significantly lower than the juvenile and adult plants in the canopy of the MF.

Table 4. Results of the analyses of covariance (ANCOVA) on the vertical distribution of epiphytic bromeliads (individuals) in three successional forest stages in a tropical premontane site, Costa Rica. Tree height was used as covariate, with species and life stages as main factors.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Early succession</th>
<th>Mid-succession</th>
<th>Mature forest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F-ratio</td>
<td>P</td>
</tr>
<tr>
<td>Tree height</td>
<td>1</td>
<td>774.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>93.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Life stage</td>
<td>2</td>
<td>17.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species x life stage</td>
<td>2</td>
<td>54.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>2684</td>
<td>1022</td>
<td>435</td>
</tr>
</tbody>
</table>
Figure 5. Vertical distribution of the most abundant bromeliad species in three successional habitats in a tropical premontane forest, Costa Rica. Data represent the proportion of individuals (y-axis) in the different life stages (SEEDLINGS: previous year and older seedlings, JUVENILES and ADULTS: sub-adults and adults) according to host tree height (x-axis). The bromeliad position is expressed as a relative measure of the total tree height divided in ten equally distributed ranges. Successional habitats (rows): early succession (ES), mid-succession (MS), and mature forest (MF).
Location on the host-tree
Bromeliads showed a significant association between life stage and ‘substrate type’; this relationship, however, was affected by the habitat condition and species ($Q_{GMH} = 40.5$, df = 6, $p < 0.001$). *Guzmania monostachia* and *C. nutans* showed the clearest difference in the ES: the first species was mostly found on stems and primary branches (marginally significant) while the second one was more common on tertiary branches (Fig. 6). As the forest became more complex, the pattern of within-tree location changed. Seedlings in the MS were comparatively more frequent on stems and primary branches than older plants, whereas in the MF all individuals of the different species were mainly found on stems and primary branches. Bromeliads’ attachment on the substrate was proportionally higher on the sides or below the branches or stems ($Q_{GMH} = 38.4$, df = 4, $P < 0.001$), irrespective of species or habitat condition. Nevertheless, the seedlings and juveniles of *C. nutans* were anchored more frequently on top of their substrates in the MS and MF stages, respectively (data not shown). Similarly, bromeliads were more common on branches or stems with steep inclinations (> 45 degrees) ($Q_{GMH} = 71.7$, df = 6, $P < 0.001$). However, the seedlings of *G. monostachia* and *T. tricolor* did not show a preference for a substrate inclination in the MS stage (data not shown).

Discussion

The lower diversity of epiphytic bromeliads in the secondary forests, when compared to the nearby mature forest (Tables 3), coincided with patterns described for vascular epiphytes from other Neotropical areas (Barthlott *et al.* 2001, Krömer & Gradstein 2003, Wolf 2005). These studies have suggested that epiphyte diversity is positively associated with the diversity of phorophytes (trees) and microclimatic conditions in each habitat. Other aspects related to the distribution of epiphytic bromeliads at each habitat were not affected or did not show any consistent or clear pattern regarding the forest successional stage. Nevertheless, the specific community composition and recruitment rates of the dominant species were noticeably different between secondary and mature forest.

The uneven bromeliad distribution among host trees, also reported in other studies (i.e. Zotz *et al.* 1999, Bader *et al.* 2000, Benzing 2000 and references therein, Nieder *et al.* 2000, van Dunné 2001), did not change with the successional stage (Fig. 3). In the younger vegetation, the higher frequency of occupied trees may reflect a higher availability of light due to the more homogeneous and scattered vegetation of low height (< 5.5 m). Moreover, the rough and spongy corky bark of the most abundant tree species in the area (*Acnistus arborescens*) is likely to enhance seed attachment and seedling establishment because
it retains more moisture (sensu Castro-Hernández et al. 1999, Callaway et al. 2002).
The distribution of bromeliads along the vertical gradient was clearly a function of the
vegetation structure; however, the vertical distribution of conspecifics of different age was
not determined by the forest successional condition. The tendency of bromeliad seedlings to
establish near their mother plants (Benzing 1978, Zotz 1997, van Dunné 2001) suggests that
seed dispersal mostly occurs within trees, both in secondary and mature forest.

**Figure 6.** Within-tree location (by substrate type) of the most abundant bromeliad species
in three successional habitats in a tropical premontane forest, Costa Rica. Data are
proportions per life stage on each substrate and represented by sections in each bar: SDL
(previous year and older seedlings), JUV (juveniles) and ADL (sub-adults and adults).
Successional habitats (rows): ES (early succession), MS (mid-succession) and MF (mature
forest). Above each graph: P-values after chi-squared tests were considered significant when
p<0.05; ‘nc’ are tests not computed due to zeros in rows or columns.
The lack of consistent differences in the type of substrate utilized by bromeliads among species and forest stages (Fig. 6) does not suggest a specific ability to colonize different parts of the phorophyte as proposed by Rudolph and collaborators (1998). The preference of most bromeliads to attach laterally on the substrate surface, both in secondary or mature forest, is likely the effect of excessive stem flow caused by heavy rains, which washes most seeds from the top of the branches to their sides, where they germinate. The trampling effect of arboreal mammals that travel along tree limbs (Perry 1978b), cannot be excluded, but is insufficient to explain this pattern, particularly in the younger vegetation where such routes are not yet established. Although not quantified, the prevalence of bromeliads on inclined substrates (> 45°) may just reflect the higher availability of such substrates as reported by Rudolph et al. (1998).

We argue that the specific composition of the bromeliad community in secondary and mature forests is probably not the result of structural and microclimatic differences in the arboreal vegetation. The static data presented here do not offer any information on dynamic processes of seed dispersal, establishment, plant growth and reproduction to elucidate the mechanisms of community assembly. Although these issues are the subject of experiments reported in the following chapters of this thesis, this paper provides the material to hypothesize on the relative importance of such processes.

Adaptations to a light gradient and water-stress have been postulated to explain the distribution of epiphytic bromeliads (Pittendrigh 1948). The higher light exposure in the canopy of younger vegetation is expected to favour species with CAM-photosynthesis and morphological adaptations to avoid desiccation, as in the case of *T. fasciculata* (Benzing & Renfrow 1971, Griffiths & Smith 1983), and the similar *T. tricolor*. However, these species had small populations in that vegetation or were completely absent there and this is a common phenomenon in the entire area. Conversely, the small populations of the facultative C3-CAM species *G. monostachia* (Martin 1994) and *C. nutans* (Benzing 2000) had low recruitment rates in the mature forest, which does not match their supposed ability to deal with the more shady conditions of that habitat. This lack of a direct correlation between the species distribution and their photosynthetic pathway in the latter species has also been reported elsewhere (Zotz 1997, Zotz & Vollrath 2002).

dispersal has been proposed to influence the formation of terrestrial plant communities (see Hubbell et al. 1999, Turnbull et al. 2000, Svenning 2001, Szentesi & Jermy 2003, Verheyen et al. 2004).

All epiphytic bromeliads recorded here (subfamily Tillandsioideae) have small seeds that feature a hairy appendage or coma at one or both ends which enhances air buoyancy and dispersal by wind (Benzing 2000). The physical separation (<700 m) between the studied secondary and mature forest does not seem to represent an obstacle for seed movement among habitats. Nevertheless, wind-dispersed seeds of epiphytic bromeliads apparently reportedly don’t disperse very far from the mother plant (García-Franco & Rico-Gray 1988).

Seed-dispersal success is also bound to the reproductive capacity of the species or the number of seeds produced. The probability of establishment increases with the amount of seeds dispersed into a specific area, which in turn depends on the proximity and size of the source population, as well as on factors affecting the movement of seeds (i.e. wind currents). Comparative studies involving the evaluation of growth rates, phenological patterns, seed production, pollination rates and breeding systems are in progress to determine the role of propagule availability in the formation of epiphytic vascular plant communities.

Concluding remarks
Community assembly and distribution of epiphytic bromeliads cannot be solely predicted on the basis of physiological and morphological attributes of the species to exploit certain niches. In this study, we draw attention to the potential effect of seed availability and dispersal in shaping community composition and bromeliad distribution in successional forests. In this view, life-history differences that affect seed production, the location of seed sources, as well as the local wind patterns lead to a non-random dispersal of wind-dispersed epiphytic bromeliads. Therefore, species diversity and composition of the surveyed bromeliad communities is expected to change during forest succession according to site-specific factors that affect seed availability and dispersal, in addition the performance and survival of individual plants, their ability to flower and the time it takes them to do so.

Acknowledgements

We thank the staff of the Ecolodge San Luis & Research Station (UGA) for their logistic support. P.H. van Tienderen and two anonymous reviewers provided valuable comments on an earlier draft. This is a project funded by WOTRO, The Netherlands (grant W85-34), in collaboration with the National Museum of Costa Rica.
References


CHAPTER 3

Germination and Seedling Establishment of Epiphytic Bromeliads in a Tropical Premontane Forest, Costa Rica

With J. Gerard B. Oostermeijer, Jan H. D. Wolf & Joannes C. M. den Nijs

Summary

The assembly of plant communities is the combined result of inter-specific competition (niche perspective) and the availability of propagules (dispersal perspective). For epiphytes, dispersal-related conditions has been proposed as main factor. By controlling seed dispersal constraints, we experimentally examined whether community composition of epiphytic bromeliads in successional forests are determined during the early phases of seedling recruitment and whether those differences are related to eco-physiological traits of the species. A total of 7200 seeds was artificially affixed and distributed on several host trees in two secondary forest patches and in a mature forest stand in a tropical premontane area in Costa Rica. Four bromeliad species with contrasting physiological characteristics (i.e. CAM and C₃-CAM) and habitat preference (secondary vs. primary forest) were selected. The results indicated that species had similar germination rates, which were not affected by the habitat. After two years, seedling survival and development was comparatively higher in the early-successional vegetation, although this was only statistically significant for Werauhia gladioliflora (survival) and Catopsis nutans (growth). Seedling establishment success was not associated with specific physiological or morphological adaptations or habitat preference (secondary vs. primary forest) of the species. Our results did not agree with the documented patterns of community composition and rates of population recruitment described for the studied species in the same successional habitats. These findings support the hypothesis that chance and historic events related to seed availability have a major influence on community assembly of epiphytic bromeliads.
Introduction

The composition and distribution of epiphytic bromeliad communities in the forest canopy has been related to the species’ ability to exploit a gradient of light and humidity (i.e. Pittendrigh 1948). Thus, among the factors determining epiphyte establishment, light and moisture along with host-tree bark characteristics are considered critical (Benzing 2000). During successional processes of forest recovery, the microclimatic conditions associated to light and humidity are expected to vary according to the successional stage of the vegetation (Brown & Lugo 1990). Therefore, it is reasonable to expect that the composition of the community will reflect the species abilities to exploit the specific habitat conditions. Alternative approaches to community assembly of epiphytes attribute a major role to propagule availability and dispersal in determining the species colonization success (Benzing 1981, Yeaton & Gladstone 1982, Wolf 1994, van Dünné 2001, 2002). Current models assign a higher importance to recruitment limitation than to niche differentiation (Dispersal- versus Niche-assembly perspectives) (cf. Hurtt & Pacala 1995, Hubbell 2001).

The failure of a species to recruit in all available favorable sites (recruitment limitation) due to limited dispersal has been documented for several terrestrial plant communities in different ecosystems (Hubbell et al. 1999, reviewed by Turnbull et al. 2000, Svenning 2001, Tofts & Silvertown 2002). In probably the only available study related to limited recruitment in epiphytes, Ackerman et al. (1996) demonstrated that seed supply and dispersal rather than availability of suitable micro-sites influenced the recruitment levels of *Tolumnia variegata*, an epiphytic orchid.

Bromeliads represent an important component of the epiphytic communities of several montane tropical rain forests (Gentry & Dodson 1987) and participate in ecological interactions in the ecosystem by providing food, shelter or brood-sites to other organisms (Benzing 2000). Although secondary forest areas are increasing in tropical regions (FAO 1993), there exists a limited understanding of the processes involved in determining the composition of epiphyte communities during forest recovery. The literature on this topic has documented changes in diversity and composition of epiphytes between secondary and nearby mature forests (Dunn 2000, Barthlott et al. 2001, Krömer & Gradstein 2003, Merwin et al. 2003). Many bromeliad species constitute an important part of indigenous cultures in tropical American countries in the form of fibers, food, forage, medicine and ritual/mythical uses (Bennett 2000). They represent a potential source of exotic ornamental plants for the international market; however, habitat destruction and uncontrolled harvesting is threatening many local populations (Wolf & Konings 2001). Conservation practices orientated to the recovery or sustainable use of epiphytic communities can benefit from increased knowledge of the factors affecting their establishment.
In a previous study, we found marked differences in species richness and composition of epiphytic bromeliad communities between secondary and mature forests (Chapter 2). We hypothesized that dispersal and seed availability were more likely to determine the species distribution and recruitment rates than specific abiotic conditions of the habitat or differences in the species’ ecophysiology. In the present work, we tested this hypothesis by examining establishment success of four epiphytic bromeliads in two secondary forests of different age and a mature forest stand in a tropical premontane area.

We artificially sowed seeds of a series of different species in field conditions, and thus controlled for the constraints imposed by seed availability and dispersal. We recorded germination and monitored seedling survivorship and growth over a two-year period. This experiment specifically tested 1) whether seedling establishment of epiphytic bromeliads is affected by the forest successional stage, 2) whether differences in the analyzed variables among species are related to species-specific habitat preferences or physiological characteristics, and 3) whether seedling establishment success is correlated with the species presence and abundance in the studied secondary and mature forest.

Materials and Methods

Study site

The Upper Watershed of the San Luis River Valley is located on the Pacific slope of the Tilarán mountain range in the zone of Monteverde, Province of Puntarenas, Costa Rica (latitude: 10° 17’ 10” N, longitude: 84° 47’ 40” W), at 1100 m a.s.l. The vegetation is considered as seasonal and is classified as premontane humid forest, bmh-P (Haber 2000). Average annual rainfall is 3282 (± 489) mm and nearly 80% of the vertical rainfall is collected during the rainy season (from May to November). Annual temperature fluctuates between 17—25 °C (Records from the Ecolodge San Luis and Research Station). Trade winds carrying moisture from the continental divide are a constant feature in the study area, especially during the dry season.

Study species

Catopsis nutans (Sw.) Griseb., Guzmania monostachia (L.) Rusby ex Mez, Tillandsia fasciculata Sw., and Werauhia gladioliflora (H. Wendl.) J. R. Grant belong to the Tillandsioideae subfamily. The species fit into Benzing’s (2000) ecophysiological Type Four, they have a moderate to well-developed phytotelm and C$_3$ is the most common photosynthetic pathway. Facultative C$_3$-CAM has been documented for G. monostachia (Martin 1994); however, and is suspected in C. nutans (Benzing 2000), while T. fasciculata...
is an obligate CAM species (Griffiths & Smith 1983). The geographic distribution of these species encompasses a large area of the Neotropics, from South Florida and Mexico to Colombia, Ecuador and Brazil, and some Caribbean islands (Morales 2003). Seeds of all taxa are small, have a hairy appendage or coma, and are clearly adapted for anemochory (Smith & Downs 1977). Dispersal occurs during the dry season (from February through April). Table 1 summarizes information about seedling form, metabolic pathway, habitat preference and reproduction.

**Forest successional stages**

Based on an earlier study of the bromeliad communities (Chapter 2), we selected three forest areas of different successional stage. The youngest of these areas consisted of an early successional vegetation (8-12 y) on an abandoned pasture field (c. 3 ha). The vegetation cover was scattered and composed of an herbaceous understory beneath a low canopy of between 5.5—6.0 m tall, predominantly composed of *Acnistas arborescens* (Solanaceae), *Psidium guajava* (Myrtaceae), and *Sapium glandulosum* (Euphorbiaceae) trees. *Guzmania monostachia* and *C. nutans* were the dominant bromeliad species here, showing high recruitment rates, while the *T. fasciculata* population was mainly composed of juvenile plants. The second area was a mid-successional vegetation patch (35-40 y) on an abandoned shaded coffee plantation (c. 1 ha). The understory contained a.o. *Heliconia* sp. (Heliconiaceae) and shrubs and treelets of *Hamelia patens* and *Randia calycosa* (Rubiaceae). The canopy reached a height of 12-16 m and the predominant trees were *Beilshmiedia brencisi* (Lauraceae), *Cordia eriostigma* (Boraginaceae), *Dendropanax arboreus* (Araliaceae), *Hasseltia floribunda* (Flacourtiaceae), and *Lonchocarpus oliganthus* (Fabaceae). This area was also dominated by the bromeliad species mentioned above and additionally comprised *T. tricolor*. The third area was a mature forest remnant (ca. 20 ha and older than 100 y) on a hilly terrain, surrounded by pastures and secondary vegetation areas but connected to other forest fragments in the area. There is a well-developed understory and sub-canopy. Canopy trees were between 18—22 m tall, and some characteristic species are *Billia colombiana* (Hippocastanaceae), *Exothea glabra* (Sapindaceae), *Diospyros conzatii* (Ebenaceae), *Ficus pertusa* and *F. tuerckheimii* (Moraceae), *Nectandra salicina* (Lauraceae), *Ormosia cruenta* (Fabaceae), and *Sideroxylum stenospermun* (Sapotaceae). *Tillandsia fasciculata* and *T. tricolor* are the dominant bromeliads here, but exhibited low rates of recruitment, whereas the populations of *G. monostachia* and *C. nutans* were much reduced. *Werauhia gladioliflora* did not occur in any of the study sites, but occurs at scattered early to mid-successional locations in the surroundings.
<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat preference</th>
<th>Seedling form</th>
<th>Metabolic pathway</th>
<th>Adult rosette diameter (cm)</th>
<th>Longest leaf length (cm)</th>
<th>Reproductive periodicity per ramet</th>
<th>Seeds per fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. nutans</em> (Sw.) Griseb.</td>
<td>early &amp; mid-succession</td>
<td>tank</td>
<td>$C_3$ – CAM</td>
<td>14.2 (3.1)</td>
<td>12.6 (1.9)</td>
<td>annually</td>
<td>137 (20)</td>
</tr>
<tr>
<td><em>G. monostachia</em> (L.) Rusby ex Mez</td>
<td>early &amp; mid-succession</td>
<td>tank</td>
<td>$C_3$ – CAM</td>
<td>41.1 (11.4)</td>
<td>31.2 (7.6)</td>
<td>annually</td>
<td>321 (71)</td>
</tr>
<tr>
<td><em>T. fasciculata</em> Sw.</td>
<td>mid-succession &amp; mature forest</td>
<td>atmospheric</td>
<td>CAM</td>
<td>31.2 (5.4)</td>
<td>22.9 (3.2)</td>
<td>bi-annually</td>
<td>240 (46)</td>
</tr>
<tr>
<td><em>W. gladioliflora</em> (H. Wendl.) J. R. Grant</td>
<td>mid-succession &amp; forest edges</td>
<td>tank</td>
<td>$C_3$</td>
<td>79.7 (13.7)</td>
<td>61.2 (9.7)</td>
<td>annually</td>
<td>1679 (259)</td>
</tr>
</tbody>
</table>

*a at the study site (Chapter 2)

*b mean values (standard deviation), unpublished data
Experimental design
We obtained the seeds from five to seven fruits from each of 10—15 plants per species that were collected in all habitat types during February and March 2002. The seeds were pooled into a large, mixed batch from which random samples were taken for the experimental replicates. At each successional stage, six host trees were selected according to accessibility (by using an aluminum ladder or rope climbing techniques) and presence of bryophytes or lichens. On each tree, rectangular sections covering an area from 100—140 cm$^2$ were delimited on the main trunk or on a big branch in the inner part of the crown, where they could be easily located and monitored later on. On each section (replicate), fifty randomly selected seeds from each species were manually affixed by gluing their coma on the bark surface with a small droplet of non-toxic transparent glue (Resistol ®), during the end of April and early May 2002.

Since the presence of bryophytes and lichens on the bark has been suggested to facilitate colonization and further establishment by vascular epiphytes (Nadkarni 2000), we included this factor in our experiment. Two substrate conditions were defined: (a) presence of a natural bryophyte and lichen cover, and (b) absence of it, either by manually removing the bryophytes and lichens from a specific section of the bark or by selecting a naturally bare area.

Each host-tree contained eight sections (replicates) representing the four bromeliad species in the two substrate conditions. The sections were located next to each other in order to avoid the effect of small-scale climatic conditions derived from selecting separated sites within the tree crown. The vertical position on the host tree varied according to the vegetation height and was within the observed vertical distribution range of bromeliads in the study site (Chapter 2): at 1.5—2.5 m in the early successional vegetation, 4.0—12.5 m in the mid-succession and 5.5—16.0 m in the mature forest. A total of 7200 seeds from the four study species was distributed among the three forest habitats.

Data analysis
Germination rate (maximum number of germinated seeds) was calculated per replicate as a proportion after subtracting the amount of missing seeds in the first month of which germination was unknown. A seed was recorded as germinated when the hypocotyl elongation was visible to the naked eye. Survival probability was considered as the proportion of seedlings present relative to the original sample of planted seeds per replicate. The number of surviving seedlings was monitored monthly during the first year and then at the 18th and 24th month in the second year. As measure of seedling development, we recorded for each plant the maximum leaf length to the 0.5 mm and the number of leaves at the end of the first and second year.
For all the measured variables, we examined the differences with a Factorial Analysis of Variance using successional stage, species and substrate condition as main fixed factors. The proportions of surviving seedlings were compared at three time intervals after seed planting: (i) three months, (ii) one year, and (iii) two years. For seedling size we compared the absolute growth (actual size) and the relative growth as a percent of the seedling size relative to the adult reproductive size of the species. Growth parameters were analyzed at six month intervals, separately. To examine the effect of host tree identity on the abovementioned variables, an ANOVA was carried out including tree identity as a nested factor within habitat. Data on seed germination and survivorship were arcsine-transformed, while data on seedling size and number of leaves were transformed with a square-root and \( 10 \log \) transformation, respectively, in order to increase normality and reduce heteroscedasticity (Zar 1999). For significant ANOVAs (\( P < 0.05 \)), the means for the main factors were compared with a Tukey’s Honest Significant Differences (HSD) Test, and comparisons among habitats per species were performed with Contrast Analyses. Computations were carried out using STATISTICA for Windows® (GLM module, StatSoft Inc. 2000).

**Results**

**Germination**

The successional stage of the forest did not affect the germination rate of the studied bromeliads (\( P = 0.54 \)) and the differences among species were not statistically significant either (\( P = 0.45 \)) (Table 2). The substrate condition had a significant effect (\( F_{1,120} = 4.5, P = 0.037 \)). More seeds germinated when bryophytes and lichens were present on the bark surface (62.2% vs. 55.2%, respectively), although this difference was only significant in the mature forest. The mature forest registered the highest overall germination percentage (61.1%) followed by the early (58.7%) and mid-successional habitats (56.3%); whereas *G. monostachia* showed the lowest overall germination (54.6%) as opposed to *C. nutans* (61.9%); however, these differences were not significant (Table 2). Most of the germination occurred during the first month of the experiment (May), which represented the onset of the rainy season, but several *W. gladioliflora* seeds germinated during the second month. The germination rate significantly varied among host-trees (\( F_{15,122} = 3.8, P < 0.001 \), Fig. 1A) at each habitat, but there was not a significant interaction between host-tree and the bromeliad species.
Table 2. Seed germination rate of epiphytic bromeliads under field conditions in successional forest habitats in a premontane area, Costa Rica. Data are untransformed mean percentages (SE). N = number of replicates of 50 seeds each per habitat. No significant differences were found among habitats or species.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Early succession</th>
<th>Mid-succession</th>
<th>Mature forest</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. nutans</em></td>
<td>12</td>
<td>56.8 (5.9)</td>
<td>61.0 (3.9)</td>
<td>68.1 (5.5)</td>
<td>61.9 (3.0)</td>
</tr>
<tr>
<td><em>G. monostachia</em></td>
<td>12</td>
<td>52.6 (6.4)</td>
<td>54.2 (5.8)</td>
<td>56.9 (5.7)</td>
<td>54.6 (3.4)</td>
</tr>
<tr>
<td><em>T. fasciculata</em></td>
<td>12</td>
<td>63.9 (3.3)</td>
<td>50.3 (4.6)</td>
<td>59.6 (6.4)</td>
<td>57.9 (2.9)</td>
</tr>
<tr>
<td><em>W. gladioliflora</em></td>
<td>12</td>
<td>61.8 (3.1)</td>
<td>59.7 (6.6)</td>
<td>59.8 (8.8)</td>
<td>60.4 (3.7)</td>
</tr>
<tr>
<td>Pooled</td>
<td>48</td>
<td>58.7 (2.5)</td>
<td>56.3 (2.7)</td>
<td>61.1 (3.3)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Variation among host trees in (A) germination rate and (B) seedling survivorship after 24-months of epiphytic bromeliads in different successional forest habitats in a tropical premontane area, Costa Rica. Data pooled from four species and presented by host tree (bars) within each successional stage. Horizontal lines are 1 SE.
The number of seeds of which germination was unknown that disappeared during the first month was lower in the younger vegetation, 6.0 ± 0.9% ($F_{2,120} = 8.0, P < 0.001$), compared to the mid-succession (14.5 ± 2.2%) and mature forest (17.1 ± 2.6%). In all species, the amount of missing seeds was significantly lower in the early succession habitat, except for *T. fasciculata* (Table 3). When pooling the data from the different habitats, *T. fasciculata* recorded the overall lowest percent of missing seeds, 9.6 ± 1.7%; followed by *C. nutans* 12.5 ± 2.7%, *G. monostachia* 13.2 ± 2.5%, and *W. gladioliflora* 14.9 ± 2.8%, but these differences were not statistically significant ($P = 0.50$). The substrate type did not influence the number of missing seeds ($P = 0.18$) and all interactions were not statistically significant either.

Table 3. Proportion of seeds lost during the first month of a field germination experiment of epiphytic bromeliads in successional forest habitats in a premontane area, Costa Rica. The germination of these seeds was unknown. Data are untransformed mean percentages (SE). Statistical differences between habitats per species (ANOVA, Contrast Analysis test) are indicated with different superscript letters.

<table>
<thead>
<tr>
<th>Species</th>
<th>Forest habitat</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early succession</td>
<td>Mid-succession</td>
</tr>
<tr>
<td><em>C. nutans</em></td>
<td>3.3 (0.8) a</td>
<td>14.0 (3.9) b</td>
</tr>
<tr>
<td><em>G. monostachia</em></td>
<td>9.5 (2.5) a</td>
<td>18.2 (5.7) bc</td>
</tr>
<tr>
<td><em>T. fasciculata</em></td>
<td>5.2 (1.4) a</td>
<td>9.8 (2.7) a</td>
</tr>
<tr>
<td><em>W. gladioliflora</em></td>
<td>6.0 (2.0) a</td>
<td>16.2 (5.0) ab</td>
</tr>
<tr>
<td>Pooled</td>
<td>6.0 (0.9) a</td>
<td>14.5 (2.2)</td>
</tr>
</tbody>
</table>

**Seedling survival**

The forest successional stage had a significant effect on the survival probability in the periods analyzed (3 months: $F_{2,120}, P = 0.011$; 12 months: $F_{2,120}, P < 0.001$; 24 months: $F_{2,120}, P = 0.003$). In overall, species survival did not differ between mid-successional and mature forest conditions but was usually higher in the early successional vegetation (Fig. 2). However, only for *W. gladioliflora* were these differences statistically significant along
the experiment (Table 4). Neither the species identity nor the substrate condition related significantly to seedling survivorship ($P >> 0.05$), and these factors did not interact.

When all species were lumped together, the accumulated curves of survivorship followed the same trend among habitats (Fig. 2A). Individually, species displayed similar patterns among each other and across habitats, except for $W. gladioliflora$ where survival in the younger vegetation clearly deviated from that in other habitats (Fig. 2E). There was an important fall in survival during the first three months. At that time, the percent of seedlings was 28.6% in $C. nutans$, 23.5% in $G. monostachia$, 25.2% in $T. fasciculata$, and 30.9% in $W. gladioliflora$. The higher reduction recorded during the first months is the combined effect of missing seeds for which germination is unknown and the remaining seeds that failed to germinate. By the end of the experiment, two years after seed sowing, the species survival probability was reduced to 3.8, 4.1, 5.7 and 4.8%, respectively.

**Figure 2.** Accumulated survival probability curves of epiphytic bromeliads in successional forest habitats in a tropical premontane area, Costa Rica. Values represent averaged monthly proportions during the two-year monitoring period (2002-2004). In each graph the three successional stages are denoted by curves: early succession (open circles), mid-succession (solid circles) and mature forest (triangles).
Table 4. Seedling survivorship of epiphytic bromeliads in successional forest habitats in a premontane area, Costa Rica. Data are untransformed mean percentages (SE) after 3, 12 and 24 months of seed sowing. Statistical differences between habitats per species (ANOVA, Contrast Analysis test) are indicated with different superscript letters.

<table>
<thead>
<tr>
<th>Species</th>
<th>Months</th>
<th>Early succession</th>
<th>Mid-succession</th>
<th>Mature forest</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Habitat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pooled</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C. nutans</strong></td>
<td>3</td>
<td>32.8 (7.5) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.7 (4.5) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.2 (5.9) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.6 (3.5)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>10.3 (3.5) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.3 (2.4) &lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.3 (2.1) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.3 (1.6)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>5.2 (2.3) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0 (2.2) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3 (1.0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8 (1.1)</td>
</tr>
<tr>
<td><strong>G. monostachia</strong></td>
<td>3</td>
<td>24.5 (6.0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.3 (6.1) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.7 (6.6) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.5 (3.5)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>12.3 (4.3) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.2 (3.3) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7 (2.2) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.7 (2.0)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>8.0 (4.5) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5 (2.0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 (1.5) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1 (1.7)</td>
</tr>
<tr>
<td><strong>T. fasciculata</strong></td>
<td>3</td>
<td>30.3 (5.9) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.7 (4.8) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.7 (4.8) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.2 (3.0)</td>
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<tr>
<td></td>
<td>12</td>
<td>10.8 (3.8) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.2 (2.8) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.8 (3.1) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.9 (1.8)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>7.3 (3.1) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2 (1.3) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7 (2.3) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7 (1.3)</td>
</tr>
<tr>
<td><strong>W. gladioliflora</strong></td>
<td>3</td>
<td>50.8 (3.1) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.7 (5.3) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.2 (6.3) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.9 (3.7)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>17.3 (3.2) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3 (2.8) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5 (0.7) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.7 (1.8)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>12.0 (3.7) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 (1.3) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5 (0.5) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8 (1.3)</td>
</tr>
</tbody>
</table>
The proportion of surviving seedlings among host trees was significantly different (\(F_{15,122} = 6.21, P < 0.001\), Fig. 1B). The number of seedlings present of each species on the host tree at the end of the experiment was not correlated to the number of germinated seeds (\(P > 0.05\) after a Pearson Product-Moment Correlation Test for each successional stage).

**Seedling development**

Seedling growth, expressed as plant size and as number of leaves, was significantly affected by the habitat condition and species identity at the different intervals analyzed (Table 5). Analyses based on absolute seedling size and relative growth (not shown) yielded similar significant results. In overall, seedlings consistently reached a larger size in the younger vegetation, while no differences between mid-succession and mature forest were detected, except in the first interval analyzed. Nonetheless, at the experiment’s termination, these differences were significant only for *C. nutans* (Fig. 3). The substrate type influenced seedling growth (Table 5), indicating a higher size increase for those seedlings on the bryophyte/lichens substrate. The analysis of this factor was restricted to the first census because missing seedlings resulted in an incomplete statistical design in following censuses. Seedling size per species after two years placed *W. gladioliflora* with the largest absolute mean size (29.4 ± 3.2 mm), followed by *G. monostachia* (15.6 ± 0.4 mm), and *C. nutans* and *T. fasciculata* with small and similar sizes, 13.9 ± 0.8 and 11.5 ± 0.3 mm, respectively (Fig. 3). In terms of relative growth, the species showed a different pattern. We found that *C. nutans* attained a higher relative size, eleven percent of its mean adult size compared to 4.8 – 5% in the other species (\(F_{3,319} = 42.9, P < 0.001\)). The specific response of some species was modified either by the habitat or substrate type as indicated by the significant interactions at the six- and 18-month census (Table 5).

The number of leaves per rosette showed a comparable tendency to plant size (Table 5), except for the substrate type which did not produce a significant effect (\(P = 0.69\)). In overall, seedlings tended to develop more leaves in the early successional stage and after two years all species, except *W. gladioliflora*, exhibited significant differences among habitats (Fig. 3). In some cases species responded differently in relation to the habitat as suggested by the significant interaction (Table 5, Fig. 3). By the end of the experiment, seedlings of *G. monostachia* had produced most leaves (mean = 11.7 ± 0.5), *W. gladioliflora* the fewest (mean = 6.3 ± 0.3), and *T. fasciculata* and *C. nutans* showed intermediate values (8.7 ± 0.2 and 8.7 ± 0.3, respectively). The differences among species were again more apparent in the early successional stage (Fig. 3).
Table 5. Results of the Univariate Factorial ANOVAs on the parameters of seedling growth of epiphytic bromeliads in different successional forest habitats and substrates in a tropical premontane area, Costa Rica.Analyses were performed at 6-month intervals after seed sowing.

<table>
<thead>
<tr>
<th>6 MONTHS</th>
<th>Seedling size</th>
<th>Number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( df )</td>
<td>( MS )</td>
</tr>
<tr>
<td>Habitat (hab)</td>
<td>2</td>
<td>4.67</td>
</tr>
<tr>
<td>Species (sp)</td>
<td>3</td>
<td>12.04</td>
</tr>
<tr>
<td>Substrate (st)</td>
<td>1</td>
<td>0.77</td>
</tr>
<tr>
<td>Hab x Sp</td>
<td>6</td>
<td>0.56</td>
</tr>
<tr>
<td>Hab x St</td>
<td>2</td>
<td>0.16</td>
</tr>
<tr>
<td>Sp x St</td>
<td>3</td>
<td>0.67</td>
</tr>
<tr>
<td>Hab x Sp x St</td>
<td>6</td>
<td>0.08</td>
</tr>
<tr>
<td>Error</td>
<td>975</td>
<td>0.09</td>
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</tbody>
</table>

<table>
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<th>12 MONTHS</th>
<th>Seedling size</th>
<th>Number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>( MS )</td>
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<tr>
<td>Habitat (hab)</td>
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<td>2.12</td>
</tr>
<tr>
<td>Species (sp)</td>
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<td>11.40</td>
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<tr>
<td>Hab x Sp</td>
<td>6</td>
<td>0.26</td>
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<tr>
<td>Error</td>
<td>537</td>
<td>0.17</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>18 MONTHS</th>
<th>Seedling size</th>
<th>Number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( df )</td>
<td>( MS )</td>
</tr>
<tr>
<td>Habitat (hab)</td>
<td>2</td>
<td>6.52</td>
</tr>
<tr>
<td>Species (sp)</td>
<td>3</td>
<td>3.36</td>
</tr>
<tr>
<td>Hab x Sp</td>
<td>6</td>
<td>0.32</td>
</tr>
<tr>
<td>Error</td>
<td>404</td>
<td>0.13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>24 MONTHS</th>
<th>Seedling size</th>
<th>Number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( df )</td>
<td>( MS )</td>
</tr>
<tr>
<td>Habitat (hab)</td>
<td>2</td>
<td>6.32</td>
</tr>
<tr>
<td>Species (sp)</td>
<td>3</td>
<td>5.25</td>
</tr>
<tr>
<td>Hab x Sp</td>
<td>6</td>
<td>0.27</td>
</tr>
<tr>
<td>Error</td>
<td>319</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Discussion

Seed germination
The environmental conditions in the secondary and mature forest habitats did not affect the germination capacity of the species. Moisture availability is considered the most important factor for germination of non-dormant seeds (Bewley & Black 1994), as in the case of most of the epiphytic bromeliads (Benzing 2000). This suggests that humidity conditions among habitats were similar enough to trigger germination. Since the rainy season starts after the seed dispersal period in the study site, any of the frequent rains will provide the necessary humidity. We conclude that seed germination did not influence seedling establishment.
success among successional habitats or species. Additionally, our findings indicated a small, but positive effect of bryophytes and lichens presence. Nadkarni (2000) has suggested that their presence would facilitate seed germination and establishment of vascular epiphytes because they retain humidity and nutrients longer. The higher germination on patches with bryophytes and lichens in the mature forest is possibly related to the observation that the bryophytic layer is more developed there than in the other habitats.

Disappearance of seeds from the substrate before the germination phase reduces the number of colonizing seeds and affects the eventual survival probability. We were not able to determine the specific causes of seed loss or explain why the proportion was lower in the younger vegetation. Bromeliad seeds are subjected to removal from the substrate by wind and water during storms or heavy rains, or due to the action of herbivores and some birds that use them as nesting material (Nadkarni & Matelson 1989, personal observations JGBO). Taller canopy trees in older forest stages may experience more stem flow than small trees in the younger vegetation, increasing the probability of the seeds being washed off the substrate. There is the chance that seeds dislodged by wind or water landed and germinated on a different part of the phorophyte. Since we artificially affixed the seeds on the substrate, the figures on missing seeds must be considered as conservative.

Seedling survival and development
The term ‘survival’ as defined in this study represents the probability of a seedling reaching a given age from the moment it attached to the substrate as a seed. This probability is determined by the combined effect of several potential causes of mortality: seed and seedling dislodgement, herbivory and physiological causes acting before and after germination. The changes in the number of surviving seedlings were usually associated with plant disappearance from one census to the next, either in secondary or mature forest habitat. Some authors have suggested that dislodgement is the main cause of seedling mortality in epiphytic bromeliads (see Bennett 1986, Castro-Hernández et al. 1999). Although epiphytic bromeliads develop roots with morphological adaptations that promote anchorage (Benzing 2000), the root system during the early stages of seedling development is probably insufficient to provide a reliable adherence.

We found a general effect of the habitat condition on survival, although it was significant for a single species (W. gladioliflora). The higher survival probability in the younger vegetation does not have a straightforward explanation. It was not our goal to determine the specific causes of seedling mortality and it represents a difficult task to separate the factors acting at each successional habitat or species. One could argue that in older stages of forest recovery there is a bigger array of herbivores and the activity of arboreal mammals
(capuchin monkey, coatis, porcupines, and others) that use trunks and limbs as transit routes (sensu Perry 1978) have a higher impact on seedling permanence. Nevertheless, it does not fully explain why only for *Werauhia gladioliflora* the survival probability was significantly different among habitats.

Similarly, it is difficult to identify the probable causes behind the higher seedling development recorded in the younger vegetation (only significant for *C. nutans*), which might result from a higher availability of light in that habitat, a limiting factor for epiphyte establishment (Benzing 2000). The lower stature of the younger vegetation with its scattered trees and small crowns possibly promote more homogeneous and favorable conditions for seedling growth compared to the more shaded and heterogeneous environment under the closed-canopy of older forests.

**Recruitment limitation during community assembly**
Recent models of plant community assembly emphasize the importance of recruitment limitation over the existence of competitive differences among species (cf. Hurtt & Pacala 1995, Hubbell 2001). For epiphytic bromeliads, community composition and species distribution are presumed to be determined by physiological and morphological adaptations to different degrees of light exposure and water stress (Pittendrigh 1948). Our results indicated that seedling establishment success does not correlate with the presumed eco-physiological adaptations of the species. To illustrate this point, we expected *T. fasciculata* seedlings to be more successful in the younger vegetation due to its metabolic CAM system (Benzing 2000) and thicker leaves with a denser trichome layer adapted to high exposure to light and low water availability (Benzing & Renfrow 1971). Similarly, but more in agreement with our results, we expected the facultative C₃-CAM species, *G. monostachia* (Martin 1994) and *C. nutans* (Benzing 2000) to show similar survival probabilities in high light irradiance (i.e. in younger vegetation) as well as under increased shaded conditions (i.e. in older forests). Nevertheless, the supposed C₃ species *W. gladioliflora* performed better in the youngest vegetation. It is likely that factors influencing mortality through dislodgement from the substrate obscure any potential competitive advantage among species during the early phase of seedling establishment. The effect of those presumed adaptations may be more important if they are expressed later during the plant life cycle (Zotz *et al.* 2001). However, the exact ecophysiological response of epiphytic bromeliads in relation to forest recovery and as a function of plant size remains to be investigated.

In this study, species were able to establish in those successional habitats where they are nearly absent or show low rates of seedling recruitment, not correlating those results with the observed pattern of species presence and abundance at a particular habitat (Chapter 3).
The lack of clear differences in seedling establishment between secondary and mature forest species further suggests that community composition in the study site is recruitment limited (Hurtt & Pacala 1995, Hubbell 2001). Remarkably in this respect is *W. gladioliflora* which establishment success was comparable to the other species (Fig. 2), contrasting with the fact that this species is almost absent from the studied habitats (Chapter 2). The nearest population of this species was located more than one kilometer away and seed influx into the study site was low as shown by seed-trapping experiments (Chapter 4 & 5). Hence, localized seed dispersal rather than competitive limitations during establishment apparently explains its rarity in the study site. The seed-trapping experiment also showed that the amount of seeds caught inside the mature forest of *Catopsis* and *Guzmania* that dominated the secondary forest was also low, in spite of the proximity of the successional habitats (<600 m) and the similar wind-dispersal mechanism (Chapter 4). Wolf (2005) found that variation in the composition of epiphyte communities on a regional scale was significantly explained by the geographic position in the landscape and attributed that result to seed dispersal.

To conclude, the differences in bromeliad abundance and distribution are not likely the result of competitive interactions during seedling establishment and are best explained by seed dispersability and availability. Assuming that dispersal characteristics of the plumed seeds do not strongly differ among species, seed availability mainly depends on growth rate, fecundity, and other life-history traits of species, which are currently being investigated. For conservation purposes, the recovery process of epiphytes in successional or degraded habitats can be improved by increasing the amount of seeds available for germination and further seedling establishment in a given habitat, thus eliminating the constraints to seed dispersal in natural conditions. One way to attain this is to spare large trees with a considerable diversity of epiphytes during logging, as proposed by Wolf (2005).

**Acknowledgements**

We thank the staff of the Ecolodge San Luis and Research Station (UGA) for their logistic support. Adam Fuentes and Cristian Lobo provided invaluable help during the field-experiments. Electra Enslow collaborated during the seed planting in the field and Rodrigo Solano kindly provided extra climbing gear. This is a project funded by WOTRO, The Netherlands (Grant W85-34), in collaboration with the National Museum of Costa Rica.
References


Germination and Establishment of Epiphytic Bromeliads


Summary

Seed dispersal has often been proposed as the major determinant of the spatial aggregated pattern of epiphytic vascular plants. In order to evaluate this hypothesis, we examined the seed rain of wind-dispersed epiphytic bromeliads (subfamily Tillandsioideae) in forests of different successional condition in a premontane area in Costa Rica, during the seed-dispersing season of 2002. After a monitoring period of nine weeks, using seed traps in the forest canopy, we found the highest number of seeds in the younger vegetation, which coincided with the higher abundance of epiphytic bromeliads in that habitat. In contrast, the poor-bromeliad mature forest received the lowest amount of seeds. The sampled areas within the same forest type showed significant variation in the magnitude of the seed rain but differences among forest types were not statistically significant. The most frequent species in the seed rain was Guzmania monostachia (85%): the most common species in secondary forests in the area. Most seeds tended to disperse within a few meters from the mother plant, with a significant high frequency of seeds on traps located within one meter from the dispersing plant. This pattern of seed dispersal coincided with the reportedly aggregated distribution of bromeliad seedlings around the mother plants. Although an important factor, dispersal limitation is not enough to account for the differences in species composition among successional forests since long distance dispersal events are possible. It is likely that environmental factors affecting the growth and reproductive capacity of the species play an additional role in the formation of a stable population in the mature forest.
Introduction

The distribution patterns of epiphytic bromeliads and epiphytes in general, have often been described as clumped and patchy (e.g. Benzing 1981, Bennett 1986, Nieder et al. 2000). Studies to explain these patterns have mainly focused on environmental factors, such as light, temperature, humidity and altitude (e.g. Bennett 1987, Wolf 1994, Castro-Hernández et al. 1999, Zotz & Vollrath 2002, van Dunné 2002). However, such habitat characteristics can in general only partly explain the observed distribution of epiphytes. It has been concluded repeatedly that epiphytes occur only in a small portion of what seems suitable habitat. For instance, Johansson (1974) found that the vascular epiphytes he investigated inhabited 10% of their potential growing sites, and Rossi et al. (1997) observed that Costa Rican epiphytic bromeliads were present only in about half of the life zones where they were expected to grow according to the Holdridge’s classification system. Also, epiphytic communities can differ greatly in their composition at apparent identical habitats (Barkman 1958, Johansson 1974, Wolf 1994, 2005). Moreover, epiphyte communities are commonly presumed to be composed of ecologically similar species (Schuster 1957, Benzing 1981, Wolf 1994, Kernan & Fowler 1995, Zotz & Vollrath 2002). According to the classic competitive exclusion principle, species can only coexist when they occupy different niches. However, an increasing number of theoretical and empirical studies propose that local dispersal can promote coexistence of ecologically similar species and it is an important determinant of community composition and species abundances (e.g. reviewed by Turnbull et al. 2000, Hubbell 2001 and references therein).

Various studies have suggested that local and random colonization processes (i.e. propagule supply) are the main determining factor in the distribution and community composition of ecologically equivalent epiphytes (e.g. Benzing 1981, Yeaton & Gladstone 1982, Oksanen 1988, Wolf 1994, Zotz et al. 1999, Zotz & Vollrath 2002). For example, Barkman (1958) and Yeaton & Gladstone (1982) found that epiphyte composition is more similar between trees that stand next to each other than between trees that are farther apart. Hietz-Seifert et al. (1996) reported a negative correlation between epiphyte numbers on isolated forest trees and distance from the forest border. Van Dunné (2002) observed a significant effect of the proximity of older conspecifics on the presence of juvenile bromeliads. In spite of these indications that bromeliad seed dispersal may drive community structure, knowledge of dispersal-related life-history traits and other non-physiological attributes that possibly can account for the spatial distribution of epiphytes, such as reproductive biology, breeding system, population genetics and demography, is still scarce (but see e.g. Soltis et al. 1987, Palací 1991, Izquierdo & Piñero 2000).
The present study concerns an investigation of seed rain and dispersal distance of wind-dispersing epiphytic bromeliads (subfamily Tillandsioideae) in primary and secondary montane cloud forest in the Monteverde region, Costa Rica. The study area is characterized by a mosaic of forest patches of different successional stages, which show considerable differences in bromeliad species composition and abundances. For example, some areas of young secondary forest support very high densities of epiphytic bromeliads, while mature forest stands are generally nearly devoid of these plants (Chapter 2). The main objective of this study is to determine the importance of seed supply for colonization and distribution of epiphytic bromeliads in secondary and mature forest. In order to test this, we measured bromeliad seed rain by placing and monitoring seed traps in each of three forest types: young secondary, old secondary and primary forest. We investigated dispersal distance by relating trapped seed numbers to the distance of the nearest dispersing (fruiting) bromeliad.

**Materials and Methods**

**Study site and species**

Fieldwork was carried out in the San Luis River Valley in the northwest of Costa Rica, on the pacific slope of the Monteverde region (latitude: 10° 17’ N, longitude: 84° 48’ W). The sampling plots were laid down on a 67 ha property belonging to the San Luis Ecolodge and Research Station at 1100-1200 m. The study area comprises a mosaic of active pastures, patches of primary forest and secondary forest of different ages, and some small coffee- or other agricultural plantations. The annual temperature fluctuates between 17-25°C and annual rainfall is approximately 3300 mm. Two main seasons with a defined transition period are recognized in this area: a rainy season from May to October, a transition period from November to January with strong north-easterly trade winds, and a dry season from February to April with moderate trade-winds and during which rain falls sporadically (Nadkarni & Wheelwright 2000). The forests in this area are considered premontane wet, according to Holdridge’s Life Zone classification (Bolaños & Watson 1993).

The Tillandsioide bromeliads typically produce small seeds that feature a plumose coma at one or both sides, which enables the seed to stay in the air longer and promotes adhesion to bark-like structures (Benzing 2000). The species at the study site disperse during the dry season. During our study, we trapped seeds of the following species: *Guzmania monostachia* (L.) Rusby ex Mez, *Tillandsia fasciculata* Sw., *Tillandsia tricolor* Schltldl. & Cham., *Catopsis nutans* (Sw.) Griseb., and *Werauhia sp.*, in addition to a small number of seeds (<1%) that could not be identified to the species level with confidence. The identifiable seeds showed small but consistent differences in morphology (e.g. shape and length of the coma, colour and size).
Experimental set-up
The experiment was conducted from January to March in 2002, during the dry season. We chose two sampling areas in each of three forest types: young secondary (YS), old secondary (OS) and mature (M) forest. Distance between sample areas in the same forest type was from 200 to 400 meters. Per sampling area 15 seed traps were placed, resulting in a total of 90 traps. A trap consisted of a 60×60 cm piece of 3 mm thick synthetic carpet supported at the edges by a PVC pipe frame, to which it was attached with nylon cord. The traps were tied to trees at various heights with nylon cord and positioned perpendicular to the prevailing E-NE (255°) wind direction. Access to the trees was achieved by using single rope climbing techniques (Perry 1978, Whitacre 1981) or with the help of a 5-meter extendable aluminum ladder. The distance between adjacent traps varied between 3 and 10 meters. Heights of the traps were appointed arbitrarily according to the trees’ height and architecture, with height ranges of 1.1-4.5 m in the young secondary, 3.4-14.0 m in the old secondary, and 4.7-18.9 m in the mature forest. The distance of the nearest dispersing or fruiting bromeliad to each trap was recorded. In order to avoid seed lost due to detachment or being washed away by sporadic rains, each trap was monitored for bromeliad seeds weekly and for a period of nine weeks. Trapped bromeliad seeds were removed and identified to the species in the laboratory.

Description of the sampling areas
Young secondary forest - Both sampling areas in this forest type were located in a 3 ha area of former pasture. For the last seven years natural succession has taken place, resulting in young secondary vegetation with a relatively low plant species diversity (compared to older forests in the area).

Sampling area YS1 - The tree species composition consisted mainly of Acnistus arborescens (Solanaceae), with a few Sapium sp. (Euphorbiaceae). Tree density was high and the average canopy height was around five meters. Most of the A. arborescens trees were densely covered with Guzmania monostachia. Other bromeliad species present in this area, but sparser than G. monostachia, were Catopsis nutans and Tillandsia fasciculata.

Sampling area YS2 - Nearly the entire sample area was covered with guava trees (Psidium guajaba, Myrtaceae), with only a few A. arborescens and Sapium sp. individuals at the edges. The density of bushy guava trees was high, and the average canopy height was around three meters. The guava trees were practically devoid of bromeliads, apart from a few reproducing C. nutans and some G. monostachia seedlings. The A. arborescens on the edges supported G. monostachia, C. nutans and very few T. fasciculata.
Old secondary forest - The secondary forest of both sampling areas was approximately 40 years old. The two sampling areas were not located within one bigger forest patch of the same age, but were separated by forest patches of different ages.

Sampling area OS1 - A small 20×30 meter patch of old secondary forest which was surrounded mainly by (disturbed) older forest. Among the tree species were *Inga punctata* (Fabaceae), *Dendropanax arboreus* (Araliaceae), *Nectandra salicina* (Lauraceae) and *Rondeletia brenesii* (Rubiaceae). The average canopy height was around 10 meters. Bromeliads were relatively scarce and only occurred in the canopy or on fallen branches on the forest floor. We observed *G. monostachia*, *T. fasciculata*, *T. tricolor*, *T. juncea*, *C. nutans*, and *C. nitida*.

Sampling area OS2 - This sampling area was placed within a 40×100 meter patch that used to be a shaded coffee plantation. Among the tree species were *Cordia eryostigma* (Boraginaceae), *Inga* sp. (Fabaceae), *Hasseltia floribunda* (Flacourtiaeae) and *Diospyros digyna* (Ebenaceae) at the edge of the patch. The average canopy height was about 10 meters. On the northeast side the sampling area was bordered by an open ‘edge’: a road beyond which there were pastures and the young secondary patch described above. Especially at this edge, bromeliads were abundant. The following bromeliad species were present: *G. monostachia*, *T. fasciculata*, *T. tricolor*, *T. juncea*, *T. multicaulis*, *T. bulbosa*, *C. nutans*, *C. nitida*, and *W. werckleana*.

Mature forest - Both mature forest sampling areas were located within one large patch which was selectively logged for Meliaceae and Lauraceae tree species about 50 years ago.

Sampling area M1 - Tree species composition was diverse and represented various families. Observed species were e.g. *Pouteria reticulata* (Sapotaceae), *Diospyros digyna* (Ebenaceae), *Nectandra salicina* (Lauraceae), *Dendropanax arboreus* (Araliaceae), *Drypetes lateriflora* (Euphorbiaceae), and *Ficus* sp. (Moraceae). Trees were bigger but in lower density when compared to the old secondary forest. Canopy height was around 20 meters, with some emergent trees up to 25 meters tall. Bromeliad density was remarkably low, and most plants were growing in clumps higher on the trees. Species observed were *T. fasciculata*, *T. tricolor* and *C. nutans*.

Sampling area M2 - Vegetation composition and height were similar to sampling area M1. Bromeliad density was very low, with only one or a few individuals found of the following species: *G. monostachia*, *T. fasciculata*, *T. tricolor*, and *C. nutans*.
Statistical analysis
To test for differences between the total amount of seeds trapped per forest type and sampling area over the nine weeks of the experiment, a two-level nested ANOVA was carried out for each species. The factor ‘forest-type’ contained three groups (young secondary, old secondary and mature forest) and the factor ‘sampling area’ nested within ‘forest-type’, contained 6 groups (sampling area: YS1, YS2, OS1, OS2, M1, M2).

To analyse the relationship between the total number of Tillandsioid seeds found on a trap (regardless of the species) and the distance of the nearest dispersing bromeliad to that trap, an ANCOVA was carried out. To eliminate a possible effect of differences in reproducing bromeliad densities between sampled areas, these densities were included into the analysis as covariate (Cascante-Marín, unpublished data). This was done because the shorter distance classes were overrepresented by traps from sampling areas with a higher reproductive bromeliad density, compared to the longer distance classes. Seed traps from OS2 and M2 were excluded from the distance analysis because data on reproducing bromeliad density was not available for these sample areas. The factor ‘distance’ contained four classes: <1m (n=8), 1-5m (n=13), 5-10m (n=12) and >10m (n=27). Post-hoc multiple comparisons were made with a Bonferroni correction. For all statistical tests the variable ‘number of seeds per trap’ was natural log (+1) transformed, which resulted in a normal distribution of the data. All analyses were carried out with the computer program SPSS11 for Apple MacIntosh ®.

Results
Seed rain
During the 9 week period, a total of 5927 Tillandsioid seeds were trapped in 5 species: G. monostachia (85%), C. nutans (7%), T. fasciculata (6%), T. tricolor (1%) and Werauhia sp. (<1%). In addition, several unidentifiable seeds were trapped. The high proportion of G. monostachia was mainly caused by its high density in YS1, where we could not avoid placing traps close to dispersing plants. Consequently, the largest number of Tillandsioid seeds was trapped at YS1 (67%), followed by OS2 (17%), YS2 (12%), OS1 (2%), M1 (<1%) and M2 (<1%). In the mature forest, a number of traps did not receive any seeds; this was the case in three traps in M1 and eight traps in M2.

The average number of seeds caught on each trap varied considerably between species and between the sampled areas (Fig. 1). Differences in seed numbers between sampled areas within the same forest type were highly significant (P<0.001) for all species except...
for *Werauhia* sp. (P<0.05, Table 1). In contrast, none of the species showed significant differences between forest-types.

**Figure 1.** Average number (natural log-transformed) of wind-dispersed seeds per trap of epiphytic bromeliads at different successional forest areas. YS = young succession, OS = old succession and M = mature forest. Each graph presents data from one species. Error bars represent ± 2SE. Non-transformed average numbers are displayed above the data-points in the graph.
Table 1. Summary of the ANOVA's performed for each of the five bromeliad species, comparing seed rain per sample area and forest type. F- and p-values are given.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Guzmania monostachia</th>
<th>Catopsis nutans</th>
<th>Tillandsia fasciculata</th>
<th>Tillandsia tricolor</th>
<th>Weraubia sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Among forest types</td>
<td>2</td>
<td>6.13</td>
<td>0.09</td>
<td>1.65</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.16</td>
<td>0.42</td>
<td>1.72</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.73</td>
<td>0.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among sampled areas within forest types</td>
<td>3</td>
<td>23.68</td>
<td>&lt; 0.001</td>
<td>16.87</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39.16</td>
<td>&lt; 0.001</td>
<td>20.73</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.96</td>
<td>0.037</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within sampled areas (error)</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Of the dominant *G. monostachia*, most seeds were trapped in the young secondary forest, especially in YS1 where an average of 265.7 seeds of this species was caught per trap. In the other young secondary forest, YS2, the numbers of trapped *G. monostachia* seeds was lower and similar to that of OS2, and again fewer seeds were trapped in OS1. Least *G. monostachia* seeds were trapped in the mature forest: only 3 in M1 and 2 in M2. The pattern of seed rain density showed by *G. monostachia* was not repeated in the other species. For example, while most seed of the latter species were trapped in YS1, *C. nutans* numbers were highest in YS2 and OS2, for *T. fasciculata* in OS2, and for *T. tricolor* in OS1. Noteworthy is, however, that few seeds were trapped for all species in the mature forest sites (M1 and M2) (Fig. 1).

**Dispersal distance**

To test for the effect of the distance to the nearest dispersing bromeliad on the number of seeds per trap, we divided the traps into 4 distance classes: (I) closer than 1 m, (II) between 1 and 5 m, (III) between 5 and 10 m and (IV) more than 10 m from the nearest dispersing bromeliad. As expected, the closer a trap was located to a dispersing bromeliad, the more seeds were trapped (Fig. 2). In class (I) an average number of 401.1 seeds was found per trap, in (II) an average of 86.4, (III) an average of 21.3 and in (IV) an average of 13.7. The ANCOVA revealed that the covariate ‘density of reproductive bromeliads per sample area’ was significantly related to the variable ‘distance classes’ (P<0.001). It also showed a significant difference between distance classes, after controlling for the effect of the covariate (P<0.001) (Table 2). Bonferroni corrected post-hoc comparisons revealed a significant difference between class I and class II (P=0.011), III (P<0.001) and IV (P<0.001). Differences between class II and III, and class III and IV were not significant (Table 3).

![Figure 2. Average number of bromeliad seeds per trap in four distance classes from the nearest dispersing plant. Error bars represent ±1 standard error.](image-url)
Table 2. ANCOVA-table comparing average number of bromeliad seeds per trap in four distance classes. Bromeliad density per sample area was included into the analysis as covariate.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>$F$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromeliad density at the sampled areas (covariate)</td>
<td>43.97</td>
<td>1</td>
<td>43.98</td>
<td>48.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distance classes</td>
<td>57.48</td>
<td>3</td>
<td>19.16</td>
<td>21.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>50.14</td>
<td>55</td>
<td>0.91</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. $p$-values of paired post-hoc comparisons between distance classes (see text for definitions). An asterisk indicates a significant difference between two categories below the confidence level of $p=0.05$ after a Bonferroni correction.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I – II</td>
<td>0.011*</td>
</tr>
<tr>
<td>II – III</td>
<td>0.069</td>
</tr>
<tr>
<td>III – IV</td>
<td>0.739</td>
</tr>
</tbody>
</table>
Discussion

Seed rain
The seed rain measured during the dry season of 2002 differed significantly between sampling sites, but not between forest types. For *G. monostachia*, *C. nutans*, *T. fasciculata*, and *T. tricolor* the difference between sampling areas was highly significant and for *Werauhia* sp. it was less, but still significant. Especially the sampled areas within the young and old secondary forest showed a larger variation (Fig. 1). For example, comparable numbers of *G. monostachia* seeds were trapped in YS2 and OS2, while the differences between the replicate sampled areas within the young and old secondary forest were much larger. Thus, the successional condition of the forest was not a good predictor of seed density for all species. In general, the seed rain distribution seems to reflect the occurrence and abundance of reproducing plants in the species pool of the sampled areas. Accordingly, the observed variation in numbers of trapped seeds is probably a direct consequence of the patchy distribution of the species, both within and between forest types. Some of the few *T. fasciculata* seeds that were trapped in YS1 probably came from individuals on several large isolated remnant trees near the sampled area, which supported many fruiting *T. fasciculata* plants. Numbers of trapped seeds were very low for all species in the mature forest, which corresponds to the low abundances or absence of reproducing plants in this forest type.

Dispersal distance
During the seed trapping experiment a very large part of the seeds was trapped in the close vicinity of dispersing plants. This is in agreement with earlier suggestions (*e.g.* Zotz *et al.* 1999, van Dunné 2002) and observations (Garcia-Franco & Rico-Gray 1988). To rule out a possible effect of differences in densities of reproducing bromeliads surrounding the traps, the reproducing bromeliad density of the sampled areas was included into our analysis as a covariate. The analysis revealed that, after controlling for the density effect, traps located within one meter of a dispersing Tillandsioid bromeliad received significantly more seeds than traps beyond a distance of one meter (*P*=0.011), on which the numbers of trapped seeds decreased sharply (Fig. 2). We cannot be sure that the nearest dispersing bromeliad was indeed the source of the trapped seeds but the clear negative distance-seed density correlation suggests that long distance dispersal is indeed relatively rare, as predicted by models on wind dispersal (*e.g.* Greene & Johnson 1989, Bullock & Clarke 2000). In agreement also, the branches and stems of the host tree as well as other plants directly around dispersing bromeliads were often covered with seeds (de Jong, personal observation). Moreover, it is likely that a considerable part of the seeds never becomes airborne. Sporadic rains, which become more frequent at the end of the dry season, cause the seeds to cluster and form masses of seeds that are deposited either on the own inflorescence or on vegetative parts of
the mother plants, where they sometimes even germinate (de Jong, personal observation). Similar observations were reported by Castro-Hernández and collaborators (1999).

**Seed dispersal in relation to bromeliad distribution**

Local seed dispersal, as shown by this study, can very well explain the clumped distribution of Tillandsioïd epiphytic bromeliads that is often reported in previous studies (e.g. Benzing 1981, Bennett 1986, van Dunné 2002) including for our study site (Chapter 2). The strong variation in bromeliad species composition and abundance between patches of the same forest type, as observed for our young secondary and old secondary patches, is likely caused by random and local propagule supply.

So far, the statistical results have shown that in our research the Tillandsioïd bromeliad seeds were mainly dispersed on a very local scale, but rare long distance dispersal (LDD) events did also occur. For instance, in all sampling areas *Werauhia* sp. seeds were found while no dispersing plants of this species were present in or directly around any of them. Likewise, *G. monostachia* seeds were found in the mature forest sampling areas and *T. fasciculata* in OS1. It should be taken into consideration that only a very small part (5.2 m²) of the total tree bark surface of the sampling areas was considered, and thus the total seed rain on the tree should be much larger than the numbers of seeds that we trapped during this study suggests. Nevertheless, the behaviour of the tail of a seed-dispersal curve can be very important in colonising new sites (Portnoy & Wilson 1993) and extending the range of a species (Nathan *et al.* 2002). An extreme example of this is the transatlantic dispersal event that resulted in the establishment of the bromeliad *Pitcarnia feliciana* in West Africa (Benzing 2000). Malanson & Armstrong (1996) modelled dispersal and establishment probabilities in a fragmented forest landscape, and found that exclusion of very rare dispersal events had a severe negative effect on tree species richness. However, the probability of landing on a “safe site” represents a major bottleneck in the establishment of epiphytes (Hietz *et al.* 2002). The low survival probability recorded from an experiment on seedling establishment success in the same study site supports the previous assumption (Chapter 3)

The strong winds during the first half of the dry season may have affected the recorded LDD events of our study, since turbulence can have a considerable effect on LDD (Portnoy & Wilson 1993). However, it should also be taken into account that lighter seeds are uplifted by wind more easily (Nathan *et al.* 2002) and can stay in the air longer. Strykstra *et al.* (1998) observed a negative correlation between flying capability and seed quality of *Arnica montana* seeds: lighter seeds dispersed further but had lower germination rates, probably because empty, non-viable seeds were included in the experiment. The *Werauhia* seeds
found on the traps had an identical appearance but were often much smaller than the average *W. gladioliflora* seed. It is possible that only the smallest seeds of this species travelled this far. An attempt to germinate these seeds in the laboratory failed. Another option is that the seeds came from another *Werauhia* species (*W. werckleana*) that occurs at a slightly higher elevation. Unfortunately, we do not have a detailed description of the seeds of this species, although we do know that they are smaller than *W. gladioliflora* seeds, but generally not as small as most of the trapped *Werauhia* seeds (Cascante-Marín, personal observation).

It is unlikely that the sparse bromeliad growth in the studied mature forest can be attributed to dispersal limitation alone, considering the abundant bromeliad seed sources in the area. Taking the age of the mature forest into account, in time there should have been plenty of opportunities for bromeliad colonisation. Barthlott *et al.* (2001) did one of the first studies on epiphyte diversity and species composition with regard to both secondary and primary forest. They observed more bromeliad species in secondary and disturbed forest than in primary forest, in contrast to the other epiphyte genera included in their study. A similar result was reported by Dunn (2000) in a survey of epiphytic bromeliads. Interestingly, *G. monostachia* seeds apparently were able to reach the mature forest of our study, and we found some juvenile plants of different sizes (Chapter 2), but no reproducing individuals. Perhaps too few *G. monostachia* seeds reach the forest and seedling numbers are too low to compensate for the normal rates of seedling mortality. On the other hand, the occurrence of juvenile *G. monostachia* plants could be caused by a “mass effect”. This is the establishment of species in habitats in which they cannot reproduce due to niche differences, depending on the presence of dispersing individuals in adjacent zones (Shmida & Wilson 1985).

There are various environmental factors influencing the distribution of bromeliads and epiphytes in general. The canopy can be a very heterogeneous environment to epiphytes with respect to ecophysiological conditions (Zotz 1997). Differences in height and relative position in the tree crown give rise to different microclimates (Freiberg 1996). Several studies divided epiphyte communities into guilds of different zones in the canopy, following gradients of light and humidity (e.g. Pittendrigh 1948, Johansson 1974). In a study on the distribution of the bromeliad species *G. monostachia*, *T. fasciculata* and *Vriesea sanguinolenta* on the host tree *Annona glabra*, Zotz (1997) found significant differences between the species with respect to height, inclination and periphery.

A possible reason for this might be a difference in tolerance to drought-stress between the species. Epiphytes that grow in the more exposed part of the tree crown, have the benefit of abundant light, but experience more frequent drought stress (Hietz 1997). Zotz & Andrade (1998) discovered that juvenile *G. monostachia* plants are much more vulnerable
to desiccation than *T. fasciculata* plants of equal size. This is caused by a lower ratio of plant leaf area to plant water content for *T. fasciculata*, so that similar area-based transpiration rates result in lower relative water losses in this species. The researchers suggested that these findings may be responsible for the differences in the spatial distribution in host trees. In a recent study, Zotz & Vollrath (2002) tested whether the vertical distribution on the host *A. glabra* of *G. monostachia, T. fasciculata* and *V. sanguinolenta* (discussed above) is determined at a very early ontogenetic stage. The researchers attached more than 1000 first-year seedlings of the three species to substrates varying in orientation and position within the crown of the host, but found no differences in mortality rates between the species. Their results suggested that the spatial distribution patterns are determined very early in the plants’ life cycle, probably during seed dispersal or germination. However, no consistent differences were found among the studied bromeliad species after an experiment in germination and establishment in our study area regarding the forest type (Chapter 3). This basically only leaves dispersal (i.e. seed arrival), and the timing thereof, as explanations for the differences in spatial distribution among species.

Another factor contributing to the failing establishment of *G. monostachia* in the mature forest could be the frequent strong wind in the canopy during the dry season. Wind can have a drying effect during cloud-free periods (Sugden 1981), intensifying drought-stress in exposed parts of the canopy, but it can also dislodge bromeliads from their substrate (Hietz 2002). Sugden (1981) found that *G. monostachia* favoured leeward slopes and was not found on windward slopes at all on his study site in Colombia, in contrast to some other bromeliad species. In the secondary forest of our study site, *G. monostachia* plants were regularly found lying on the forest floor. Perhaps, they were dislodged from trees by wind easier than *e.g.* *T. fasciculata*. Epiphytes that fall on the ground may not die immediately but their survival is very low and the chance of reproducing is almost nil (Matelson *et al.* 1993).

Finally, various studies have demonstrated that epiphytes differ in abundance among potential host species (*e.g.* Benzing 1981, Ter Steege & Cornelissen 1989, Kernan & Fowler 1995). Tree species can differ in their suitability as hosts for epiphytes (Callaway *et al.* 2002). Bark texture, porosity, chemical composition and stability are some of the characteristics that may influence the presence and abundance of epiphytes on a host tree (Bennett 1987). In a study on two vascular epiphytes (*T. usneoides* and a fern) epiphyte growth was highly correlated with the water-holding capacity of the host tree’s bark (Callaway 2002). In our study, the two sampled areas of the young secondary forest differed greatly in bromeliad abundance. The dominant tree species of sampling area YS1, *A. arborescens*, was densely covered with *G. monostachia*. The bark of this tree has a rough, corky structure that holds
water well and facilitates bromeliad seed adhesion (personal observation). In contrast, sampling area YS2 was dominated by Guava trees, which have a smooth and exfoliating bark. Only a few bromeliad seedlings and no adult plants were found on these trees. Not only does this tree species’ bark seems to retain no or little moisture, the regular exfoliation would dislodge any established seedlings.

Conclusion
We have shown that despite their adaptation to wind dispersal, seeds of Tillandsioid epiphytic bromeliads are mostly dispersed over very short distances of one to several meters. This dispersal limitation can for a large part explain the observed variation in species composition and abundance in secondary and mature forest patches in the study site. However, we have also recorded long distance dispersal events and therefore it is unlikely that the sparse bromeliad growth in the mature forest is entirely caused by dispersal limitation. We expect that environmental factors also play a role in this. Bromeliad dispersal by wind is clearly a limiting factor in colonization processes and can slow down the colonization of new sites and forest regeneration considerably, but it cannot solely explain the bromeliad distribution in our study area. Our study indicates that a combination of dispersal limitation and environmental factors determines bromeliad distribution, with a preference of Tillandsioid bromeliads for successional secondary forest over mature forest.

Acknowledgements
We thank the staff of the Ecolodge San Luis & Research Station (UGA) for their logistic support. Financial support was received from the Stichting Het Kronendak and WOTRO (grant W85-34) from The Netherlands. This is a project in collaboration with the National Museum of Costa Rica.

References


Seed dispersal patterns of epiphytic bromeliads


CHAPTER 5

Dispersal Limitation in Epiphytic Bromeliad Communities in a Fragmented Landscape

With Noemi von Meijenfeldt, Hanneke de Leeuw, J. Gerard B. Oostermeijer, Jan H. D. Wolf & Joannes C. M. den Nijs

Summary

The transformation and reduction of tropical forests is likely to affect the patterns of seed dispersal of wind-dispersed epiphytes and seedling survival in the altered habitats. We tested this hypothesis by carrying out a comparative study of seed influx, survival and growth of transplanted seedlings of epiphytic bromeliads among bromeliad-poor forest interiors, forest edges, and isolated pasture trees in a premontane area in Costa Rica during 2003 and 2004. Inside the forest, the number of seeds collected on carpet-made traps was significantly lower compared to forest edges and pasture trees. The most abundant genera collected in each habitat were Guzmania and Tillandsia. The number of seeds per trap was positively correlated with the number of fruiting plants in its vicinity, which suggests a predominance of short-range dispersal. Variation in seed rain distribution is likely the effect of local patterns in abundance and spatial distribution of the species pool. Seedling survival after one year showed no differences among habitats in Catopsis nutans, Guzmania monostachia and Tillandsia fasciculata; nevertheless, the latter species showed the highest survival rate in each habitat. The relatively more severe daily fluctuations in air temperature and humidity recorded in forest edges and pasture trees during the end of the dry season were not clearly associated with an increase in seedling mortality. After one year, growth rate was higher of seedlings in forest interiors, also providing no explanation for the low bromeliad densities there. Assuming that germination and establishment rates are similar between habitat types and that adult mortality is relatively low, we hypothesize that the distribution of epiphytic bromeliads is recruitment limited due to limited seed dispersal rather than being determined by specific microclimatic conditions of each habitat.
Introduction

Tropical forests in mountain areas are increasingly fragmented into patches of heterogeneous structure that are part of a complex and changing landscape, due to human activities related to agriculture and cattle farming (i.e. Sánchez-Azofeifa et al. 2001). The biological consequences of forest fragmentation have been the subject of several reviews (i.e. Lovejoy et al. 1986, Saunders et al. 1991, Murcia 1995, Schelhas & Greenberg 1996). Few studies have so far addressed the fate of epiphytic plant populations in anthropogenically disturbed forests (Barthlott et al. 2001, Merwin et al. 2003, Wolf 2005). Epiphytes represent a diverse and important group among the vascular plants of tropical areas (Madison 1977, Gentry & Dodson 1987) and often highly contribute to the diversity (reviewed in Wolf & Flamenco-S. 2003) and biomass (Nadkarni 1985, Hofstede et al. 1993) of montane forests. Given the inherent dependency of epiphytes on trees as their substrate, the transformation of the natural forest is expected to have a direct impact on this life form.

A result of forest fragmentation is the creation of forest edges, which experience an immediate change in microclimatic conditions compared to the forest interiors. Particularly, there is an increase in light, air temperature and exposure to winds (Lovejoy et al. 1986, Saunders et al. 1991, Murcia 1995). The drier conditions at the forest edges are likely to favour species adapted to water-stress and the increase in wind-exposure is expected to favour the influx of wind-borne seeds from the surrounding matrix into the edges. An extreme form of forest fragmentation that is related to cattle farming is represented by remnant trees from the original forest in pastures. These remnant isolated trees represent important stepping-stones for birds and bats that deposit seeds under the crown, which eventually germinate and contribute to the forest regeneration (Guevara et al. 1992, Harvey & Haber 1999). Epiphytes on remnant trees represent an important source of propagules for re-colonization of surrounding secondary forests. Preliminary reports, however, indicate a reduction in epiphyte diversity between remnant trees and comparable trees within the primary forest (Barthlott et al. 2001). Remnant or isolated trees are expected to experience similar or more drastic microclimatic conditions than forest edges, which is likely to limit the establishment and persistence of epiphytes of more mesic habitats. Alternatively, the establishment on pasture trees may be dispersal-limited, which is suggested by the negative correlation between epiphyte species richness and distance from the forest edge in lowland pastures in Veracruz, Mexico (Hietz-Seifert et al. 1996).

To evaluate the importance of dispersal as a force that drives community structure, i.e. the dispersal assembly perspective sensu Hubbell (2001), we examined factors related to the establishment of epiphytic bromeliads (Bromeliaceae, Tillandsioideae) in forest edges.
and on pasture trees in a tropical premontane area in Costa Rica. Different habitats were chosen to obtain a long gradient in microclimatic conditions (forest interior – pasture trees). Moreover, species densities that are presumed to relate to seed output differed greatly on this gradient of anthropogenic disturbance. In the forest interior, all bromeliad species occurred in lower densities (Chapter 2). First, we compared the influx of seeds on artificial substrates in each of the three forest habitats. Next, a manipulative experiment was carried out to compare the survival probability and growth of transplanted seedlings from selected species into forest edges, pasture trees and forest interiors. Seedling survival and growth were related to measured microclimatic conditions at each habitat.

This paper addresses the following questions: (i) Does seed influx in the forest interiors differ from that in nearby altered habitats such as edges and pasture trees and how is this correlated with local and regional species densities? (ii) Is seedling survival and growth differentially affected by the forest habitat or the physiological and morphological adaptations of the selected species? (iii) Are the fluctuations in the microclimatic conditions of the canopy different among forest edges, pasture trees and forest interiors? and (iv) if so, do these fluctuations correlate with seedling survival and growth in the studied forest habitats?

Materials and Methods

Research area and experimental design
The San Luis River Valley is located on the pacific slope of the Monteverde region in the Tilarán mountain range, Costa Rica (latitude: 10° 17’ N, longitude: 84° 48’ W). The area is considered as humid premontane (bmh-P), according to Holdridge’s life-zone classification (Haber 2000) and elevation ranges from 950 to 1200 m. Average annual rainfall is 3300 mm, 80% of which falls from May to November; temperature varies from 17-25 °C (data from the Ecolodge San Luis and Research Station, 1050 m). The topography of the terrain is irregular and the distribution and conservation status of the forest is heterogeneous, as a consequence of several decades of dairy farming and coffee production. Pastures are intermingled with secondary forest patches, and a few relatively undisturbed forest remnants of different sizes and shapes are randomly distributed over the landscape. In the pastures, both remnant trees from the original forest and relatively younger, planted trees may be present, which is a common pattern in the area (Harvey & Haber 1999). The study was carried out on properties belonging to the Ecolodge San Luis and Research Station, the “Buen Amigo” farm, and the Children’s Eternal Forest Reserve of the Monteverde Conservationist League.
We included three forest habitat types: (i) forest interiors that were part of mature or slightly disturbed forest remnants (>10 ha), (ii) forest edges adjacent to agricultural fields or roads (“maintained edges”, sensu Fraver 1994), and (iii) isolated pasture trees, either remnant trees from the original forest or planted trees.

Forest interiors (6) consisted of two sites in each of three forest remnants (two near the Ecolodge, 960-1050 m; and one at the Conservationist League’s Reserve, 1100-1150 m). Sites within a forest were separated by at least 150 m and did not include natural gaps. At each site, we arbitrarily selected five canopy trees, separated by 10 to 100 m. Forest edges (6) were separated >200 m and up to c. 1.0 km from one another and were part of forests with similar structure and physiognomy. At each edge, five trees were arbitrary selected over the full length of the edge and no more than 5 m into the forest. The distance between trees within an edge varied from 7.5 to 100 m. Because of the landscape’s heterogeneity, the edges differed in their orientation and exposure to wind currents. The pasture trees (12) were dispersed over several active pastures and equally distributed among remnant trees (6) and younger planted trees (6). We used rope-climbing techniques to access the tree-crowns (Perry 1978, Mitchell et al. 2002).

Seed dispersal experiment
The number of bromeliad seeds arriving to the forest canopy of the studied habitats was investigated by means of seed traps. The traps consisted of a 0.60 x 0.60 m frame constructed from PVC plastic tube (ø 1/2”), covered by a piece of synthetic carpet (dark-grey in colour, 3 mm thick) that was affixed to the frame with polyethylene strings. Previously, this carpet’s material proved to have a suitable degree of seed adhesion and retention (Chapter 4). We utilized a single trap per host tree: 30 in forest interiors, 30 in forest edges and 12 in pasture trees, resulting in a total of 72 traps.

The traps were tied to main branches or trunks and positioned facing the predominant wind direction to increase the probability of catching seeds; at the forest edges the traps were located facing the border. The trap height ranged from 10.1 to 21.2 m in forest interiors, from 6.2 to 22.5 m in forest edges and from 7.0 to 23.2 m in pasture trees. We avoided (i) the presence of nearby leaves or big branches directly in front of the trap that might represent an obstacle to seeds, and (ii) the presence of a fruiting bromeliad directly in front of, or close to, the trap. For all traps, the number of fruiting plants in the vicinity (max. 10 m in all directions) was recorded.

The number of seeds caught on each trap was monitored at two-week intervals to minimize loss due to predation, detachment by wind or washing away by sporadic rains.
Monitoring took place during March and April 2003, which represents the major period of bromeliad seed dispersal in the study site (Cascante-Marín et al., unpubl. data) and coincides with the peak of the dry season. With the help of a reference collection, we identified the seeds to genus level on the basis of coma shape and length, seed size, colour and shape. Species could not be recognised with confidence.

**Seedling survival and growth experiment**

The effect of forest habitat on seedling survival and growth was tested with a transplantation experiment that lasted one year, from the mid-dry season of 2003 (March) to the next dry season (March 2004). We selected the three dominant species in the area: *Catopsis nutans*, *Guzmania monostachia* and *Tillandsia fasciculata* (Table 1). A sample of 1500 established seedlings of each species was collected from secondary vegetation patches, trees in living fences and some solitary trees in the study site. Seedling sizes measured in a sub-sample (N=2160) were similar among the studied species (Table 1), varying from 3 to 17 mm. Seedling age varied from about 6 to 18 months, based on results from a previous experiment on seed germination and early seedling growth (Chapter 3).

Seedlings were kept in laboratory conditions at the study site (average of 10 days) before being placed in the field. In the laboratory, they were kept in Petri-dishes on tissue paper that was regularly wetted. During that period a small fraction (2—5 %) of the collected plants died. In order to provide a homogeneous substrate, seedlings were affixed to pieces of synthetic carpet (19 x 19 cm and 3 mm thick, dark-grey colour) using a small droplet of non-toxic “White Craft ®” glue. The seedling positions were systematically alternated among species to avoid position-related effects. Individuals (20 per species) were separated by 1-cm and distributed over eight rows with the edge positions at least 2 cm from the carpet’s margin. The combination of row and column number was used to identify each plant for further monitoring. In total, 3660 seedlings were distributed among 61 pieces of carpet.

The seedlings were placed on a selection of the same 72 trees utilised for the seed dispersal experiment, and per tree we placed only one carpet to avoid spatial dependence. The carpets were firmly attached to the trunk or major branches inside the tree-crown using polyethylene strings and followed the bark surface’s contour. Twenty-four carpets were placed in forest interior sites (4 carpets per site), at heights from 10.2 to 21.2 m (mean = 14.3 ± 2.6 SD); 25 carpets among the six forest edges, at heights from 5.2 to 22.7 (mean = 12.1 ± 4.0 SD) and one carpet at each pasture tree at heights from 7.2 to 23.1 m (mean = 15.0 ± 4.9 SD).
Table 1. Ecological characteristics of the epiphytic bromeliad species utilized in the transplantation experiment of seedling survival and growth in forest interiors, forest edges and pasture trees in a premontane area, Costa Rica

<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat preference</th>
<th>Seedling form</th>
<th>Metabolic pathway</th>
<th>Initial seedling size (mm)</th>
<th>Adult rosette diameter (cm)</th>
<th>Longest leaf length (cm)</th>
<th>Reproductive periodicity per genet</th>
<th>Seeds per fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. nutans</em> (Sw.) Griseb.</td>
<td>early &amp; mid-</td>
<td>tank</td>
<td>C₃ – CAM</td>
<td>8.9 (1.7)</td>
<td>14.2 (3.1)</td>
<td>12.6 (1.9)</td>
<td>annually</td>
<td>137 (20)</td>
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<tr>
<td></td>
<td>succession</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. monostachia</em> (L.) Rusby ex Mez</td>
<td>early &amp; mid- success</td>
<td>tank</td>
<td>C₃ – CAM</td>
<td>10.4 (1.9)</td>
<td>41.1 (11.4)</td>
<td>31.2 (7.6)</td>
<td>annually</td>
<td>321 (71)</td>
</tr>
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<tr>
<td><em>T. fasciculata</em> Sw.</td>
<td>mid-</td>
<td>atmospheric</td>
<td>CAM</td>
<td>8.9 (1.7)</td>
<td>31.2 (5.4)</td>
<td>22.9 (3.2)</td>
<td>tri-annually</td>
<td>240 (46)</td>
</tr>
<tr>
<td></td>
<td>succession &amp;</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>mature forest</td>
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</tr>
</tbody>
</table>

*a* at the study site (Chapter 2)

*b* of the collected sample for the survival and growth experiment (mean and SD)

*c* mean values and SD (unpubl. data)
Survival was monitored once a month. At every census, the number of seedlings present per carpet was recorded and classified as (1) living (all the leaves green), (2) drying (some leaves dried out but the inner leaves of the rosette still green), (3) dead (all the leaves dried and shrivelled) and (4) missing (disappeared in between censuses). Seedling size measured as the maximum leaf length from the rosette base to the leaf tip was recorded at the beginning of the transplantation and again after 12 months (except for those that had dried out).

**Canopy temperature and humidity conditions**

We measured the microclimatic variables of air temperature and relative humidity in the canopy of the forest habitats using data loggers (StowAway® series; Onset Computer Company, MA, USA). A plastic non-transparent casing was designed to protect the devices from direct exposure to sunlight and rainfall, with the bottom open to allow wind entrance. The data loggers were affixed in pairs (temperature and humidity) next to the pieces of carpet that carried the seedling transplants.

Data were obtained during four non-consecutive census rounds of four days each at the end of the dry season (March 23 through April 25) and during two more rounds at the beginning of the rainy season of 2004 (May 16 to 27). The data loggers were programmed to start at the same time at each round, recording measurements at 10-minute intervals. We placed 9 pairs of data loggers in forest interiors, 9 in forest edges and 6 in pasture trees. Between census rounds, we rotated the loggers among trees within the same forest habitat to include a higher variation and to negate any bias in the equipment’s readings. The weather conditions appeared to affect the instrument’s functioning, and by the last round the number of reliable data loggers had declined to 12 for temperature and 9 for humidity.

**Statistical data analysis**

Differences in the number of seeds arriving to the studied forest habitats were analysed with a Factorial Analysis of Variance (Type III SS). Habitat and genus were included as fixed factors and the number of seeds per trap (replicate) as response variable. The total number of seeds per trap was expressed as a proportion relative to the total number of seeds caught in the three habitats. A non-parametric correlation test by ranks (Spearman $r_s$) was used to evaluate the relation between the number of seeds per trap and the number of fruiting plants in the vicinity of the trap. The significance of the correlation coefficient ($r_s$) was assessed with a $t$-test procedure.

Seedling survival in response to forest habitat was examined using a similar statistical design as described for seed dispersal. The response variable was the proportion of surviving seedlings per carpet (replicate). Tests were performed at three stages that reflected...
major changes in the climatic conditions along the experiment: (1) beginning of the rainy season 2003 (May), (2) end of the rainy season 2003 (December) and (3) middle of the dry season 2004 (March, end of the experiment). Using Contingency Table Analyses, we evaluated whether the frequency of dead and alive seedlings per species (at the first stage) was independent of their initial size. Seedlings were assigned to three size categories; the intermediate interval was defined as the mean initial size ±1 SD. We additionally performed independent Nested Analyses of Variance, with site or edge and species as main factors and ‘tree’ nested within site, to examine the variation on seedling survival within forest interiors and forest edges.

Analyses of seedling growth were based on the proportional size increment calculated as: (final size–initial size)/initial size. The statistical design followed the described procedure for analysing seedling survival. Since initial size was negatively correlated with size increase ($r =-0.34$, $t =-7.62$, $P<0.001$), it was considered as a covariate in the analysis.

Differences in temperature and relative humidity were analysed by using a Factorial Analysis of Variance with forest habitat and season (end of the dry season and beginning of the rainy season) as fixed factors. The response variables examined were the average minimum and maximum daily temperature and relative humidity, as well as the amplitude of the mean daily fluctuation (maximum minus minimum). Each data logger was considered as a replicate.

Proportions were submitted to an angular transformation (Arcsin $\sqrt{p}$, Zar 1999) to improve normality and reduce heteroscedasticity of the dataset. After detecting a significant result, differences among main factors were examined with a Tukey’s test (HSD) and differences among levels of the factors were compared by using a series of post-hoc contrast analyses. Computations were performed using the program STATISTICA for Windows ® (StatSoft, Inc. 2000).

Results

Seed dispersal
Traps in the forest interiors recorded the lowest absolute number of bromeliad seeds (N=205) compared to pasture trees (N=331) and forest edges (N=762). The overall mean number of seeds per trap in the forest interior (6.8 ± 2.1 SE) was significantly lower than in the altered habitats ($F_{2, 276} =7.9$, $P<0.001$). Pasture trees (27.6 ± 8.5 SE) and forest edges (25.4 ± 10.4 SE) were not different from each other. From the total 1298 seeds collected, the most abundant genera were Guzmania (49%) and Tillandsia (39%) ($F_{3, 276} = 19.2$, $P<0.001$;
Fig. 1). To a lesser extent, there were representatives of *Catopsis* (5.9%) and *Werauhia* (4.5%) and a small percent (1%) that could not be identified. When analyzed per genus, *Guzmania* and *Tillandsia* were significantly underrepresented inside the forest compared to the altered habitats (Fig. 1). Differences among habitats in *Catopsis* and *Werauhia* were not significant. The habitat type and species identity did not interact (P=0.12).

High heterogeneity in seed rain distribution among traps and genera was recorded in the studied forest habitats (Table 2). A high proportion (93%) of all traps received at least one bromeliad seed; however, the amount of seeds on a given trap ranged from a single seed to 299 seeds (mean=18, SD=39, CV=217%). The genus most frequently caught on the traps was *Guzmania*, which was present on 70 percent of the traps inside the forest and >90% of traps in the altered habitats; whilst the other genera were present in half or less of the traps in a given habitat (Table 2). In all species, the magnitude of the seed influx into a given trap was low, usually there were fewer than five con-specific seeds in a single trap (Table 2). The proportion of traps with more than five seeds of the same genus was highest in *Guzmania* (43%), followed by *Tillandsia* (8%) and *Catopsis* (4%), whereas for *Werauhia* none of the traps contained more than five seeds. In an exceptional case, 260 seeds of *Tillandsia* were recorded on a single trap, which was caused by a high density of dispersing plants in the vicinity of the trap.

Figure 1. Seeds caught of four epiphytic bromeliad genera in three forest habitats in a premontane area, Costa Rica. Data collected from March to April 2003. Shown are LS means of the transformed proportion of seeds per trap; vertical lines represent 1 SE. Letters above bars show among-habitat comparisons per genus and letters inside the bars show among-genera comparisons per habitat.
The frequency of dispersing plants inside the forest seemed lower than in forest edges and pasture trees, as suggested by the mean numbers of fruiting plants in the vicinity of the traps (i.e., within a 10 m radius) (Table 2). We detected a positive correlation between the number of seeds caught per trap and the number of nearby fruiting plants in 6 out of 9 individual tests that could be performed by genus and habitat (Table 2).

### Table 2. Descriptive parameters of the seed dispersal experiment of epiphytic bromeliads into three forest habitats in a premontane area, Costa Rica. See parameters’ description below the table.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Parameter</th>
<th>Forest interior</th>
<th>Forest edges</th>
<th>Remnant or isolated tree</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Catopsis</em></td>
<td>Total of seeds a</td>
<td>13</td>
<td>52</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Traps with seeds b</td>
<td>23 %</td>
<td>50 %</td>
<td>50 %</td>
</tr>
<tr>
<td></td>
<td>Seed range per trap c</td>
<td>0—5</td>
<td>0—11</td>
<td>0—5</td>
</tr>
<tr>
<td></td>
<td>Mean seeds / trap d</td>
<td>0.4 (1.0)</td>
<td>1.7 (3.0)</td>
<td>0.9 (1.4)</td>
</tr>
<tr>
<td></td>
<td>Nearby fruiting plants e</td>
<td>0.0 (0)</td>
<td>1.2 (0.6)</td>
<td>3.6 (2.1)</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>n.c.</td>
<td>0.49**</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>Guzmania</em></td>
<td>Total of seeds</td>
<td>124</td>
<td>287</td>
<td>227</td>
</tr>
<tr>
<td></td>
<td>Traps with seeds</td>
<td>70 %</td>
<td>93 %</td>
<td>92 %</td>
</tr>
<tr>
<td></td>
<td>Seed range per trap</td>
<td>0—57</td>
<td>0—56</td>
<td>0—66</td>
</tr>
<tr>
<td></td>
<td>Mean seeds / trap</td>
<td>4.1 (10.3)</td>
<td>9.6 (12.1)</td>
<td>18.9 (23.4)</td>
</tr>
<tr>
<td></td>
<td>Nearby fruiting plants</td>
<td>0.3 (0.2)</td>
<td>2.8 (0.9)</td>
<td>8.7 (4.0)</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>0.37*</td>
<td>0.69***</td>
<td>0.58*</td>
</tr>
<tr>
<td><em>Tillandsia</em></td>
<td>Total of seeds</td>
<td>43</td>
<td>393</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Traps with seeds</td>
<td>17 %</td>
<td>40 %</td>
<td>25 %</td>
</tr>
<tr>
<td></td>
<td>Seed range per trap</td>
<td>0—4</td>
<td>0—260</td>
<td>0—56</td>
</tr>
<tr>
<td></td>
<td>Mean seeds / trap</td>
<td>1.4 (2.7)</td>
<td>13.1 (48.7)</td>
<td>6.3 (16.0)</td>
</tr>
<tr>
<td></td>
<td>Nearby fruiting plants</td>
<td>0.5 (0.2)</td>
<td>2.5 (0.8)</td>
<td>2.4 (1.2)</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>n.s.</td>
<td>0.65***</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>Werauhia</em></td>
<td>Total of seeds</td>
<td>20</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Traps with seeds</td>
<td>43 %</td>
<td>40 %</td>
<td>29 %</td>
</tr>
<tr>
<td></td>
<td>Seed range per trap</td>
<td>0—4</td>
<td>0—4</td>
<td>0—3</td>
</tr>
<tr>
<td></td>
<td>Mean seeds / trap</td>
<td>0.7 (0.9)</td>
<td>0.8 (1.2)</td>
<td>1.2 (1.4)</td>
</tr>
<tr>
<td></td>
<td>Nearby fruiting plants</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.8 (0.4)</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>n.c.</td>
<td>n.c.</td>
<td>0.61*</td>
</tr>
</tbody>
</table>
Seedling survival and growth

Seedling survival of epiphytic bromeliads was significantly affected by the type of forest habitat (Table 3). Significant differences in survival were detected nine months after seed sowing (end of the rainy season, \( P=0.029 \)) and at the end of the experiment, one year after seed sowing (\( P=0.047 \)). At the end of the rainy season, the overall percent of surviving seedlings was higher inside the forest (31.3\%) compared to edges (22.6\%) and isolated trees (28.3\%) which did not differ among each other. Three months later, at the end of the experiment, the forest interior had more surviving seedlings (27.7\%) than edges (20.1\%), while isolated trees showed an intermediate survival rate (23.3\%). Two trees were knocked down during heavy storms, one from a forest edge and the other was a pasture tree; excluding these trees from the analyses did not change the final outcome.

Species exhibited significant and constant differences in their survival likelihood (Table 3, Fig. 2). *Tillandsia fasciculata* exhibited the highest survival across all three habitats. At the end of the experiment, the percent of surviving seedlings of the latter species ranged from 37—48\% compared to 13—19\% in *C. nutans* and 9—16\% in *G. monostachia*. The survival rates of the last two species were not statistically different. Variation within habitats (interior sites and forest edges) in seedling survival rates, including the tree effect, was also significant (\( F_{5, 46} =24.8, P<0.001 \) and \( F_{5, 46} =10.4, P<0.001 \), respectively).

Species reacted in different ways. *Catopsis nutans* and *G. monostachia* showed a steeper decline in the number of surviving seedlings during the first three months as compared to *T. fasciculata* (Fig. 2). Species decreased to 51.3, 42.7 and 70.9 percent of the original sample during that period. At this stage of the experiment, seedling mortality of each species was independent of their initial size (chi-square tests, \( P\)-values>0.05). After a year, no differences among habitats were found for *C. nutans* and *G. monostachia* (\( P>>0.05 \) after Contrast analyses). For *T. fasciculata*, the forest interior recorded more surviving seedlings...
(P=0.038) than the edges at the end of the rainy season, although it did not differ from pasture trees. However, this difference disappeared by the end of the experiment.

Table 3. Results of the Factorial ANOVA on seedling survival of epiphytic bromeliads transplanted into different forest habitats (Forest interiors, edges and pasture trees). Analyses were performed at three different stages of the experiment.

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>MS</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start of the rainy season</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td>2</td>
<td>796.1</td>
<td>2.3</td>
<td>0.11</td>
</tr>
<tr>
<td>Species</td>
<td>2</td>
<td>6164.8</td>
<td>16.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Habitat x species</td>
<td>4</td>
<td>82.7</td>
<td>0.2</td>
<td>0.95</td>
</tr>
<tr>
<td>Error</td>
<td>171</td>
<td>363.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of the rainy season</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td>2</td>
<td>882.3</td>
<td>3.6</td>
<td>0.029</td>
</tr>
<tr>
<td>Species</td>
<td>2</td>
<td>8298.2</td>
<td>34.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Habitat x species</td>
<td>4</td>
<td>60.2</td>
<td>0.2</td>
<td>0.91</td>
</tr>
<tr>
<td>Error</td>
<td>171</td>
<td>243.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of the experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td>2</td>
<td>719.6</td>
<td>3.1</td>
<td>0.047</td>
</tr>
<tr>
<td>Species</td>
<td>2</td>
<td>7788.0</td>
<td>33.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Habitat x species</td>
<td>4</td>
<td>40.8</td>
<td>0.2</td>
<td>0.95</td>
</tr>
<tr>
<td>Error</td>
<td>171</td>
<td>231.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Seedling growth was significantly affected by habitat type (F_{2, 486} =8.2, P<0.001) and the response also differed among the species (F_{2, 486} =4.8, P=0.009). Consistently, in forest interiors seedlings grew significantly more, followed by pasture trees and forest edges (Fig. 3). *Guzmania monostachia* showed the highest growth increment, but this was only significant when compared to *T. fasciculata* in pasture trees (Fig. 3).
Figure 2. Seedling survivorship of three epiphytic bromeliads transplanted into different forest conditions in a premontane area, Costa Rica. Data are un-transformed means for the accumulated proportion of surviving seedlings over the experimental period, with vertical bars = 1 SE. Curves represent the habitats: forest interior (empty circles), forest edges (filled circles) and pasture trees (squares).

Figure 3. Relative growth of transplanted seedlings of epiphytic bromeliads into different forest conditions after 12 months. Shown are mean values with vertical lines representing 1 SE. Letters above bars show among-habitat comparisons per species and letters inside the bars show among-species comparisons per habitat.
Canopy temperature and humidity conditions

The daily cycles of temperature and humidity followed a very similar pattern in the three forest habitats and the magnitude of the peaks and valleys of the curve (i.e., the maximum and minimum values) significantly changed with the season (Fig. 4). Generally, both variables showed an opposite behaviour, which was more evident during the dry season. The highest temperature peaks, which usually occurred just after mid-day, coincided with the lowest values of relative humidity; conversely, the decrease in temperature during the afternoon and night was associated with an increase in humidity. Maximum temperatures were higher at the end of the dry season ($F_{1, 438} = 66.9, P < 0.001$); and the minimum values showed their lowest records during that same period ($F_{1, 438} = 195.2, P < 0.001$). Therefore, the daily fluctuations in temperature were wider during the dry season ($F_{1, 438} = 73.5, P < 0.001$, Fig. 5A). Pasture trees registered higher maximum temperatures, which accounted for their higher amplitude in the daily fluctuations (Fig. 5A). Inside the forest, the temperature change was less drastic and the forest edges were intermediate (Fig. 5A). During the beginning of the rainy season, the three forest habitats experienced lower and similar temperature fluctuations (Fig. 5A).

Figure 4. Daily cycles of air temperature and relative humidity at three forest habitats (interiors, edges and pasture trees) in a premontane area, Costa Rica. Data are averaged values recorded at 10-minute intervals during two 4-day periods at (1) the middle of the dry season (graphs on the left) and (2) beginning of the rainy season 2003 (graphs on the right).
The mean relative humidity, as expected, significantly increased during the beginning of the rainy season ($F_{1, 438} = 195.2, P < 0.001$) due to a raise in the minimum values, from $63 \pm 1.1\%$ (in the dry season) to $83 \pm 2.2\%$. Similar to temperature, the daily fluctuations were higher during the dry season ($F_{1, 438} = 182.6, P < 0.001$, Fig. 5B). Forest edges and pasture trees experienced higher changes in daily humidity compared to the forest interiors (Fig. 5B); however this difference disappeared as soon as the rainy season started.

**Discussion**

In the study area, bromeliad densities are generally lowest in the forest interior as compared to the more exposed forest edges and pasture trees for all investigated genera (Table 2). Species such as *G. monostachia, T. fasciculata* and *C. nutans* are generally common in secondary forest patches, on pasture trees and in living fences around the studied site. This is surprising since the more stable climatic conditions in the forest and the protection offered by the surrounding trees during occasional storms (i.e. Lovejoy *et al.* 1986, Saunders *et al.* 1991, Murcia 1995) would seem to enhance epiphyte survival inside the forest as compared with the edge of the forest and with solitary trees. However, the increased wind speed after forest alteration is also likely to increase seed transportation. The amount of seeds
dispersed and the probability of landing in a “safe site” have been assumed to represent the bottleneck in the establishment of epiphytes (Hietz et al. 2002). High winds may not only dislodge more seeds from the seed pods, they may also increase the airborne time, which would enhance the probability to collide with a suitable bark surface area. The seed input in forest edges and on pasture trees may, moreover, be enhanced because there is less or no surrounding vegetation that filters out airborne seeds.

In our study we found that forest interiors indeed recorded a lower influx of bromeliad seeds compared to the altered forest habitats. This difference between habitat types is, however, not due to differences in long distance dispersal, but can largely be explained by the local variation in the distribution of reproductive plants. For most of the genera in each forest habitat, there was a positive and significant correlation between the number of fruiting plants in the vicinity of a trap and the number of seeds on it (Table 2). Thus, dispersal over distances of more than 10 m is a relatively rare event, contributing little to the seed rain. Seed dispersal experiments (i.e. García-Franco & Rico-Gray 1988) and correlative studies of seedling distribution also point to the prevalence of short-range dispersal in epiphytic bromeliads (i.e. Benzing 1978, Hietz & Hietz-Seifert 1995, Zotz 1997, van Dunné 2001). Short distance dispersal contributes to the aggregated distribution of epiphytes, which is well documented for natural and secondary forest habitats (Benzing 2000 and references therein, Nieder et al. 2000, van Dunné 2001, Chapter 2). Nevertheless, we also documented the existence of “long-distance” dispersal events at a local scale. For instance, seeds of Weranuahia and Catopsis were collected inside the forest interiors where adult plants are rare or absent (pers. obs.) and no reproductive plants were present near the traps (Fig. 1, Table 2).

Seeds that are trapped on trees need to germinate, attach and survive until fruiting in order to contribute to the survival and growth of the population. Castro-Hernández et al. (1999) reported germination rates over 90% of the epiphytic Tillandsia guatemalensis under wet-season field conditions. The same study showed high seedling mortality in the following dry season, corroborating other studies that showed high seedling germination and mortality in epiphytic bromeliads (Benzing 2000). Once past the seedling stage, however, mortality drops substantially and many juvenile plants develop into the adult flowering stage. Also in our study, seedling mortality was high, particularly during the first 6 months (Fig. 2).

The fluctuations in temperature and humidity showed a decreasing trend from pasture trees to forest edges and were more buffered inside the forest (Fig. 5). Seedlings transplanted into the altered forest habitats did not experience a lower survival relative to the less disturbed conditions of the forest interiors. This despite the fact that our microclimatic data supported the initial expectation concerning the more rigorous conditions in the altered habitats but
only during the dry season. Apparently, these differences were not large enough to influence the survival rate; however, seedling growth was apparently influenced in a positive way under the conditions inside the forest (Fig. 3). The wide geographic distribution of the studied species, from southern-Florida to northern South America (Smith & Downs 1977), suggests a great versatility in adapting to different habitat conditions. They possess physiological and morphological adaptations to deal with the light exposure and water limited conditions either in the canopy inside the forest or in the edges or pasture trees. *Guzmania monostachia* is a facultative C\textsubscript{3}-CAM species (Martin 1994) and a similar condition is suspected in *C. nutans* (Benzing 2000). Both species are capable of developing an impounding tank to store water, while the CAM-species *T. fasciculata* (Griffiths & Smith 1983) has thicker leaves covered by trichomes. However, the interactions of such physiological and morphological traits with the prevalent conditions at each forest habitat is not clear enough to provide a sound explanation for the recorded differences in growth rates among habitats (Fig. 3). On the other hand, the leaf morphology and physiology of *T. fasciculata* seem to provide an advantage in survival under the fluctuating microclimatic conditions in forest edges and pasture. Yet, this has not resulted in its higher representation in the bromeliad community in these habitats (Chapter 2). Differences in growth rate, demographic turnover from seedling to flowering, breeding system and fecundity might explain more and are addressed in Chapter 6.

Apart from the species’ adaptations to microclimatic conditions, particular host-tree characteristics may account for the variation in seedling survival and growth. There is evidence that the stability of the tree-bark and its capacity to maintain moisture have a positive influence on epiphyte establishment (Castro-Hernández *et al.* 1999, Benzing 2000, Callaway *et al.* 2002). Forest habitats may differ in the relative abundance of trees with different bark types, a source of potential variation not considered here. Although, we attempted to bypass the effect of bark using a homogeneous substrate, our results are probably conservative because the carpet’s material could have retained some moisture.

In conclusion, low bromeliad densities in the forest interior as compared with forest edges or pasture trees may not be explained by higher mortality or differences in growth rates of seedlings, which is a crucial life-history phase of epiphytic bromeliads. Additional evidence indicates that germination rates and early establishment of seedlings inside the forest were not different (Chapter 3). In contrast, we found evidence for several species that the size of populations is limited by the low quantity of the seed supply that determines the establishment rate, another demographic bottleneck. The question why there are fewer fruiting adults in the forest interior, however, can not be fully answered. Even with low initial seed supply it would seem that enough time has past to establish an equally dense
population as in anthropogenically altered habitats. Increased shade within forest interiors may prevent many seedlings from reaching the fertile condition and produce the necessary seed input to maintain the population. In older forest, the unmeasured effect of arboreal mammals, such as capuchin monkeys, which trash adult rosettes in search for insects and which avoid isolated trees (pers. observ.) may help to explain the reduced abundance of adult bromeliads in that habitat.

Acknowledgments

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Chapter 5


CHAPTER 6

Reproductive Strategies and the Colonizing Ability of Two Sympatric Epiphytic Bromeliads in a Tropical Premontane Area

With Maaike de Jong, Ethan D. Borg, J. Gerard B. Oostermeijer, Jan H. D. Wolf & Joannes C. M. den Nijs

(Submitted to International Journal of Plant Sciences)

Summary

Life-history characteristics of tropical vascular epiphytes are expected to affect their colonizing ability. We studied several pre-dispersal characteristics related to growth and reproduction in two sympatric epiphytic bromeliads, Guzmania monostachia and Tillandsia fasciculata, in relation to their colonizing strategies in a premontane forest in Costa Rica. We found that these species differed in most of the analyzed traits. A higher growth rate of G. monostachia genets allows this species to reach fertility 6 y after germination compared to 13 y in T. fasciculata. Similarly, asexually produced ramets reproduced faster in the former species. Guzmania monostachia also exhibited a shorter time from flowering to seed dispersal (8-9 vs. 16-20 months). Consequently, an established and reproducing G. monostachia ramet may sexually reproduce annually by growing a new ramet, whereas a T. fasciculata individual requires at least three years to disperse a new batch of seeds. Seed production by spontaneous autogamy was high in both species; however, in natural conditions G. monostachia produced significantly more flowers (29 vs. 22) and fruits per inflorescence (89 vs. 79% fruit set) and more seeds per fruit (321 vs. 240) than T. fasciculata. The estimates of the mating system were indistinguishable from complete selfing in both species. Fixed heterozygosity in the pattern of microsatellite bands in T. fasciculata suggests polyploidy. In all, our findings indicate greater seed availability in G. monostachia, which correlates with the high rates of seedling recruitment in the studied region. Life-history traits that enhance seed availability are important for colonizing species. The community composition of epiphytic bromeliads in secondary and mature forests is likely the combined result of seed availability and dispersal, next to factors affecting adult survival and the likelihood of flowering.
Introduction

Tropical vascular epiphytes occupy unpredictable and transient substrates (Benzing 1990). Branches may detach from the host-tree or even whole trees may fall down during extreme, but not infrequent, climatic conditions in the tropics (Oberbauer et al. 1996). The instability of the substrate threatens the survival of individuals and local populations and presumes the pre-adaptation of epiphytes to recurrent colonization. Hence, the existence of variation in life-history traits among species is likely to influence their rates of seed production, seedling recruitment, and consequently, their colonizing success.

Following classical theory on plant colonization (MacArthur & Wilson 1967), in the epiphytic life the production of many easily-dispersed seeds to abet the colonization of new or empty sites is expected. Ground-rooted colonizing plant species are often self-compatible and spontaneously autogamous to compensate for the absence of conspecific mates and pollinators in newly colonized habitats (Baker 1955, Opler et al. 1980). While these mechanisms guarantee the supply of seeds for population growth and colonization, the degree to which they are expressed influences the amount and distribution of genetic variation. Autonomously autogamous, primarily inbreeding species are expected to have low genetic variation and higher population subdivision due to reduced gene flow and drift in comparison to out-breeders (Loveless & Hamrick 1984). As consequence, seed production success in any plant in terms of quantity and (genetic) quality of the progeny depends upon the combination of several factors acting before (i.e. growth rate) and during the flowering period, such as the pollination mechanism, the self-(in)compatibility system, and the fruit maturation process. The available data on the reproductive success of vascular epiphytes and its relation to their presumed colonizing capacity is inadequate, yet epiphytes contribute to the diversity of neotropical humid forests (Kress 1986, Gentry & Dodson 1987a, b, reviewed by Wolf & Flamenco-S. 2003).

The Bromeliaceae family is a highly diverse group (ca. 2450 species) restricted to the New World Tropics (Smith & Downs 1974, 1977, 1979) and most species in the subfamily Tillandsioideae are obligate or facultative epiphytes. The reproductive biology of most of them is unknown. Relatively recent field studies are starting to document a tendency toward autonomous autogamy in Tillandsioid species (Martinelli 1994, Bush & Beach 1995, Chapter 7), although some exceptions have also been documented (García-Franco & Rico-Gray 1991). The scanty reports on the breeding system of bromeliads are based on inferences from population genetic data in a few Tillandsia species: *T. ionantha* and *T. recurvata* (Soltis et al. 1987) and *T. achyrostachys* var. *achyrostachys* (González-Astorga et al. 2004).
This study evaluates the contribution of life-history traits to the reproductive success and colonizing ability of two sympatric epiphytic bromeliads in a premontane forest in Costa Rica: *Guzmania monostachia* and *Tillandsia fasciculata*. The breeding system of both species was determined by analyzing progeny arrays from maternal plants with microsatellite markers. Both species are found growing together in different forest habitats from young successional vegetation to mature forest in the study region and exhibit different colonizing strategies, as suggested by differences in abundance and rates of seedling recruitment in different successional forest stages (Chapter 2). Previous experiments on seedling establishment suggested that recruitment limitation is partially responsible for the differences in species presence and abundance between habitats (Chapter 3). Here, we focus on the role of the following pre-dispersal stages in the reproductive success of both species: (i) plant growth, (ii) flower production, (iii) flowering behavior at the individual and population level, (iv) levels of autonomous self-pollination and self-compatibility, (v) levels of fruit set and seed production, and (vi) the mating system.

**Materials and Methods**

**Study site and species**

Fieldwork was carried out in the mixture of secondary and mature forest areas intermingled with pasture and early successional vegetation patches that occur in the Upper San Luis River Valley, in the Pacific slope of the Tilarán mountain range in the northwestern part of Costa Rica (latitude: 10˚ 17’ N, longitude: 84˚ 47’ W). The area is considered as humid premontane (bmh-P) according to Holdridge’s life zone system (Haber 2000). Weather data from the Ecolodge San Luis and Biological Station (1050 m) indicate a mean annual rainfall of 3300 mm and a temperature range from 17-25 °C, with a dry season from December through April.

*Guzmania monostachia* (L.) Rusby ex Mez and *Tillandsia fasciculata* Sw. (Bromeliaceae, Tillandsioideae) are epiphytic bromeliads with a wide geographic range, from southern Florida to South America and the Antilles (Smith & Downs 1977). The species exhibit sympodial branching with determinate ramets and usually 1-3 offshoots arise from the leaf axils of flowering ramets. The rosette leaves form a moderate foliar water impoundment, of which the storing capacity is larger in *G. monostachia* than in *T. fasciculata*. Physiologically, *Tillandsia fasciculata* appears an obligate CAM-species in contrast to *Guzmania monostachia*, which is C₃-CAM (Benzing & Renfrow 1971, Griffiths & Smith 1983, Martin 1994, Benzing 2000). Flower morphology is clearly different between both species. The flowers of *G. monostachia* are short and white (length: 2.8 ± 0.1 cm, N=25),
and have inserted reproductive organs, and the stigma is capped by the whorl of anthers. In *T. fasciculata*, the corolla is longer and purple (length: 6.5 ± 0.2 cm, N=22) and the anthers and stigma are exerted from the tube (Fig. 1). Herkogamy was rare in *T. fasciculata*; generally, the receptive stigma and ripe anthers were positioned more or less at the same level. Refer to Table 1 for additional ecological traits of the species.

In the study region, *G. monostachia* shows an aggressive colonizing behavior. It is more abundant in secondary forests and shows high rates of seedling recruitment, whereas *T. fasciculata* is more dominant in mature forest and has low rates of recruitment (Chapter 2). For convenience, we will refer to the studied species by their genus name throughout this paper.

**Figure 1.** *Plant habit and flower drawings of Guzmania monostachia (A, D) and Tillandsia fasciculata (B, D). Bar scales = 1 cm*
Table 1. Ecological and morphological characteristics of two sympatric epiphytic bromeliads in a premontane area, Costa Rica. Standard deviations in parenthesis.

<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat preference</th>
<th>Seedling form</th>
<th>Metabolic pathway</th>
<th>Adult rosette diameter (cm)</th>
<th>Longest leaf length (cm)</th>
<th>Inflorescence length (cm)</th>
<th>Flowers per inflorescence</th>
<th>Flower size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. monostachia</em> (L.) Rusby ex Mez</td>
<td>early &amp; mid-succession</td>
<td>tank</td>
<td>C₃ – CAM</td>
<td>41.1 (11.4)</td>
<td>31.2 (7.6)</td>
<td>25.8 (4.6)</td>
<td>28.6 (10)</td>
<td>2.8 (0.1)</td>
</tr>
<tr>
<td><em>T. fasciculata</em> Sw.</td>
<td>mid-succession &amp; mature forest</td>
<td>atmospheric</td>
<td>CAM</td>
<td>31.2 (5.4)</td>
<td>22.9 (3.2)</td>
<td>24.4 (3.2)</td>
<td>20.5 (8.7)</td>
<td>6.5 (0.2)</td>
</tr>
</tbody>
</table>
Growth

Growth rate differences between species were examined on genets (i.e. single rosettes originating directly from seed) and vegetatively produced side-rosettes (hereafter referred to as ‘ramets’) distributed over 30 trees in an early (8-12 y) and a mid-successional (35-40 y) forest area. In 2001, we marked 374 genets with plastic tags (c. 7 x 12-15 mm) attached with thin plastic-coated wire tied loosely around the rosette. For small plants (c. 0.5 cm in size), the tag was attached around the branch. One year later (2002), we measured their size as the maximum leaf length from the rosette base to the leaf tip. In that same year, we marked 866 additional genets. In 2003, we measured all plants a second time. Over the monitoring period, fifty percent of the marked genets (mostly seedlings) were missing due to dislodgement, tag removal or relocation problems. We pooled the data from each of the two census years and calculated the relative growth of each individual as 

\[
\frac{(\text{size}_{(t+1)} - \text{size}_{(t)})}{\text{size}_{(t)}}
\]

Then, rosettes were categorized into seven size classes of five centimeter intervals before comparing mean growth rates between species and size classes with a Factorial Analysis of Variance.

The relationship between the size of genets in two successive years was estimated with a non-linear regression by means of the Nonlinear Estimation Module of STATISTICA © (StatSoft Inc. 2000). A growth model was computed for each species using the regression equation and setting the initial size (year 1) at 0.5 cm, which usually represents the size of a seedling <1 y old (ACM, pers. observ.). The final dataset on genet growth comprised 638 observations for Guzmania and 275 for Tillandsia. The growth of vegetative ramets developing from the previously marked genets was analyzed with a student t-test by comparing their mean annual growth (217 Guzmania and 57 Tillandsia).

Flowering

Floral traits, related to the number of flowers per inflorescence, inflorescence size measured from the base of the peduncle to the uppermost part of the main axes, and the number of lateral branches (spikes) per inflorescence was measured on a sample of 109 Guzmania and 105 Tillandsia.

We followed the flowering behavior of individual plants (34 Guzmania, 32 Tillandsia) which were taken to a shade-house constructed in the study area, at the premises of the San Luis Biological Station. We recorded (i) the number of open flowers per day, (ii) the number of days that passed between two consecutive days with open flowers and (iii) the time between the first and the last flower of an inflorescence to open. On individual flowers and fruits, we registered (a) the anthesis time, i.e. the moment a flower opened, (b) the flower lifespan, i.e. the number of hours a flower remained open, and (c) fruit maturation period, i.e. the time from flower senescence until first day of seed dispersal.
Population flowering was followed on 410 ramets (269 genets) of *Guzmania* and 148 ramets (121 genets) of *Tillandsia*, from April to October 2002. We monitored the plants along a walking transect through the entire area every five days and recorded for each individual whether it belonged to any of the following phenological stages (i) non-reproductive, (ii) with developing inflorescence, (iii) opening flowers and (ii) developing fruits. We selected genets with a minimum leaf length of 18 cm as potentially reproductive, based on previous field observations.

**Autonomous selfing and self-compatibility**

We examined the ability to produce fruits and seeds by autonomous self-pollination by excluding pollinators from 25 *Guzmania* and 12 *Tillandsia* inflorescences. We kept the plants in the shade-house and isolated the inflorescences with mesh gauze bags, which were kept from folding and touching the reproductive organs by using an aluminum-wire frame in the case of *Tillandsia*. We measured the degree of autogamy by comparing the fruit set (developed fruits / total flowers) and the number of seeds per fruit (on 2-3 ripe fruits per plant) of the manipulated plants against a random sample of naturally pollinated plants (50 *Guzmania* and 40 *Tillandsia*). We examined the differences between main factors, species and pollination mode, with a Factorial ANOVA. Specific differences between pollination modes per species were analyzed with Contrast Analyses. All statistical analyses were performed in STATISTICA ® (GLM module, StatSoft Inc. 2000).

**Breeding system**

We estimated the outcrossing rate by determining the genotypes of seedlings in progeny arrays on the basis of four microsatellite markers previously developed for both species (Boneh *et al.* 2003). Using 20 maternal plants per species from all habitats, we randomly collected two or three fruits per plant during February and March 2002 and stored the fruits in paper envelopes. Simultaneously, leaf tissue from the mother plants was collected, carefully washed with tap water and stored in plastic tubes (2 ml) containing a 6M Guanidine buffer solution (Guanidine thiocyanate, 50 mM Tris-HCL, 20 mM EDTA, pH 6.4), before sending them to the laboratory of the University of Amsterdam. For each mother plant, seeds were combined and a sample of 100 was germinated on Petri dishes with filter paper. To promote growth, seedlings were transplanted into plastic containers with a layer of soil and sprayed once with Benomyl® (1%) to prevent fungal contamination. Germination rates were high and no obvious differences among maternal families were noticed. Two to three months after germination, from 21 to 27 seedlings per maternal family were collected for DNA-isolation following Wang *et al.*’s (1993) method. The seedlings were cleaned with water and their roots removed. The leaf material from the mother plants was dried with tissue paper to remove the excess of buffer solution and cut into small thin pieces before grinding.
the tissue with liquid nitrogen. We used about 170-200 mg for genomic DNA extraction using a CTAB extraction buffer, following a slightly modified version of Doyle and Doyle’s protocol (1990). PCR reactions and product visualization followed the procedure described by Boneh et al. (2003).

Estimates of multilocus \((t_m)\) and single-locus \((t_s)\) outcrossing and the parental fixation index \((F)\) were obtained with the maximum-likelihood, mixed-mating procedure of Ritland and Jain (1981) by using the program MLRT version 3.0 (Ritland 2004). Pollen gene frequencies were constrained to equal ovule frequencies. Sampling among families was used to calculate the standard errors after 500 bootstraps.

We further examined the reproductive pathway (sexual or apomictic) of seeds from both species by means of flow-cytometer screening (Matzk et al. 2000). For each species, we sampled seeds from manually pollinated plants. After removal of the coma, 30 seeds were chopped with a razor blade in DAPI staining buffer and the extract filtered. The DNA content of nuclei (C values) was measured with a Partec PA flow cytometer. Embryo and endosperm DNA peaks can be distinguished by their height and position in the histograms owing to differences in the amount of nuclei, which is lower in the endosperm than in the embryo (Matzk et al. 2000). In diploid sexual plants the embryo is diploid and the endosperm triploid, thus the ratio of the DNA content between embryo and endosperm cell nuclei or “peak index” (Matzk et al. 2000) can be used to infer the reproductive pathway, independently of the ploidy level.

**Results**

**Growth**

_Guzmania_ rosettes originated from seeds grew significantly faster than _Tillandsia_ genets \((F_{1,899} = 24.6, P<0.001)\), especially in size classes below 25 cm (Fig. 2). The relative increase in size differed significantly between size classes in both species \((F_{6, 899} = 31.9, P<0.001)\) and showed a decreasing linear trend relative to plant size (Fig. 2).

The estimated regression equation for each species appropriately reflected the size increment between two successive years (Fig. 3A and 3B). The non linear regression fitted to the data of _Guzmania_ was: 

\[
s_{\text{year } (t+1)} = \frac{2.589 \times s_{\text{year } (t)}}{1 + 0.043 \times s_{\text{year } (t)}}
\]

\((R^2=0.87, P<0.001)\) and in _Tillandsia_: 

\[
s_{\text{year } (t+1)} = \frac{1.459 \times s_{\text{year } (t)}}{1 + 0.0128 \times s_{\text{year } (t)}}
\]

\((R^2=0.94, P<0.001)\). Using these equations we modeled the vegetative growth for both species (Fig. 3C). The time needed to reach the minimum reproductive size (i.e.
20 cm of maximum leaf length) was estimated as 6 y for Guzmania and 13 y for Tillandsia. A rosette needs 8 y in Guzmania and 14 y in Tillandsia to grow to the average size for the species (see Table 1).

Figure 2. Mean relative growth rate (RGR) of genets from Guzmania monostachia and Tillandsia fasciculata in a premontane area. Pooled data from 2001-2002 and 2002-2003 census. Data are presented according to size classes (upper limits are indicated on x-axes). Vertical lines are 1 SE.

Ramets produced vegetatively by reproductive genets showed a greater growth rate than primary genets. Guzmania ramets grew significantly faster than those of Tillandsia (t-test = 1.98, P<0.001). In less than a year, a new ramet of Guzmania reached a size of 18.5 ± 0.5 cm (mean ± SD) in contrast to 9.9 ± 0.8 cm (mean ± SD) in Tillandsia.
Figure 3. Relationship between rosette size (measured as maximum leaf length) between two successive years in (A) Guzmania monostachia (N = 638) and (B) Tillandsia fasciculata (N = 275) and (C) model growth curves for each species using the fitted regression equations indicated above graphs A and B. Plant size at year 1 was set as 0.5 cm.
Flowering

All genets of *Guzmania* produced a single-spiked inflorescence (25.8 ± 4.6 cm long ± SD) with an average of 28.6 ± 10.0 (SD) flowers. The inflorescences in *Tillandsia* consisted of a single (80%), two (14.3%) or three (5.7%) spikes. On average, the latter species produced fewer flowers per inflorescence (20.5 ± 8.7 SD; t-test = 6.33, P<0.001).

The flowering turnover and effort, both at the individual and population level, were noticeably higher in *Guzmania* (Table 2, Fig. 4). Plants of this species opened more flowers per day (1—3) in a continuous rhythm, completing their flowering period in less than two weeks (average 12 ± 3 SD days). In *Tillandsia*, the almost six-times longer flowering period per ramet (82 ± 23 SD days) was the result of a single flower being open on each day, and longer intervals of inactivity (5 ± 2 SD days). The flower life-span of *Guzmania* was also shorter (12—14 h) than that of *Tillandsia* (22—24 h), which is likely to affect the individual rates of outcrossing.

The population flowering period of *Guzmania* lasted a total of three months, with a peak between June and July (Fig. 4). Conversely, the flowering of *Tillandsia* lasted longer than the monitoring period, with a peak in July and August (Fig. 4), and finished in December (not shown). From all the potentially reproductive ramets (≥ 20 cm) monitored, a higher proportion flowered in *Guzmania* compared to *Tillandsia* (65.8 versus 22.3%, respectively).

---

**Table 2.** Summary of flowering characteristics of two sympatric epiphytic bromeliads in a premontane forest, Costa Rica. Standard deviations in parenthesis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th><em>Guzmania monostachia</em></th>
<th><em>Tillandsia fasciculata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthesis time</td>
<td>early morning</td>
<td>before dawn</td>
</tr>
<tr>
<td>Flowers open per day</td>
<td>1—3</td>
<td>1</td>
</tr>
<tr>
<td>Flower life-span (hours)</td>
<td>12—14</td>
<td>22—24</td>
</tr>
<tr>
<td>Interval between days of flower production (days)</td>
<td>0</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Flowering period (days)</td>
<td>12 (3)</td>
<td>82 (23)</td>
</tr>
<tr>
<td>Fruit maturation (months)</td>
<td>8—9</td>
<td>16—20</td>
</tr>
</tbody>
</table>
Fruit maturation also presented a remarkable contrast between the species. Fruits attained their full size within 8-9 weeks after pollination in both species. Fruit ripening and seed dispersal took place 8-9 months after flowering in *Guzmania*, coinciding with the following dry season (March-April). In contrast, fruits of *Tillandsia* required c. 16-20 months to reach the dispersal stage. This pattern explains the proportion of ramets with developing fruits that we observed at the beginning of the flowering census (Fig. 4); which represented those plants that flowered the previous year and that will disperse until the next dry season. This difference in fruit maturation time greatly reduces the amount of dispersing seeds in each dry season, but the adaptive value of the long maturation time - if it has any - is not at all clear.

Figure 4. Reproductive phenology of (A) *Guzmania monostachia* and (B) *Tillandsia fasciculata* during 2002 in a tropical premontane forest, Costa Rica. Values are the proportion of ramets developing inflorescences (open circles), flowering (solid circles) and developing fruits (squares).
Autonomous selfing and self-compatibility

When pollinators were excluded, both species showed high levels of fruit production by autonomous selfing: *Guzmania* 92.4 ± 1.7% versus 90.9 ± 2.0% in *Tillandsia* (mean ± SE). The difference was not statistically significant. Nonetheless, under natural pollination condition, plants of *Guzmania* developed significantly more fruits than *Tillandsia*: 88.9 ± 1.5% versus 78.9 ± 2.4%, respectively (F<sub>1,138</sub> = 16.3, P<0.0001; Fig. 5A).

The levels of fruit set in *Guzmania* from both pollination treatments, natural and pollinator-excluded, were similar; while in *Tillandsia* the proportion of recorded fruits per plant was significantly lower in natural conditions (F<sub>1,138</sub> = 8.3, P = 0.005, Fig. 5A). This is an interesting result considering the flower morphology of the latter species, which suggests predominant cross-pollination. There was no significant interaction between species and pollination mode (P = 0.11).

Figure 5. Effect of autogamy on fruit and seed production of the epiphytic bromeliads *G. monostachia* and *T. fasciculata* in a premontane area, Costa Rica. Data are mean values for (A) fruit set and (B) number of seeds per fruit between autonomous self-pollination (white bars) and natural pollination condition (grey bars). Vertical lines are 1SE. Letters above the bars show comparisons between pollination conditions per species and letters inside the bars show comparisons between species per pollination condition.
Seed production per fruit was significantly higher in *Guzmania* than in *Tillandsia* in both pollination conditions \((F_{1,254} = 188.8, P<0.001)\). In *Guzmania*, the number of seeds from naturally pollinated fruits \((321 \pm 10)\) and from autonomous selfing \((349 \pm 10)\) were not statistically different. In *Tillandsia*, on the contrary, fruits produced by autonomous selfing developed fewer seeds: \(209 \pm 9\) versus \(240 \pm 5\) \((F_{1,254} = 7.0, P=0.009; \text{Fig. 5B})\). In all, the results of fruit set and seed production suggest a high level of self-compatibility in both species, and place *Guzmania* as the species with the highest seed production in natural conditions at the study site.

**Breeding system**

The levels of outcrossing were indistinguishable from zero (Table 3), suggesting a high selfing rate in both species. These results are in agreement with the detected high rates of autogamous fruit set. Additionally, the estimated contribution of biparental inbreeding to the estimated selfing \((t_m - t_s)\) was not significant.

<table>
<thead>
<tr>
<th>Species</th>
<th>Families</th>
<th>Seeds</th>
<th>Loci</th>
<th>(t_m)</th>
<th>(t_s)</th>
<th>(F)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. monostachia</em></td>
<td>20</td>
<td>510</td>
<td>3</td>
<td>0.007</td>
<td>0.008</td>
<td>0.918</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.006)</td>
<td>(0.007)</td>
<td>(0.096)</td>
</tr>
<tr>
<td><em>T. fasciculata</em></td>
<td>20</td>
<td>453</td>
<td>4</td>
<td>0.028</td>
<td>0.013</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.030)</td>
<td>(0.013)</td>
<td>(0.018)</td>
</tr>
</tbody>
</table>

The majority of the assessed progeny in *Guzmania* exhibited homozygous genotypes for the loci analyzed, which correlates with the high value of the parental fixation index \((F=0.918)\) that indicated a deficiency of heterozygotes. The allelic segregation of the few heterozygous loci (\(e6b\) and \(CT-5\) in mother-plant 5 and \(e19\) in mother-plant 20) in *Guzmania* approximated that expected for a diploid selfing species under Hardy-Weinberg
assumptions (Fig. 6). On the contrary, most of the offspring genotypes in *Tillandsia* were heterozygous, which correlated with the low $F$-value = 0.001 and in all cases where the mother was heterozygous, the offspring genotypes were identical to the maternal genotype ("fixed heterozygosity") (Fig. 7).

**Figure 6.** Gel (locus e6b) of *Guzmania monostachia* showing the genotypes of three maternal plants (MP) each with six offspring. On the left, segregation of a heterozygous maternal genotype in the offspring after selfing; in the centre, a homozygous selfed mother, and on the right, a homozygous mother with a heterozygous offspring from out-crossing.

**Figure 7.** Gel (locus e6b) of *Tillandsia fasciculata* showing the genotypes of five maternal plants (MP) each with six offspring. In all cases, offspring are identical to the heterozygous mother. This pattern of segregation is not expected in a diploid selfing, but is apparently possible in a polyploid species with fixed heterozygosity.
The flow-cytometer analysis ruled out an apomictic origin of seeds from both species (Fig. 8). The DNA content of endosperm cells (peak 3C) in relation to embryo cell (peak 2C) or ‘peak index’ was 1.49 for *G. monostachia* and 1.33 for *T. fasciculata* (Fig. 8) and approximate the value of 1.5 expected for sexually produced seeds.

**Discussion**

Several of the life-history traits examined in *Guzmania monostachia* and *Tillandsia fasciculata* showed significant differences that can help to explain their contrasting colonizing strategies in the studied forests. Firstly, genets of *G. monostachia* at different size classes grew much faster than those of *T. fasciculata* (Fig. 2). The growth models estimated from size increments between successive years (Fig. 3) further supported the notion that in *G. monostachia* the time from germination to flowering is much shorter compared to *T. fasciculata* (6 y vs. 13 y, respectively). Our estimate for the latter species is within the range of estimations for other *Tillandsia* species from Mexico (*T. deppeana*, *T.
Reproductive strategies in two epiphytic bromeliads

juncea, T. multicaulis and T. punctulata), which ranged from 11 – 18 y (Hietz et al. 2002). Furthermore, these results suggest that G. monostachia grows even faster than some smaller species of Catopsis (size: 15.8 cm) for which Hietz et al. (2002) estimated 9 y to reach fertility. These findings are consistent with the general perception that epiphytes grow slow; however, such long times to flowering are not what is usually expected for a colonizer plant species. On the other hand, offshoots produced by both species grew faster than genets originated from seed; which is not surprising, given that ramets receive an additional input of metabolites from their parental genet. However, ramets of G. monostachia seem to reach the reproductive size after one year and most of them flowered in between censuses during the monitoring period (ACM, pers. obs.).

In summary, an established genet of G. monostachia will flower faster while producing fast-growing offshoots that enter the reproductive condition sooner than in T. fasciculata. The lower turnover in Tillandsia might be compensated through differences in plant survival. From field experiments in the same studied forests, we found that survival rates of artificially planted seeds to the 2-y old seedling stage showed no differences between species: G. monostachia = 4.1 ± 1.7 % and T. fasciculata = 5.7 ± 1.3% (mean ± SE) (Chapter 3). In contrast, survival of established seedlings from the same species in a transplantation experiment resulted in a lower mortality in T. fasciculata after a 1-y period in the studied forests (Chapter 5). For some Tillandsia species in Mexico, high survival rates of established plants (> 2 y old) were recorded (0.90 – 0.96) during a 5 y experiment (Hietz et al. 2002). These results underscore the need for long term experiments on survival of epiphytic bromeliads.

The patterns of flowering and seed maturation were quite remarkable and are quite likely affecting the temporal variation in seed availability in both species (Fig. 4). A reproductive Tillandsia fasciculata genet takes nearly two years to complete its sexual reproductive cycle, from flowering to seed dispersal. Because vegetatively produced ramets develop slower, a given genet of this species will experience longer intervals (at least three years) between reproductive periods. In contrast, the shorter reproductive period in G. monostachia (less than a year), in combination with the annual contribution of offshoots that enter the reproductive stage, produces a more continuous year-to-year seed production at the individual level, thus increasing the reproductive population size and availability of seeds. This scenario is accentuated when the differences between the species in fruit and seed production in natural conditions are considered. In average, a naturally pollinated fruit of G. monostachia contained 34% more seeds than T. fasciculata (Fig. 5B). Translating the results of flower production, fruit set and number of seeds per fruit into the reproductive output of an average (primary or secondary) ramet in natural condition, we obtain a total of 8166 seeds in G. monostachia versus 3879 seeds in T. fasciculata.
Chapter 6

The variation in the individual flowering strategies is more likely to affect the levels of outcrossing. The number of open flowers per day and their longevity influences the plant's floral display and determines the pollinator visitation rate (Primack 1985). The shorter flowering period of *G. monostachia* and its flower morphology suggested a lower rate of outcrossing in contrast to the longer-lived flowers of *T. fasciculata*, which open over an extended period and have exerted stigma and anthers exposed to pollinators. Nevertheless, the high levels of selfing detected with the breeding system analysis (Table 3) showed that autonomous selfing is the prevalent mode of pollination in natural conditions in both species. The self-compatibility detected in our studied species seems a common reproductive trait among vascular epiphytes, and in particular in bromeliads (see Catling 1980, Lumer 1980, Bush & Beach 1986, Martinelli 1994, Chapter 7).

This study demonstrated that seed production in natural conditions of the studied species is fully independent of pollinators, as initially assumed for colonizer species. The lower fruit set under natural conditions that we observed for *T. fasciculata* (Fig. 5B) contrasts with its ability to produce fruits by autogamous means, and is possibly the effect of factors unrelated to pollination. Cockroaches were observed at night removing the pollen and anthers of several flowers after anthesis (ACM, pers. obs.). In *Werauhia gladioliflora*, another epiphytic bromeliad at the study region with self-compatibility and autonomous pollination, fruit set was reduced due to flower-bud predation by a wasp species (Chapter 7). In *T. deppeana* the low fruit set (22%) was attributed to pollinator limitation and inability to produce seeds autonomously (García-Franco & Rico-Gray 1991); this poor reproductive success together with its slow growth (Hietz et al. 2002) partially explains the restricted distribution of this endemic species. These findings underline the importance of life history traits in explaining the distribution and colonization ability of epiphytic bromeliads.

An interesting finding was the putative polyploidy of *T. fasciculata* (Fig. 7). The fixed heterozygosity could be caused by apomixis, but the flow cytometer results largely excluded this possibility, although we performed no formal experimental test (e.g. by checking seed set after stigma removal). Fixed heterozygosity or duplication of the heterozygous condition of the maternal genotype in the offspring, without apparent recombination, is known from several polyploid plant species (Richards 1997). Although polyploidy is mentioned for the Bromeliaceae, chromosome counts have yielded different results for *T. fasciculata* (Benzing 2000 and references therein). *Tillandsia fasciculata* has a wide distribution and comprises several morphologically variable subspecies (Smith & Downs 1977).

*Guzmania monostachia* is a common epiphytic bromeliad in secondary forests at the study region, showing high rates of seedling recruitment. Seventy-six percent of the
populations in young successional vegetation were seedlings or juveniles (less than 5.0 cm in size) and in mid-successional forest they comprised 49% of the total number of individuals. In contrast, *T. fasciculata* was more frequent in the mature forest where it exhibited low proportions of seedlings and juveniles (28%) (Chapter 2). Although the availability of seeds in a population is influenced by the species’ local abundance, it is clear from this study that differences in life-history traits affect the reproductive success of the species and consequently the availability of propagules. *Guzmania monostachia* clearly has reproductive characteristics expected for a colonizer species. The higher and more continuous seed production is congruent with the high rates of seedling recruitment in the studied region. The low recruitment inside the mature forest is probably the effect of limited dispersal, since we showed experimentally that the probability of seedling establishment was not limited in that habitat (Chapter 3 & 5). An important number of dislodged adult plants were found on the ground in the mature forest; however, whether this species’ abundance in that habitat is controlled by an increase in mortality or a reduced reproductive output under the more shaded conditions inside the forest needs to be determined. The lower recruitment in *T. fasciculata* in the studied region is probably due to the combination of low seed availability and dispersal. Similar to *G. monostachia*, seedling establishment of *T. fasciculata* is equally feasible in mature and secondary forest (Chapter 3 & 5) but the lower fecundity in *T. fasciculata* might significantly affect its colonization capacity.

The present study shows the influence that life-history traits may have on growth rate, flowering, fruit set and seed production per fruit. Hence, they can explain the colonizing strategies of epiphytic bromeliads by causing differences in seed availability. The main effect consists of a reduction (or increase) in the amount of propagules available to colonize empty sites or to expand the existing population, which is eventually reflected in differences in the rate of seedling recruitment. However, the colonization at a local scale is also governed by the distribution of established populations as potential seed sources, and factors (*i.e.* prevailing wind currents, landscape heterogeneity) that are affecting the non-random dispersal of propagules.

**Acknowledgements**

We thank the Ecolodge San Luis and Research Station (UGA) for their logistic support. Adan Fuentes and Cristian Lobo provided invaluable support during the field work. Rob Bregman kindly carried out the flow-cytometer analyses. This is a project funded by WOTRO, The Netherlands (Grant W85-34), in collaboration with the National Museum of Costa Rica.
References


REPRODUCTIVE STRATEGIES IN TWO EPiphytic Bromeliads


CHAPTER 7

Reproductive Biology of the Epiphytic Bromeliad
Werauhia gladioliflora in a Premontane
Tropical Forest

With J. Gerard B. Oostermeijer, Jan H. D. Wolf & Joannes C. M. den Nijs

(Published in: Plant Biology 7: 203-209, 2005)

Summary

The floral phenology, fruit and seed production, and self-compatibility of Werauhia gladioliflora, an epiphytic bromeliad with a wide range distribution, was studied in a premontane forest in the Monteverde area in Costa Rica. The species presents the pollination syndrome of chiropterophily, and it is visited by the small bats Hylonycteris underwoodi and Glossophaga commissarisi (Glossophagidae). The population flowering period extended from October to early December (end of rainy season) and seed dispersal occurred from February to April (dry season). Most plants opened a single flower per night, either every day or with one-day intervals during the flowering period. In natural conditions, the average fruit set amounted to almost half the potential output, but individual fecundity (number of seeds) remained high. Seed number per fruit and germination capacity after artificial selfing and out-crossing treatments did not differ from natural pollination conditions. Werauhia gladioliflora exhibited high levels of autonomous self-pollination and self-compatibility at the individual and population level, characters associated to the epiphytic habitat. These reproductive traits are also associated to early colonizer species, yet life-history traits such as seed dispersal, seedling establishment success and growth are likely to have a major role in determining the presence of this species in the successional vegetation patches scattered over the studied premontane area.
**Introduction**

Epiphytic plant populations display a patchy spatial distribution in tropical forests and individuals tend to aggregate in a relatively few host trees (Tremblay 1997, Benzing 2000 and references therein, Nieder et al. 2000, van Dunné 2002). Consequently, the flowering individuals are distributed in hyper-dispersed groups throughout the forest. These conditions may determine the effective pattern of gene exchange among individuals and populations. In order to cope with this situation, some epiphytes, mainly orchids, have developed specialized pollination strategies based on traplining, deception and supplying male euglossine bees with pheromones (Ackerman 1986). However, mechanisms to reduce pollinator dependency (i.e. autonomous selfing and self-compatibility) may be favored in some cases.

In epiphytic bromeliads, different groups of pollinators have been proposed, but only few studies have provided detailed information about how they affect the magnitude and quality of the resulting offspring (Benzing 2000). Field observations and inferences from floral morphology of representatives of the Tillandsioideae subfamily suggest birds and moths as main pollinators of several Tillandsia species (Gardner 1986) and bats for several representatives of the genus Werauhia (Vogel 1969, Sazima et al. 1995).

In direct association with the pollination mechanisms, the breeding system constitutes an important element of the reproductive biology of any plant species. The few studies that have been carried out on bromeliads under natural conditions suggest a prevalence of self-compatibility (Martinelli 1994, Bush & Beach 1995). Autogamy is considered advantageous in the epiphytic habitat due to the unpredictability of pollinator visitation, a limited floral display and plant isolation (Bush & Beach 1995).

Reproductive traits such as self-compatibility and autonomous selfing may also play an important role during the dispersal and establishment of the species beyond their distribution range or during (re)-colonization of new habitats (i.e. during secondary succession after forest logging) (Baker 1955, Opler et al. 1980). Although bromeliads are an important component of the regrowth forest, the mechanisms controlling the pattern and process of re-colonization and its relation to the reproductive biology are still little explored.

The present study concerns a species of the genus Werauhia (formerly Vriesea); section Xiphion, which is common in the mid- to late successional premontane and montane forests of Costa Rica. As part of a larger project on the life history strategies, breeding systems and colonization of Tillandsioid bromeliads in this region, we hypothesize that species which...
are dominant in early stages of forest succession will be relatively short-lived, with an predominantly selfing breeding system, whereas species present in mature forest or late-successional stages would be longer lived with a predominantly out-crossing breeding system. As a typical mid-successional species, we suspect that our study species, *Werauhia gladioliflora*, is intermediate or belongs to the latter type.

The specific aims of this study are to examine the floral phenology of *W. gladioliflora* at the population and individual level, the compatibility system and to investigate the components of its reproductive success in relation to modes of pollination.

**Materials and Methods**

**Study site**
This study was conducted on the Pacific slope of the Monteverde Area in Costa Rica, which is located on the Tilarán Mountain Range in the north-western part of the country (latitude: 10° 17' 10" N, longitude: 84° 47' 40" W). The forest is considered as “Tropical Montane Cloud Forest” (Nadkarni & Wheelwright 2000). The study population was located in the Upper San Luis River Watershed at about 11100 m a.s.l., near the Alondra and the Bruja creeks. The landscape is composed of riparian vegetation, small patches of successional vegetation and pastures. The area borders the Monteverde Cloud Forest Preserve and the Children’s Eternal Rain Forest Reserve. Weather conditions during the fieldwork days were variable, characterized by an increase in rainfall due to the termination of the rainy season (October-mid November), and an increase in wind velocities as part of the transition into the dry season (January through April), during which trade-winds come from the Caribbean side of the country.

**Study species**
*Werauhia gladioliflora* (H. Wendl.) J. R. Grant (= *Vriesea gladioliflora*) is an epiphytic and facultative terrestrial bromeliad, with a medium-sized rosette showing the typical tank form. In the study population, rosette diameters ranged from 54.5 to 114 cm (mean = 79.7 ± 13.7 SD, *N* = 50) and maximum leaf length ranged from 36.0 to 84.0 cm (mean = 61.2 ± 9.7 SD, *N* = 50). Most plants consisted of a single (or rarely two) rosettes, which develop a single-spike inflorescence that produces from 9 to 39 flowers (mean = 26 ± 6 SD, *N* = 50). The flowers are arranged distically and mature upwardly, opening the corolla toward the inner side of the spike. The corolla petals (3) are free (mean length = 45.7 ± 2 mm, *N* = 10), white colored with the tip maroon or purplish (Fig. 1A and B) and each petal has two scales at the base (Fig. 1C). The antipetal stamens (3) are basally adnated to the petal scales, the
alternipetal stamens (3) are free (mean length $45.7 \pm 5.7$ mm, $N = 10$) (Fig. 1C). The style is simple (mean length $33.5 \pm 1.5$ mm, $N = 10$) with a tri-lobed stigma (Fig. 1D). Fruits are dry capsules with wind-dispersed plumed seeds. The geographic distribution of *W. gladioliflora* expands from México to French Guyana, Northern Brazil and Ecuador. In Costa Rica, it grows in humid forests from sea level up to 1200 m, and it is a very common bromeliad in the Caribbean lowlands (Morales 2003). In the study site, *W. gladioliflora* has a patchy distribution, occupying natural forest edges and some isolated remnant trees in pastures, as well as small trees in living fences in the wetter areas. It is less frequent or absent in several of the early successional forest patches scattered in the area and is very rare below 1000 meters of altitude, where climatic conditions become drier.

**Figure 1.** Floral diagram of *Werauhia gladioliflora* (*A* = flower in natural position, *B* = longitudinal section, *C* = Petal with antipetal stamen adnated to the scale and alternipetal stamen free, *D* = ovary and style)

**Flower development and nectar production**

The stages of flower development were recorded on randomly selected flowers and times over a period of 32 days and comprised observations on 408 flowers. At each census day, time was recorded and flower condition classified as (1) bud enlargement (the floral bud begins to grow and emerges outward from its bract), (2) anthesis (the corolla lobes begin to spread outward), (3) open flower (corolla is fully opened, with the stamens and stigma visible), (4) corolla fading (the corolla lobes begin to curl up), and (5) flower closed (the corolla lobes are closed and begin to wither). Nectar volume of a random sample of unvisited flowers was measured after anthesis or the following morning during the 2001 season. The flowers were protected from visitors with a mesh bag and the nectar harvested by inserting a calibrated pipette in mL. Nectar concentration (expressed in % w/w sucrose) was measured with a pocket refractometer, range 0-50% (Bellingham & Stanley, Ltd., England).
Population floral phenology and individual flowering

To describe the phenological pattern of flowering at the population level we randomly selected forty plants with emergent inflorescences in two consecutive blooming seasons. During the months of October and November of 2001 and 2002, we conducted daily censuses and recorded the number of open flowers per individual. The censuses were carried out during the morning (7:00-9:00 h) when it was still possible to identify the flowers that opened the previous night. The plants were located along a non-linear transect, and at a suitable distance for observation with the naked eye or with the help of binoculars. At the individual level, we used the following parameters to describe the periodicity and magnitude of the flower production per plant (1) flowering magnitude, as the number of flowers going into anthesis in a given day, and (2) flowering frequency, as the time interval (in days) between two consecutive days of new flowers going into anthesis.

Fruit and seed production under spontaneous selfing and natural pollination conditions

In order to determine the probability of producing fruits without pollinator visits we tested for spontaneous self-pollination (autonomous autogamy). During the flowering season of 2001 and 2002, in randomly selected plants the inflorescences were bagged with mesh gauze bags to exclude natural pollinators or other visitors to the flowers (11 and 14 plants, respectively). Fruit development was recorded two months after the flowering peak (in January), when the fruits had almost reached their final size, and before dehiscence and seed dispersal. The proportion of developed fruits to the total number of flowers per inflorescence was used as a measure of the degree of spontaneous self-pollination. Simultaneously, fruit production under natural pollination condition was recorded in an additional group of plants (31 and 40 plants, respectively). Differences in the proportion of developed fruits between pollination modes were analyzed with a Nested Analysis of Variance, with flowering season nested into pollination condition (GLM module, STATISTICA; StatSoft Inc. 2000). An Arcsine-square root (or angular) transformation was performed to achieve normality of the dataset (Zar 1999).

Seed number per fruit was examined on two or three randomly selected fruits from a total of 23 (natural pollination) and 18 (spontaneous selfing) plants from both seasons. Sixty-two natural-pollinated fruits and 53 fruits from spontaneous-pollination were counted. Differences in seed number per fruit between pollination treatments were examined with the statistical procedure described for the analysis of fruit set.
Self-compatibility and effects of inbreeding on seed production and germination

To test for self-compatibility, hand pollinations were performed on an additional group of twelve maternal plants during the flowering season of 2001. Flowers were pollinated at night (18:00 to 22:00 hours) from October 10 to November 14. For self-pollinations, pollen was removed from the anthers by using the tip of a flat plastic stick, and then applied gently to the stigma of the same flower, making sure to completely cover the stigma surface. Before and after the manipulation, the treated flowers were protected using mesh cloth bags, in order to prevent visitors or flower predators. Bags were removed the following day, after corolla senescence had occurred. Writing a specific code number on the surface of the respective floral bract with a non-toxic marker identified the manipulated flowers in the inflorescence for further data collection. Self-compatibility was determined as the proportion of developed fruits to self-pollinated flowers and recorded after two to three months (on January 19, 2002) when all the fruits in the inflorescence had almost reached their final size, and before the fruit dehiscence and seed dispersal period.

As a control for possible effects of selfing on fruit set and seed number per fruit, a similar amount of randomly selected flowers in the same plants was cross-pollinated. For these cross-pollinations, the pollen-receiving flower was emasculated and pollen from one or two different plant donors was obtained during the same night. Donor pollen was collected by removing the anthers with a pair of tweezers and then mixed and applied to the stigma surface of the receptor flower with a plastic stick. Flowers were bagged before and after manipulation, and identified in the same way as in the selfing treatment. The remaining flowers on the manipulated inflorescences were left exposed to natural pollination condition. The frequencies of developed fruits among the three pollination treatments were compared using a Contingency Table Analysis.

Any effect of inbreeding on seed production was analyzed by comparing the number of developed seeds per fruit among the three pollination treatments from a sample of 65 fruits (19 from natural condition, 23 from selfing and 23 from out-crossing). Differences in number of seeds per fruit were tested by means of a One-way Analysis of Covariance, with pollination treatment as fixed effect and fruit size, measured as the length of the capsule valves, as covariate (GLM module, STATISTICA; StatSoft Inc. 2000). Similarly, seed germination rates among treatments were compared. Twelve fruits from each treatment, representing seven maternal plants were used for this analysis. A sample of 60 seeds per fruit from each pollination treatments was placed on filter paper in the same Petri dishes (one dish per plant). They were kept under normal daylight conditions in the laboratory near the study site at the Ecolodge San Luis & Research Station and the filter paper was
wetted when necessary. The number of germinated seeds was recorded every two days during four weeks. Differences in germination capacity or maximum number of germinated seeds after four weeks were analyzed with a non-parametric Analysis of Variance (Kruskal-Wallis Test).

**Potential pollinators and flower visitors**
This species, like others within the genus, presents the characteristic pollination syndrome of “chiropterophily” (Vogel 1969). In order to identify the bat species visiting the flowers of *W. gladioliflora*, we set up mist nets for brief periods (2-3 days) and from 18:00 to 22:00 h near a group of plants in each flowering season. Once the bats were captured, they were identified and the pollen on their heads was removed and placed on a glass microscope slide for comparison with pollen from the study species. In addition, occasional observations on the presence and behavior of other animals or insects visiting the flowers were recorded when performing the hand pollinations at night or during the phenology census.

**Results**

**Flower development and nectar production**
The different phases of flower development and their estimated daily duration are shown in Fig. 2. Flower development begins with the enlargement of the floral bud (6:00-7:00 h), which provokes a visible swelling of the floral bract. Floral bud growth continues for the rest of the morning, and by mid-afternoon the bud emerges from the floral bract as a dark purplish tip. Anthesis usually occurs at dusk (around 17:00-17:30 h), and begins with the bud opening and outward spreading of the petal lobes. Around 18:00 h, the flowers have already opened with the yellow pollen visible and the stigma presents a transparent droplet of exudate. Flower lifespan extends for 12-14 h after anthesis; remaining open through the night and the first hours of the next morning. Senescence starts by mid-morning when the petals begin to fade and fold up. Petals close fully during the afternoon, and then begin to decay.

Total nectar volume of unvisited flowers ranged from 100—660 µL and was similar among flowers measured directly after anthesis and those measured the next morning (*t* = -1.53, df=21, *P*=0.14; Table 1). Nectar concentration was also similar between the two groups of flowers (*t* = 1.44, df=30, *P*=0.16) and the most common measure (mode value) was 18% in both groups.
Population flowering phenology and individual flowering

The flowering season of *W. gladioliflora* occurred at the end of the rainy season (from October through December), and coincides with the second major peak of rain during the year at the study site (Fig. 3). In both years, the flowering peak period extended for several days, from mid October to mid November (Fig. 4). The maximum daily number of open flowers at the sample population was 25 in 2001 (*N* = 23 plants) and 15 in 2002 (*N* = 13.

Table 1. Nectar volume (μL) and sugar concentration (% w/w sucrose) in unvisited flowers of *Werauhia gladioliflora* after anthesis or the following morning (*N* = number of flowers; s.d. = standard deviation.)

<table>
<thead>
<tr>
<th>Time</th>
<th>Volume (μL)</th>
<th>Sugar Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Average</td>
</tr>
<tr>
<td>After anthesis</td>
<td>100-640 (N = 12)</td>
<td>362 ± 200 s.d.</td>
</tr>
<tr>
<td>Next morning</td>
<td>280-660 (N = 11)</td>
<td>469 ± 125 s.d.</td>
</tr>
</tbody>
</table>

Figure 2. *Floral events and their estimated daily duration in Werauhia gladioliflora*
plants), representing 4.4% and 3.7% of the total flower production in each season. During the flowering peak, synchronization of plants flowering at the same time and at a given day varied from 22% to 58% (9 to 23 plants) in 2001 and from 15% to 32% (6 to 13 plants) in 2002.

Figure 3. Approximate timing of reproductive events of Werauhia gladioliflora in relation to rainfall pattern during the study periods of 2001 and 2002. Weather data recorded at the EcoLodge San Luis & Research Station. Gray areas represent overlapping of events

Figure 4. Daily flower production in the study population of Werauhia gladioliflora during the 2001 and 2002 flowering seasons (N = 40 plants in both years)
At the individual level, the mean number of flowers open per day was 0.47 ± 0.17 (SD) and 0.55 ± 0.10 (SD) in both seasons. Flower display per individual can be best described in terms of the number of flowers open daily (magnitude) and their periodicity or the intervals between days with flowers going into anthesis (frequency). *Werauhia gladioliflora* plants usually opened a single flower at any one day (98%); rarely did they open two or even three flowers in the same inflorescence (Fig. 5A). This means that geitonogamy occurs only occasionally in the studied population. The frequency of flower opening per plant did not follow a continuous rhythm. Mostly, flowers were produced on a daily basis or with one-day intervals of inactivity (Fig. 5B). Longer intervals of inactivity occurred when some flowers failed to develop, altering the normal sequence of flower opening in the inflorescence and likely due to predation. The majority of plants reproduced in both years. Unlike other epiphytic bromeliads, the rosette does not decay after reproduction; instead it resumes growth by producing new leaves. In rare cases, an extra rosette is developed.

![Figure 5. Flowering magnitude (A) and frequency (B) at the individual level in Werauhia gladioliflora. Data pooled from 40 individuals during the reproductive season of 2001 and 2002. Vertical lines are 1 SE.](image)

**Fruit and seed production under spontaneous selfing and natural pollination conditions**

Plants excluded from pollinators (spontaneous selfing) significantly produced more fruits than plants exposed to natural pollination condition ($F_{1,92} = 28.19$, $P<0.001$) (Table 2). Moreover, there was a significant effect of the flowering season ($F_{2,92} = 11.10$, $P<0.001$); plants on both pollination conditions produced more fruits during the second year (Table 2). In general, fruit set ranged from 0—100% in plants exposed to pollinators and from 33.3—100% in those excluded from pollinators. Differences in the mean number of developed
seeds per fruit between both pollination conditions were not statistically significant \((F_{1,111} = 3.47, P=0.065)\). Similarly, the effect of the flowering season was not significant \((F_{2,111} = 0.86, P=0.42)\) (Table 2).

### Table 2. Results of the autonomous-selfing and self-compatibility tests on fruit set, number of seeds per fruit and seed germination in *Werauhia gladioliflora*. Shown are mean values ± 1 SE and sample size in parenthesis.

<table>
<thead>
<tr>
<th>Pollination Test</th>
<th>Fruit set*</th>
<th>Seeds per fruit</th>
<th>Germination capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autonomous selfing test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous selfing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 2001</td>
<td>67.2 ± 6.7 % (11)</td>
<td>1 757 ± 94 (32)</td>
<td>--</td>
</tr>
<tr>
<td>- 2002</td>
<td>90.8 ± 2.4 % (14)</td>
<td>1 807 ± 81 (30)</td>
<td>--</td>
</tr>
<tr>
<td>Natural condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 2001</td>
<td>47.5 ± 3.6 % (31)</td>
<td>1 871 ± 78 (23)</td>
<td>--</td>
</tr>
<tr>
<td>- 2002</td>
<td>64.7 ± 3.5 % (40)</td>
<td>2 018 ± 92 (30)</td>
<td>--</td>
</tr>
<tr>
<td><strong>Self-compatibility test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artificial self-pollination</td>
<td>97.3 ± 2.7 % (37)</td>
<td>1 564 ± 59 (23)</td>
<td>0.78 ± 0.24 (12)</td>
</tr>
<tr>
<td>Artificial out-crossing</td>
<td>94.4 ± 3.9 % (36)</td>
<td>1 617 ± 78 (23)</td>
<td>0.74 ± 0.27 (12)</td>
</tr>
<tr>
<td>Natural condition</td>
<td>87.5 ± 5.6 % (36)</td>
<td>1 679 ± 59 (19)</td>
<td>0.91 ± 0.07 (12)</td>
</tr>
</tbody>
</table>

*In the autonomous selfing test the response variable was measured on individual plants in each treatment, while for the self-compatibility test the response variable was measured on groups of flowers for each treatment. SE on fruit set for the second test were calculated as \(\sqrt{pq/(N-1)}\).

### Self-compatibility and effects of inbreeding on seed production and germination

The percentage of developed fruits among the three pollination treatments was not statistically different \((\chi^2_{2,0.05} = 3.18, P>0.10)\). Self-compatibility at the level of fruit set was high, 97% of the manually self-pollinated flowers produced fruits compared to 94% and 87% in the
out-crossed and naturally pollinated flowers, respectively (Table 2). Emasculation during the manipulation of out-crossed flowers had no affect on fruit development. Manipulated flowers that failed to develop fruits were consumed by leaf-cutter ants (*Atta* sp, Formicidae), which managed to cut the mesh bags during the night after manipulation. The number of developed seeds per fruit was similar among treatments ($F_{2, 61} = 0.54$, $P=0.58$) (Table 2). The germination capacity after 28 days was higher than 70% for all the treatments and they were not significantly different ($H_{2, n=36} = 3.18$, $P=0.20$) (Table 2). The germination curves showed similar trends (Fig. 6) and in all treatments germinated seeds were evident after ten days of sowing, reaching their maximum number by the twentieth day.

**Figure 6.** Accumulated proportion of germinated seeds of *Werauhia gladioliflora* after four weeks in laboratory conditions.

**Potential pollinators and flower visitors**

During both seasons, we captured nectarivorous bats of the species *Hylonycteris underwoodi* and *Glossophaga commissarisi* (Phyllostomidae, Glossophaginae) near flowering plants of *W. gladioliflora*. Voucher specimens (#1374 and #1373) were deposited at the National Museum of Costa Rica. The bats were carrying abundant amounts of pollen on their heads and some pollen grains were found in the feces of the second species. Comparisons with the reference sample indicated that the carried pollen was identical to that of *W. gladioliflora*; besides, no other bromeliad species with similar pollination syndrome was present in the area. Some frugivorous bats of the genus *Carollia* sp. (Carollinae) and *Sturnira ludovici* (Stenodermatinae) were captured but these were not carrying any pollen. Leaf-cutter ants (Formicidae, *Atta* sp.) were observed predating the flowers at night in plants located near their foraging routes. Usually after the floral anthesis, the ants arrived at the flowers and cut the corolla lobes and the stamens and pistil, besides taking some nectar. We observed them visiting the same plant for several days, thus causing an important reduction of the plant’s
floral display. Stingless bees (*Trigona* sp.) were actively collecting pollen from the anthers during the next morning when the flowers were still open, while several small black ants and fruit flies were taking nectar. The latter insects use the withering corollas as brooding sites for their eggs. Effective pollination by these insects seems very unlikely.

**Discussion**

**Floral morphology, phenology and pollination**
Floral morphology and nocturnal anthesis in *W. gladioliflora* clearly fit the syndrome of bat-pollination described by Faegri & van der Pijl (1979). The capture of *Hylonycteris underwoodii* and *Glossophaga commissarisi* bats carrying *W. gladioliflora* pollen confirms that assumption. The study species produces nectar in relatively large and diluted quantities, another feature of chiropterophily (Tschapka & Dressler 2002). The nectar concentration in *W. gladioliflora* is within the range of previous reports from other bat-pollinated *Werauhia* species (Sazima *et al.* 1995). When extrapolated over the entire population, the nectar production of *W. gladioliflora* is likely to represent an important food source for nectarivorous bats. The pattern of individual flowering, usually one open flower at any given day and in a discontinuous rhythm (Fig. 5A and B) is probable to encourage a trap-lining behavior of the pollinators. Bats are able to travel long distances during their feeding activities in a single night (Tschapka & Dressler, 2002); thus a significant rate of pollen flow (out-crossing) among widely dispersed individuals and populations is expected. On the other hand, the floral morphology and behavior of *W. gladioliflora* also promotes autonomous autogamy under natural conditions. Spontaneous selfing assured comparable levels of fruit set and seed production to those obtained under natural pollination conditions (Table 2).

Several bagged flowers did not have any self pollen on the stigma the following morning. At that time, the style looked robust and a Peroxidase test suggested that the stigma was still receptive. We hypothesis that self-pollination mainly occurs at the end of the flower life span (i.e. delayed self-pollination). Pollination is likely to occur when the corolla closes and the petal lobes press the anthers against the stigma.

**Fruit and seed production**
A limited floral display (number of flowers produced) has been mentioned as a characteristic of epiphytic life (Ackerman 1986). In spite of the relatively low number of flowers per plant (mean = 26), the individual reproductive output (number of seeds) of *W. gladioliflora* is high. The number of seeds per fruit (Table 2) greatly exceeds that of other bromeliad species in the area (pers. obs., ACM) or in previous reports in the literature (see references in Benzing 2000). In natural condition, an average plant develops half of the flowers into
fruits, producing nearly 23,000 seeds. This amount, when extrapolated to the sampled population (N = 40), represents an estimated output of nearly one million seeds released in a given reproductive season. A striking result was the lower fruit set recorded under natural pollination conditions considering the potential for spontaneous-selfing (Table 2). Bats are considered effective pollinators (Fleming & Sosa 1994), thus this apparent contradiction suggests that factors other than effective pollen transfer are limiting fruit set in natural conditions. No evidence of fruit predation was detected. After examining a sample of non-developed flowers, we found that almost half of them showed evidence of damage by a previously unknown wasp species belonging to the genus *Eurytoma* (Eurytomidae). The adult wasps lay their eggs on the flower bud and a single larva develops inside while mainly feeding on the immature anthers and inhibiting further floral anthesis (Gates & Cascante-Marin 2004). Herbivory by leaf-cutter ants (*Atta* sp.) are likely to account for the specific reduction in fruit set in some plants.

**Breeding system**

The results of our experimental tests showed that *W. gladioliflora* is fully self-compatible and that selfing does not affect the progeny in terms of quantity (number of fruits and seeds) and quality (seed germination). These are advantageous traits for species that colonize new habitats (Baker 1955, Opler *et al.* 1980). The ability to produce seeds without pollinator visitation may be an advantage for *W. gladioliflora* during adverse weather conditions for pollinators, for example in rainy nights, which may reduce visitation rates. During both tails of the blooming season, the reduced availability of potential mates may increase the amount of progeny from selfing. In fragmented landscapes, patchy forested areas or successional vegetation, where the populations of its natural pollinators are reduced or their activity is limited; the possibility to produce seeds by autonomous selfing may increase survival probabilities through maintenance of dispersal and germination. On the other hand, the increased inbreeding under these conditions may reduce seedling performance and lower the chances of successful establishment (Oostermeijer *et al.* 1994). Similar reports of self-compatibility in epiphytic bromeliads (Martinelli 1994, Bush & Beach 1995) suggest a widespread tendency towards selfing in the breeding system of this group. Nonetheless, the specific role of each mechanism (autonomous autogamy versus out-crossing) on seed production and progeny performance remains to be investigated.

In conclusion, *Werauhia gladioliflora* exhibits reproductive traits that correspond to an early colonizer species, such as self-compatibility, autonomous selfing and high fecundity, but may be associated with lower performance at colonized sites if there is substantial inbreeding depression. However, additional life-history traits related to seed dispersal, seedling establishment and growth may play a major role in determining the presence or absence of this species in the successional vegetation patches in the study site.
Acknowledgements

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References

Chapter 7


Summary

The potential for genetic population structuring in plants is highly influenced by the breeding system and seed dispersal mode. In colonizing species, founder events associated with colonization are suggested to increase the amount of subdivision among recently established populations. Using six microsatellite loci, we found a significant genetic population structure in the colonizing epiphytic bromeliad Guzmania monostachia in a premontane area in Costa Rica. As expected, overall levels of genetic diversity were low. Subpopulations, or groups of individuals on the same tree, exhibited high genetic homogeneity within the same forest patch or population ($F_{st} = 0.014—0.043$) and high levels of inbreeding ($F_{is} = 0.867—0.957$). When grouped by age, younger populations of G. monostachia in early-successional forest patches displayed a significantly higher genetic differentiation than older populations in mid-successional forest patches ($F_{st} = 0.222$ vs. 0.030, respectively, $P=0.054$). Irrespective of age, populations were highly genetically differentiated. Significant $F_{st}$ values of pairwise comparisons between populations ranged from 0.055 to 0.342. The results of an analysis of the spatial dependence (autocorrelation) of genetic structure indicated high genetic similarity between individuals within populations (forest patches) at distances lower than 60 m and low genetic relatedness between individuals from populations separated by distances greater than 400 m. The distribution of genetic diversity of G. monostachia at the local scale is most likely the result of high selfing rates and limited seed dispersal. Founder events and drift explain the higher genetic variance among young populations.
Introduction

Plant populations become differentiated from each other as a result of both natural selection and random processes. Factors that affect gene dispersal largely determine the extent to which genetic changes in local populations are independent (Wright 1943, Slatkin 1985). The breeding system and seed dispersal mode have a major influence on the rate of gene flow and thus contribute to the differentiation and genetic structuring among plant populations (reviewed by Loveless & Hamrick 1984, Hamrick & Godt 1989, 1996). Limited dispersal of pollen and seeds may either reduce or increase population divergence. Long distance dispersal reduces divergence, while limited dispersal promotes local genetic structure and bi-parental inbreeding further enhances population differentiation (Loveless & Hamrick 1984). In small isolated populations, inbreeding reduces genetic variation by lowering the level of heterozygosity and increases divergence in combination with drift, leading to increased population subdivision (Loveless & Hamrick 1984).

In colonizing species the repeated colonization episodes, genetic bottlenecks, founder effects, and drift play a more significant role in influencing the genetic variation and population differentiation compared to plants with other life history strategies (Barrett & Shore 1989). Recently established (young) populations of colonizing species are subjected to founder effects that lead to population bottlenecks which cause an increase in genetic drift (Whitlock & McCauley 1990). Consequently, younger populations of colonizing species may display higher levels of genetic differentiation (Whitlock & McCauley 1990). Additionally to the founding of populations by small number of individuals, the often patchy distribution of the habitat in combination with the species limited gene flow promotes the mating among genetically related individuals and is more likely to increase the levels of genetic subdivision among local populations. The information from molecular analyses provides knowledge about the distribution of genetic diversity among local populations and may be useful to estimate the importance of factors related to seed and pollen dispersal in the re-colonization of epiphytic plants.

In this study, we examined the genetic variation and differentiation within and among local populations of a colonizing epiphytic bromeliad, *Guzmania monostachia*, in a premontane area of Costa Rica. Epiphytic bromeliads frequently display an aggregated pattern of spatial distribution (Benzing 1978, Hietz & Hietz-Seifert 1995, van Dunné 2001), and although they are capable of establishing on several different tree species (Benzing 2000), their spatial distribution does not match the distribution of their potential hosts; instead they tend to aggregate on a few trees. The same pattern has been documented for *G. monostachia* in the study site (Chapter 2), which could lead to some degree of genetic
isolation between individuals inhabiting the same tree or a group of nearby host trees and other groups of individuals in a local geographic context.

Using F-statistics (Wright 1943, 1951), we compared the genetic variance of young bromeliad populations in early successional forest patches to that of older populations in mid-successional forest patches. For species that colonize new habitats, the component of genetic variance ($F_{st}$) among recently established populations is expected to be greater than that of older populations owing to the founder effect and increased drift (Whitlock & McCauley 1990). The genetic structuring within populations in the forest patches was studied by comparing the gene frequencies among groups of individuals on separate trees (subpopulations) within the same forest patch (population). Additionally, a spatial analysis approach was used to obtain an indirect estimate of the gene flow among populations in a geographical context (cf. Vekemans & Hardy 2004).

We specifically addressed the following questions (1) Do young populations of *G. monostachia* in early successional forest patches show higher levels of genetic differentiation than older populations in mid-successional forest patches? (2) Do populations (forest patch) exhibit within-patch structuring regardless of age? and (3) are the genetic correlation between pairs of individuals a function of their geographic separation irrespective of the forest successional condition?

**Materials and Methods**

**Study species**

At the study site, *Guzmania monostachia* (L.) Rusby ex Mez (Bromeliaceae, Tillandsioideae) is a common epiphyte in secondary forest patches of early and mid-successional stages while less frequent in mature forests (Chapter 2). The species has a wide geographic distribution, from southern Florida through Mesoamerica, The Antilles to South-America (Peru, Bolivia and Brazil) (Morales 2003). The tubular white flowers are hermaphroditic and partially covered by red bracts in a spike-like inflorescence. The species is self-compatible, with a high rate of spontaneous self-pollination and fruit set in natural conditions (c. 90%). Additionally, an analysis of outcrossing rates with molecular markers showed high levels of inbreeding (Chapter 6). Seeds are small and have a hairy appendage (coma) adapted for wind-dispersal, increasing the chances of long-distance dispersal. Seed trapping experiments and the aggregation of seedlings around adult plants, however, indicate that dispersal is mostly short-range (Chapter 2 & 4)
Study location
The Upper San Luis River Valley is located on the Pacific slope of the Tilarán mountain range in the northwest of Costa Rica (latitude: 10° 17’ N, longitude: 84° 47’ W). The area is classified as humid premontane forest (bmh-P) according to Holdridge’s life zone system (Haber 2000) and borders the Monteverde Cloud Forest Preserve located on the near mountain ridges. The studied populations of *G. monostachia* were located in the surrounding of the Ecolodge San Luis and Research Station (UGA) (Fig. 1), a 62-ha private property on a former cattle farm (1000-1050 m). The station manages several areas representing a heterogeneous mosaic of vegetation patches of different ages, shapes and sizes; randomly distributed in the landscape and interspersed with active pastures and mature forest fragments; a common condition in the study area.

Sampling
We defined the group of individuals within a forest patch as a ‘population’ and the group of individuals within a tree as a ‘subpopulation’. Populations of *G. monostachia* were sampled in secondary forest patches of different successional age, which are easily recognized by their structure and tree species composition. The classification between “young” and “older” populations of *G. monostachia* was based on the successional stage of the forest patch. Young populations were located in forest patches of early successional stage, about 10—15 y old and with a maximum canopy height <6 m. The most common tree species were *Acnistus arborescens* (Solanaceae), *Sapium glandulosum* (Euphorbiaceae), *Croton draco* (Euphorbiaceae), and *Psidium guajava* (Myrtaceae). Older *G. monostachia* populations were found in forest patches of mid-successional stage, about 35—40 y and with a maximum canopy height <20 m. These forest patches were dominated among others by *Cordia eriostigma* (Boraginaceae), *Hasseltia floribunda* (Flacourtiaceae), *Inga* spp. (Fabaceae), and *Lonchocarpus oliganthus* (Fabaceae) trees. We studied three populations (forest patches) per age condition (successional stage) and denoted them by letters: A to C (young populations) and D to F (older populations) (Fig. 1). The forest patches (<1 ha in size) were located on abandoned pastures or agricultural fields and spread over a one-squared kilometer area and separated from each other from 140 to 1036 m (mean = 483 ± 243 SD). At each population (forest patch), three randomly selected subpopulations (each on a different tree) were sampled for bromeliad genetic material. Distances between subpopulations within the forest patches ranged from 11 to 55 m (mean = 26.7 ± 12.2 SD). Plant leaf tissue was collected from 18 to 22 individuals (2.0—43.0 cm in size) per subpopulation (tree). Leaves were carefully washed with tap water and stored in plastic tubes (2 ml) containing a 6M Guanidine buffer solution (Guanidin thiocyanate, 50 mM Tris-HCL, 20 mM EDTA, pH 6.4). Samples were stored at 4 °C before they were processed at the IBED laboratory (University of Amsterdam).
Laboratory procedures

Leaf samples were dried with tissue paper to remove excess buffer solution and cut into small, thin pieces before grinding with liquid nitrogen and sterile sand. About 170-200 mg was used for genomic DNA extraction using a CTAB extraction buffer following a slightly modified version of Doyle and Doyle’s protocol (1990). We used six microsatellite primers, five of them (CT-5, e19, e6, e6b and p2p19) previously reported for this species (Boneh et al. 2003) and an additional primer, CA-21. The loci amplified consist of di- or tri-nucleotide repeats: GA\( _{25} \), CT\( _{15} \), CAA\( _{14} \), CAA\( _{12} \), GAA\( _{13} \) and CAA\( _{16} \) respectively and vary in size from 151 bp to 204 bp (Boneh et al. 2003). DNA material was amplified by using PCR-reactions and followed the general procedure described by Boneh et al. (2003). PCR-products were visualized on a 6.5% KB+ LiCor polyacrylamide denaturing gel on a LiCor 4200 ® automatic sequencer. Between 0.5-0.7 µl of a mixture of 1/5 PCR-products and 4/5 loading dye (Deionised Formamide with Bromophenol-blue) was loaded into the gel.
Analysis of genetic variation

We estimated and compared the genetic variation of *G. monostachia* among young and older populations in early and mid-successional forest patches respectively. The following standard statistics were calculated: (i) the percentage of polymorphic loci, $L_p$, (ii) the average number of alleles per locus, $A$, (iii) Nei’s (1973) index of gene diversity averaged over loci, $H_s (= 1 - \sum p_i^2$), where $p_i$ is the allele frequency), which represents the proportion of heterozygotes per locus, (iv) the observed heterozygosity, $H_o$, and (v) Wright’s (1951) fixation index, $F_{is}$.

The number of multilocus genotypes ($G$) was determined for both age conditions (young and older) and for each population (forest patches) in both successional conditions. We calculated the frequency of individuals per multilocus genotype and calculated the multilocus genotypic diversity per population age group as $H_g = 1 - \sum p_i^2$, where $p_i$ is the frequency of genotype $i$.

Analysis of genetic structuring within populations

We examined the possible genetic structuring within populations (forest patches) in the two successional forest stages by means of two-level hierarchical analyses of $F$-statistics (Weir & Cockerham 1984). We were interested to determine whether groups of individuals in the same tree (subpopulations) are genetically different from those in other trees in the same forest patch (population). Following the terminology of $F$-statistics, $F_{is}$ is the correlation of genes within individuals relative to the subpopulation (tree), $F_{st}$ is the correlation of genes within subpopulations relative to the population (forest patch) and $F_{it}$ is the correlation of genes within individuals relative to the population (forest patch).

Analysis of genetic differentiation among populations

We investigated the magnitude of population genetic differentiation in *G. monostachia* from two perspectives. First, we compared the level of genetic subdivision among young and older populations by estimating their respective $F_{st}$-values. Here, $F_{st}$ statistics represent the correlation of genes within populations relative to the population age group (young and older populations). Due to founder events and drift we expect a higher genetic variance among young populations than among older populations. We evaluated the significance of the difference between the two groups by means of a randomization approach. Sampled populations were assigned at random to each of the two age groups, keeping the original size constant and the $F_{st}$ difference was calculated for each permutation. A null distribution of differences was created by performing 1500 permutations to test the hypothesis: $F_{st}$ (young populations) > $F_{st}$ (older populations) against the null hypothesis of equal or smaller $F_{st}$ values of younger versus older populations.
The second perspective of genetic differentiation analyzed the subdivision of *G. monostachia* populations in a local geographic context independently of their successional (age) condition. We calculated the $F_{st}$ values of all possible paired comparisons between populations. To test the statistical significance, a null distribution of $F_{st}$ values was generated by permuting multilocus genotypes between the two populations (not assuming Hardy-Weinberg equilibrium among samples) with 1500 randomizations. A Bonferroni correction for multiple comparisons was performed for the nominal p-level of 5%. All computations and significance tests of genetic structuring within and among populations were obtained by means of the program FSTAT version 2.9.3 (Goudet 1995, 2001).

**Analysis of genetic correlation in a spatial context**

The genetic relatedness between all individuals in relation to their geographic position was analyzed by means of spatial autocorrelation methods (Sokal & Wartenberg 1983). Genetic exchange through pollen and seed dispersal is expected to occur among subpopulations (trees) within and among populations (forest patches), regardless of their age condition. Thus, for this analysis we have assumed that genetic relatedness among pairs of individuals is only a function of their spatial position. Genetic similarity among individuals is expected to decrease with increasing spatial separation. Subpopulations were mapped to calculate the geographic distance among all pairs of individuals within and between populations. Individuals within the same subpopulation (tree) where given the same geographic position, even though their height in the tree sometimes differed considerably.

The genetic relatedness between pairs of individuals located at specific distance intervals was computed by means of a coancestry coefficient, $\rho_{ij}$ (Loiselle *et al.* 1995). This coefficient measures the correlation in the frequency of homologous alleles, $p_i$ and $p_j$, at a locus in pairs of mapped individuals, $i$ and $j$ (Loiselle *et al.* 1995). Coancestry values, $\rho_{ij}$, were obtained by summarizing data on all possible pairs of individuals and loci for a given distance interval. A mean value was calculated by combining over loci after weighting the result of each locus by its polymorphic index, $\sum p_i (1 - p_i)$, to obtain a multilocus measure of spatial genetic structure (Loiselle *et al.* 1995).

Tests of significance for the estimated values of $\rho_{ij}$ were performed using randomization procedures. For a given distance interval, 999 permutations were performed by randomizing the spatial positions of individuals, in order to generate a random distribution of coancestry values to test the hypothesis of no spatial distribution. The null hypothesis of $\rho_{ij} = 0$ was rejected when the frequency of randomized values $>|\rho_{ij}|$, the calculated value from the actual data, was higher than $\alpha = 0.05$. Computations were performed in the program SPAGeDi 1.1 (Hardy & Vekemans 2002).
The distance intervals were chosen to include pairwise comparisons of individuals located on separated subpopulations (trees) within the same population (forest patch) at 20, 40 and 60 m apart. Pairwise comparisons of individuals from subpopulations located in different populations are represented by distance intervals of 200, 400, 600, 800 and 1100 m. The initial class (distance zero) represents comparisons of individuals located in the same subpopulation (tree). Global tests of significance of correlograms for each locus were based on detecting at least one significant autocorrelation statistic by comparing the p-value for each distance interval to a Bonferroni-corrected significance level (dividing the nominal level, $\alpha = 0.05$, by the number of distance classes: 8 (Legendre & Legendre 1998).

Results

Genetic variation

Guzmania monostachia populations in early and mid-successional forest patches had similar levels of genetic variation (Table 1). Two of the microsatellite loci ($e6$ and $p2p19$) were consistently monomorphic and locus $CA-21$ was fixed for the same allele in one population from each successional stage (populations A and D). The proportion of observed heterozygous genotypes was quite low in both population groups ($H_o = 0.034$ and $0.021$, respectively) and these estimates were clearly lower than the proportion of expected heterozygotes under the observed allele frequencies or gene diversity ($H_s = 0.231$ and 0.252, respectively). The fixation index was very high in both groups ($F_is > 0.90$), suggesting a non random association of gametes.

The multilocus genotypic diversity was similar between young and older populations of $G. monostachia$ ($H_g = 0.899$ and 0.882 respectively). In overall, 39 multilocus genotypes were identified and the three most abundant ones represented almost half of the sampled individuals (178/360). The most common multilocus genotypes were homozygous for all the loci and their distribution among populations showed an aggregated pattern (Fig. 2), which was significantly different from an expected random distribution (3 x 6 contingency table, $\chi^2$-value = 53.9, P<0.001).

Genetic structure within populations

Guzmania monostachia did not display any significant genetic structuring within populations, irrespective of their successional age. The gene frequencies among subpopulations (trees) in the same population (forest patch) were similar ($F_st$ values not statistically different from zero), except for population D in a mid-successional forest patch (Table 2). $F_st$-values among subpopulations ranged from -0.014 to 0.006 in young populations and from 0.011 to
0.043 in older populations. There was a high genetic relatedness ($F_{is}$) between individuals within the same subpopulation. $F_{is}$-values calculated among individuals growing within the same tree ranged from 0.789 to 1.000 in young populations and from 0.624 to 1.000 in older populations (Table 2). The genetic correlation of two genes (loci) taken at random from the same population (forest patch, $F_{it}$) was also high, ranging from 0.797 to 1.000 in young populations and from 0.730—1.000 in older populations (Table 2).

**Genetic differentiation among populations**

The genetic differentiation among young populations of *G. monostachia* in early-successional forest patches ($F_{st} = 0.222$) was stronger than that among older populations in mid-successional forest patches ($F_{st} = 0.030$). Nevertheless, this considerable difference between the population age groups was only marginally significant ($P=0.054$). When subpopulations (individuals grouped in the same tree) were used as the sampling unit, the difference between $F_{st}$-values (young subpopulations = 0.181 and older subpopulations = 0.038) became statistically significant ($P=0.022$).

**Table 1.** Summary of genetic variation statistics of young and older populations of *Guzmania monostachia* in a premontane area, Costa Rica. Indicated are the number of sampled populations ($N$), percentage of polymorphic loci ($I_p$), mean number of alleles per locus ($A$), number of multilocus genotypes ($G$), diversity of multilocus genotypes ($H_g$), Nei's (1973) index of gene diversity ($H_s$), observed heterozygosity ($H_o$) and Wright's (1951) fixation index ($F_{it}$). Data are from six microsatellite loci.

<table>
<thead>
<tr>
<th>Population age condition</th>
<th>$N$</th>
<th>$I_p$</th>
<th>$A$</th>
<th>$G$</th>
<th>$H_g$</th>
<th>$H_s$</th>
<th>$H_o$</th>
<th>$F_{it}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>3</td>
<td>66.7</td>
<td>1.83</td>
<td>29</td>
<td>0.899</td>
<td>0.231</td>
<td>0.034</td>
<td>0.903</td>
</tr>
<tr>
<td>Older</td>
<td>3</td>
<td>66.7</td>
<td>1.83</td>
<td>25</td>
<td>0.882</td>
<td>0.252</td>
<td>0.021</td>
<td>0.944</td>
</tr>
</tbody>
</table>
Independently of their successional age, populations of *G. monostachia* exhibited significant levels of genetic differentiation (Table 3). In most of the paired comparisons (11/14) among populations (or forest patches), the $F_{st}$ values were significantly different from zero (Table 3) and ranged from low (5.5%) to high (34.2%) differentiation.

**Spatial genetic correlation among individuals**
The estimated coefficients of coancestry, $\rho_{ij}$, in *G. monostachia* suggest high and significant levels of genetic relatedness between pairs of individuals located in the same population (forest patch) (Fig. 3). The values of $\rho_{ij}$ for the first four distance intervals ranged from 0.117—0.152 and indicate high genetic similarity between individuals either from the same subpopulation (individuals in the same tree, distance interval of zero) or from different subpopulations within the same population (distance intervals ≤ 60 m) (Fig. 3). Negative and significant values of the autocorrelation coefficients, indicating very low genetic similarity, were detected for pairwise comparisons of individuals located in populations separated by distance intervals >400 m. Overall significance of the correlograms was detected for all loci separately and for the coefficients pooled across all loci (data not shown). These results suggest that gene flow among populations of *G. monostachia* is very restricted to the local geographic scale. A higher rate of gene flow is expected among subpopulations (trees) located within the same forest patch (< 60 m apart) than among subpopulations from different forest patches and separated by more than 400 m.
Table 2. F-statistics for two-level hierarchical analyses of genetic structure of *Guzmania monostachia* among subpopulations (groups of individuals on separate trees) within populations (forest patches) of different age in a premontane area, Costa Rica. A to C are “young” populations, occurring in early successional forest patches, D to F are “older” populations, occurring in mid-successional forest patches. Only polymorphic loci are shown.

<table>
<thead>
<tr>
<th>Population</th>
<th>e-19</th>
<th>e-6b</th>
<th>CT5</th>
<th>CA-21</th>
<th>All loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fis</td>
<td>0.804</td>
<td>1.000</td>
<td>0.857</td>
<td>0.867 ***</td>
</tr>
<tr>
<td></td>
<td>Fst</td>
<td>-0.039</td>
<td>0.070</td>
<td>-0.031</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>Fit</td>
<td>0.797</td>
<td>1.000</td>
<td>0.853</td>
<td>0.865 ***</td>
</tr>
<tr>
<td>B</td>
<td>Fis</td>
<td>0.931</td>
<td>0.847</td>
<td>0.936</td>
<td>0.962</td>
</tr>
<tr>
<td></td>
<td>Fst</td>
<td>-0.045</td>
<td>-0.010</td>
<td>-0.018</td>
<td>-0.013</td>
</tr>
<tr>
<td></td>
<td>Fit</td>
<td>0.927</td>
<td>0.845</td>
<td>0.935</td>
<td>0.963</td>
</tr>
<tr>
<td>C</td>
<td>Fis</td>
<td>0.789</td>
<td>0.951</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Fst</td>
<td>0.047</td>
<td>-0.047</td>
<td>0.064</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>Fit</td>
<td>0.799</td>
<td>0.949</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>D</td>
<td>Fis</td>
<td>0.624</td>
<td>1.000</td>
<td>0.942</td>
<td>0.929 ***</td>
</tr>
<tr>
<td></td>
<td>Fst</td>
<td>0.283</td>
<td>0.037</td>
<td>-0.031</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>Fit</td>
<td>0.730</td>
<td>1.000</td>
<td>0.940</td>
<td>0.932 ***</td>
</tr>
<tr>
<td>E</td>
<td>Fis</td>
<td>0.891</td>
<td>0.933</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Fst</td>
<td>-0.009</td>
<td>0.014</td>
<td>0.030</td>
<td>-0.019</td>
</tr>
<tr>
<td></td>
<td>Fit</td>
<td>0.890</td>
<td>0.934</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>F</td>
<td>Fis</td>
<td>1.000</td>
<td>0.897</td>
<td>0.946</td>
<td>0.953</td>
</tr>
<tr>
<td></td>
<td>Fst</td>
<td>-0.019</td>
<td>-0.030</td>
<td>0.066</td>
<td>-0.003</td>
</tr>
<tr>
<td></td>
<td>Fit</td>
<td>1.000</td>
<td>0.894</td>
<td>0.950</td>
<td>0.953</td>
</tr>
</tbody>
</table>

n.d.: allele fixed; *** P < 0.001, * P < 0.05, ns: P > 0.05
Table 3. Matrix of pairwise comparisons of genetic differentiation between populations of *Guzmania monostachia* in forest patches of early and mid-successional condition in a premontane area, Costa Rica. The $F_{st}$-values between population pairs are indicated. Significance was calculated after a Bonferroni correction ($P < 0.0033$) for multiple comparisons at a nominal level of 5%. ** $P < 0.01$, ns = not significant.

<table>
<thead>
<tr>
<th>Population</th>
<th>Early succession</th>
<th>Mid-succession</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>A</td>
<td>-</td>
<td>0.256**</td>
</tr>
<tr>
<td>B</td>
<td>0.256**</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>0.342**</td>
<td>0.103**</td>
</tr>
<tr>
<td>D</td>
<td>0.156**</td>
<td>0.036 ns</td>
</tr>
<tr>
<td>E</td>
<td>0.135**</td>
<td>0.056**</td>
</tr>
<tr>
<td>F</td>
<td>0.224**</td>
<td>0.056**</td>
</tr>
</tbody>
</table>

Figure 3. Correlogram of estimated coancestry values for pairwise comparisons of *Guzmania monostachia* plants at different distance intervals (see text for distance interval definitions) in a premontane area, Costa Rica. Solid squares indicate significant coancestry values ($P < 0.05$). The vertical dashed line at 60 m indicates the separation between distances among trees (subpopulations) within a forest patch (population) and among different forest patches.
Discussion

Low genetic variation in *Guzmania monostachia*
The low levels of genetic variation found in *G. monostachia* agree with reports for several colonizing terrestrial species (Brown & Marshall 1981). Young and older populations exhibited similar levels of allelic richness and in both cases the majority of analyzed individuals possessed most loci in homozygous condition as suggested by the very low $H_o$-values (Table 1). The general homozygosity of the most common multilocus genotypes further demonstrated such genetic homogeneity and suggests little sexual recombination between different genotypes and a preponderance of selfing, which is supported by the high values of the inbreeding coefficients ($F_is > 0.90$, Table 1) and the low rates of outcrossing ($t_m = 0.007$) for this species (Chapter 6).

Genetic homogeneity within forest patches
Subpopulations (trees) of *G. monostachia* within forest patches (populations) were not genetically differentiated from each other (Table 2), indicating that gene movement is not restricted among plant groups on trees separated by a maximum of 60 m in the same forest patch, either in young and older populations. The high levels of genetic similarity within subpopulations ($F_is = 0.867 – 0.957$, Table 2) suggests a common genetic origin of plants inhabiting the same tree. The absence of a clear genetic subdivision within forest patches can be accounted for when dispersal and reproductive factors are considered. Firstly, the high genetic similarity within subpopulations is likely the effect of most seeds being intercepted by adjacent branches or falling down in the direct vicinity of the reproductive plants in the absence of strong winds (ACM, pers. obs.). Several demographic studies have described the tendency of seedlings to establish in the vicinity of the mother plants (Zotz *et al.* 1999, Benzing 2000 and references therein, van Dunné 2001). After the initial colonization of a tree in a newly-created successional forest patch, the established population is likely to become the source of propagules for further colonization of nearby trees within the vegetation patch. However, seeds may also travel longer distances beyond the vegetation patch (i.e. during colonizing events or latter migration), which would explain the distribution of multilocus genotypes (Fig. 2). Indeed, we have found evidence for such long-distance dispersal events in a seed-trapping study in the same area (Chapter 4 & 5).

On the other hand, the mating system and pollination mode of *G. monostachia* contribute to its genetic homogeneity within forest patches. Since the studied species is highly selfing (Chapter 6), seeds have genotypes that are very similar or identical to the mother plant. Additionally, *G. monostachia* is visited by a territorial hummingbird, *Amazilia tzacatl* (Trochilidae); that forages on clumps of flowers on nearby plants (ACM, pers. obs.) and in

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the case of a successful cross-pollination event it is likely to involve mating among close relatives. This indicates that gene flow in *G. monostachia* within populations or forest patches is mostly mediated by dispersal of seeds of similar genotypes produced by selfing or biparental inbreeding. Since the most abundant genotypes at each population were composed by fully homozygous loci, it suggests that sexual recombination of alleles via pollination is very low.

The results from the spatial analysis of genetic similarity between individuals corroborate the previous assumption about the predominance of short-range dispersal of genetically similar seeds in *G. monostachia*. The analysis showed high and significant genetic correlations among individuals at distance intervals < 60 m (Fig. 2), which corresponds to individuals either in the same or between subpopulations (trees) located in the same forest patch. This coincides with the previous results of high genetic homogeneity within subpopulations (trees) as indicated by the very low *F*<sub>st</sub>-values (Table 2).

**Population age and levels of genetic differentiation**

Overall values of population genetic differentiation can be biased if populations have different ages. Recently established populations of colonizing species such as *G. monostachia* are likely the result of independent colonization events that increase genetic variation among new (young) populations (Whitlock & McCauley 1990). As metapopulations move toward the extinction-colonization equilibrium and most vacant patches are occupied, the migration rates increase homogenizing the initial genetic structure created by founder effects. In *Silene dioica* (Caryophyllaceae), a small colonizing herb native to island systems in Sweden, Giles and Goudet (1997) attributed the higher genetic variance among young populations with respect to populations of intermediate age to factors associated to colonization. In partial agreement with our expectations, young populations of *G. monostachia* exhibited higher levels of differentiation (*F*<sub>st</sub> = 0.222) than older populations (*F*<sub>st</sub> = 0.030), although this difference was marginally significant. The difference in the genetic variance between young and older populations became significant when in the analysis the group of individuals in the same tree (subpopulations) were considered as populations, which underscores the effect of the sampling unit in genetic structure studies. However, in our study, we prefer to consider the group of bromeliads on a tree as subpopulations, because of the high genetic similarity among groups within the same forest patch, and to consider each of them as populations would artificially increase the variance of the genetic differentiation measures.

Independently of their age, the populations of *G. monostachia* showed significant levels (*F*<sub>st</sub> = 0.123 ± 0.043) of genetic differentiation at a local scale (Table 3). This result is not unexpected, since other, mostly terrestrial, plant species with high amounts of inbreeding
also showed high levels of genetic variation among populations (Loveless & Hamrick 1984). Some pairwise comparisons between populations showed unexpected high levels of genetic differentiation ($F_{st} > 0.20$, Table 3) considering the short geographical separation among them (Fig. 1). One of the few reports in the literature on levels of population genetic differentiation in epiphytic bromeliads is that for *Tillandsia ionantha* and *T. recurvata* on a regional scale from Mexican populations (Soltis *et al.* 1987). Extremely high levels of subdivision ($F_{st} = 0.906$) were found for *T. recurvata* in apparent concordance with its supposedly high selfing, while the putative out-crosser *T. ionantha* had a much lower proportion of its genetic variation distributed among populations ($F_{st} = 0.043$) (Soltis *et al.* 1987). Apart from the mating system, population genetic differentiation is highly influenced by seed dispersal (Loveless & Hamrick 1984): small amounts of “long-distance” dispersal are expected to decrease the genetic structure among populations (Loveless & Hamrick 1984). However, this may not completely apply to wind-dispersed epiphytic bromeliads since the few data available indicate that dispersal is mostly restricted to within vegetation patches (García-Franco & Rico-Gray 1988, Chapter 4 & 5). The presumed importance of dispersal is corroborated by the spatial analysis, which shows that the relatedness between individuals decreased with distance between populations with significant negative $r_i$-values at distances greater than 400 m (Fig. 3).

To our knowledge, this study represents the first attempt to quantify the distribution of the genetic variation of an epiphytic vascular plant in relation to its colonizing behavior. The results strongly indicate that local genetic population structuring in *Guzmania monostachia* is the result of high selfing and limited seed dispersal. Vekemans and Hardy (2004) showed that stronger spatial genetic structure was generally observed in species that have high selfing rates, a breeding system that increases genetic drift and reduces the effective population size. However, in colonizing epiphytes such as *G. monostachia* the contribution of founder effects and genetic drift during colonizing events to the local distribution of genetic variation must also be taken into account. In highly selfing species, seed dispersal represents the major way of gene flow and in the case of wind-borne seeds the landscape heterogeneity and the prevalent wind currents greatly determine their movement. In the studied site, suitable habitats for *G. monostachia* are intermingled with pastures, agricultural fields and mature forest remnants, which represent a barrier to effective long distance dispersal, further increasing the genetic dissimilarity between individuals at distant successional forest patches. Our study thus also shows that habitat fragmentation will most likely have a strong impact on the genetic structure of tropical montane forest species, even for those with seeds that initially seemed to have a very effective long-distance dispersal mechanism.
Acknowledgments

We thank the staff of the Ecolodge San Luis and Research Station for their logistic support. Adan Fuentes and Cristian Lobo provided field assistance. Patrick Meirmans gave helpful advice regarding the spatial data analysis. This is a project funded by WOTRO, The Netherlands (Grant W85-34), in collaboration with the National Museum of Costa Rica.

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Summary

Epiphytes are hardly represented in the literature on genetic variation and its distribution in natural plant populations. This paper presents data on genetic variation of a well-known epiphytic bromeliad, *Tillandsia fasciculata* Sw. We used six microsatellite primers to study three populations in different successional forest habitats in a premontane area in Costa Rica. Overall values of allelic richness were \( L_p = 100, A = 4.33, H_g = 0.600, H_0 = 0.683, \) and \( H_s = 0.589 \). Estimates of genetic richness were lower in the mature forest population compared to populations in early and mid-successional forest stages. Microsatellite loci showed up to six alleles, but the number of allelic combinations was limited and suggests reduced gametic recombination. Overall, the number of multilocus genotypes was low \( (G = 14) \) and showed a non-random distribution among populations; two of them comprised 46 and 43 percent of all individuals. There was a significant excess of heterozygotes in the three populations \( (F_{is}-values = -0.492, -0.295 \) and -0.460, respectively\), which contrasted with the high selfing of the species. Overall genetic population differentiation was high \( (F_{st} = 0.247) \). However, populations in secondary forests had similar allelic frequencies while they significantly differed from the mature forest population \( (F_{st}-values = 0.366 \) and 0.268\). The distribution of the genetic variation in *T. fasciculata* may be explained by the combination of mating system, dispersal, polyploidy and events occurring during early colonization. High heterozygosity in *T. fasciculata* is probably maintained by fixed heterozygosity in a tetraploid genome whilst the high selfing rate may account for the reduced number of multilocus genotypes. Founder effects and further limited seed dispersal contributed to the differences in allele frequencies and distribution of multilocus genotypes among populations in secondary and mature forest.
Introduction

The development of molecular techniques has advanced the study of the genetic variation and its distribution in plant populations and they represent an important tool to obtain insights into the micro-evolutionary processes under natural conditions (Hamrick 1989). Specific levels of genetic differentiation among populations assayed with isozymes have been associated with particular combinations of life history traits (Loveless & Hamrick 1984, Hamrick & Godt 1989, 1996). For instance, high levels of genetic differentiation are related with annual and semelparous plants of early successional stages with an autogamous mating system and limited seed dispersal (Loveless & Hamrick 1984). These associations are based on plant species representing different life strategies; however, there was a notable absence of epiphytic species, as pointed out by Soltis and collaborators (1987). At present, the available information about genetic variation and population subdivision in epiphytic plants is insufficient: some papers are available for bromeliads (Soltis et al. 1987, González-Astorga et al. 2004), orchids (Ackerman & Ward 1999, Tremblay & Ackerman 2001) and ferns (Hooper & Haufler 1997).

Epiphyte vascular plants represent an important component of the diversity in tropical forests (Madison 1977, Kress 1986, Gentry & Dodson 1987, reviewed by Wolf & Flamenco-S. 2003). Most epiphytic species display different arrays of life history characteristics to survive the transient and ephemeral conditions of their habitat. Several attributes are aimed to ensure their reproductive success and further colonization of new and empty sites. Epiphytic orchids have developed pollination strategies to attract pollinators and avoid competition for them (Ackerman 1986). In contrast epiphytic bromeliads seem to rely on self-compatibility and self-pollination to assure reproduction without the necessity of pollen vectors (Bush & Beach 1986, Martinelli 1994, Chapter 6 & 7). The thousands of tiny dust seeds produced by orchids (Dressler 1990) and the small seeds with hairy appendages of many epiphytic bromeliads (Smith & Downs 1977) are adaptations for long distance dispersal by wind. Thus, it is expected that epiphytes showing particular trait combinations exhibit different levels of genetic differentiation among populations.

Genetic subdivision among plant populations is attributed solely to the degree of gene flow in the absence of natural selection forces and genetic drift (Wright 1951, Slatkin 1985a). Pollen and seed dispersal represent the main means through which plants can exchange genes among groups and hence these two processes greatly influence the levels of genetic population differentiation (Loveless & Hamrick 1984, Hamrick & Godt 1989, 1996). The degree of genetic variation and structuring will depend on the interplay between the reproductive system and seed dispersal as well as on the ploidy level of the species. In
species that colonize successional habitats the factors related to colonization such as founder effects and drift (Barrett & Shore 1989, Whitlock & McCauley 1990) play a significant role in the distribution of the genetic variation.

In this study, we examined the genetic variation and its distribution in an epiphytic bromeliad, Tillandsia fasciculata, among populations in forest habitats of different successional condition in a premontane area in Costa Rica. This is part of a larger project intended to determine the influence of life-history traits on the colonizing ability and genetic diversity of epiphytic bromeliads. The Bromeliaceae (pineapple family) constitutes an important group among vascular epiphytes in terms of their abundance and diversity (Benzing 2000). They also represent a valuable resource for indigenous cultures and for the international market of ornamentals (Bennett 2000), thus information about genetic variation in natural populations forms an important contribution to the development of conservation strategies and decisions on their sustainable harvesting and/or cultivation. The few genetic studies of epiphytic bromeliads have suggested a relation between the mating system and the degree of genetic heterogeneity among populations in Tillandsia ionantha and T. recurvata (Soltis et al. 1987). In T. achyrostachys, isolation due to habitat fragmentation was considered the main cause of population genetic subdivision (González-Astorga et al. 2004).

Tillandsia fasciculata is one of the most common epiphytes in mature forest patches around the study area. However, its abundance varies with the age of the forest, from less common in young vegetation to more frequent in mid-successional forest patches and occasionally dominant in mature forest, albeit at lower densities there (Chapter 2). Seedling establishment experiments have demonstrated its capability to survive in secondary as well as mature forests, so that the differences in abundance between habitats are best explained by dispersal limitation (Chapter 3). Estimates of the mating system based on molecular (microsatellite) markers indicated a very low rate of outcrossing ($t_m = 0.028$) and absence of apomixis (Chapter 6). Therefore, we expect a pronounced population genetic structure in T. fasciculata owing to limited dispersal and high inbreeding (cf. Loveless & Hamrick 1984).

The segregation pattern of microsatellite bands in T. fasciculata showed fixed heterozygosity in several loci (Chapter 6). This is a condition associated with many polyploid plant species, which possess multiple sets of homologous chromosomes. The latter have either originated by genome doubling (autopolyploidy) or are derived from different parental species after hybridization (allopolyploidy). If the plants have become homozygous for each genome owing to a long history of self-fertilization, and are heterozygous because they have a different allele at each genomic set, all gametes will have a copy of each allele and the
offspring, even if derived from selfing of a heterozygous genotype, will be identical to the mother (Roose & Gottlieb 1976). If *T. fasciculata* is indeed a polyploid species with fixed heterozygosity, then we would expect that the number of potential allelic combinations present in the progeny for a given locus is limited by the disomic inheritance, which reduces the degree of chromosome recombination. Contrary to the effect of fixed heterozygosity, in highly inbreeding species such as *T. fasciculata* it is likely a reduction in the diversity of heterozygote combinations in the offspring which may result in a low diversity of multilocus genotypes. The additional influence of limited seed dispersal is likely to create a non-random distribution of multilocus genotypes.

We addressed the following specific questions: (1) are the levels of genetic variation of *T. fasciculata* consequential with its breeding system?, (2) are the heterozygosity levels in *T. fasciculata* in accordance with the putative fixed heterozygosity?, (3) is there an association between the distribution of multilocus genotypes and the successional stage of the forest?, and (4) are populations in secondary forest genetically different from those in mature forest at a local scale?

**Materials and Methods**

**Study species and location**

The distribution of *Tillandsia fasciculata* Sw. (Bromeliaceae, Tillandsioideae) ranges from Southern Florida to Colombia and Brazil in South America and the Antilles (Morales 2003). Mean adult plants have rosettes with an average diameter of 31.2 ± 5.4 (SD) cm and leaf length of 22.9 ± 3.2 (SD) cm. The rosettes form a moderately sized water impoundment. The leaves are thick and densely covered by scale-trichomes. Adult shoots die after reproducing and are usually replaced by up to three vegetative ramets that develop from axillary buds. The tubular flowers, royal-purple coloured and 6.5 ± 2.0 cm (mean ± SD) in length, and with exerted anthers and stigma, suggest hummingbirds as potential pollinators.

The Upper San Luis River Valley is located on the Pacific slope of the Tilarán mountain range in the northwest of Costa Rica (latitude: 10° 17’ N, longitude: 84° 47’ W). The area is classified as humid premontane forest (bmh-P) according to Holdridge’s life zone system (Haber 2000) and borders the Monteverde Cloud Forest Preserve located on the near mountain ridges. The studied populations of *T. fasciculata* were located in the surroundings of the Ecolodge San Luis and Research Station (UGA) at 1000-1050 m, an area characterized by a heterogeneous mosaic of vegetation patches of different ages, shapes and sizes; randomly distributed in the landscape and interspersed with active pastures and mature forest fragments.
Population sampling
From August 2002 through March 2003, we sampled populations of *T. fasciculata* located in three forest habitats of different successional stages. Since we were interested in studying the genetic variation at a local scale, sampled populations were located within a 1.0 km² area and were separated by active pastures and forest patches of varying successional stages. The successional forests are easily distinguished by their physiognomy and tree species diversity: (1) the young successional forest area occupied an abandoned pasture (c. 3 ha) left to natural regeneration, with scattered trees < 5 m in height; (2) the mid-successional forest consisted of a vegetation patch (c. 2 ha) in a former shaded-coffee plantation, with a canopy of up to 22 m height and (3) the mature forest was a fragment (c. 10 ha) connected to other forest remnants in the area, with a developed understory and sub-canopy layer, where canopy trees reach up to 26 m. In the young vegetation patch, bromeliads were sampled over an area of about 1.0 ha due to the sparse distribution of trees and in the mid-successional and mature forest areas plant material was collected within an area c. 0.5-ha in size. Originally, sampling occurred in a spatially more explicit design, with the group of individuals growing on a tree and several of such trees per patch as subunits of sampling. The multilocus variation proved to be so low, however, that this subdivision yielded no additional information, because most, if not all, individuals on a tree were genetically identical. Hence, we omitted the sampling unit tree from the design.

Sampled bromeliads varied in size from 1.5 to 40.0 cm (as the maximum leaf length from the rosette base to the leaf tip). Small plants (<2.5 cm) were completely harvested, while one or two young leaves from the inner part of the rosette were collected from bigger plants. The leaf material was carefully washed with tap water and stored in plastic tubes (2 ml) containing a 6M Guanidine buffer solution (Guanidine thiocyanate, 50 mM Tris-HCl, 20 mM EDTA, pH 6.4) before sending them to the IBED laboratory (Universiteit van Amsterdam) where they were stored at 4 °C.

Laboratory procedure
Leaf samples were dried with tissue paper to remove the excess of buffer solution and cut into small and thin pieces before grinding with liquid nitrogen and sterile sand. About 170-200 mg was used for genomic DNA extraction using a CTAB extraction buffer following a slightly modified version of Doyle and Doyle’s protocol (1990). We used five microsatellite primers developed for this species: CT-5, e19, e6, e6b, and p2p19 (Boneh *et al.* 2003) and an additional primer, CA-21, which was also developed for this species but was not reported yet in Boneh *et al.* (2003). The loci amplified consist of di- and tri-nucleotide repeats: $GA_{25}$, $CT_{15}$, $CAA_{14}$, $CAA_{12}$, $GAA_{13}$, and $CAA_{16}$, respectively, with sizes from 151 bp to 204 bp. DNA material was amplified by using PCR-reactions and followed the general procedure described by Boneh *et al.* (2003). PCR-products were visualized on a 6.5% KB+
LiCor polyacrylamide denaturing gel on a LiCor 4200 © automatic sequencer. Between 0.5-0.7 µl of a mixture of 1/5 PCR-products and 4/5 loading dye (Deionised Formamide with Bromophenol-blue) was loaded into the gel.

**Analysis of genetic diversity**

Standard parameters of genetic variation were estimated for each population and the pooled data: percentage of polymorphic loci ($L_p$), mean number of alleles per locus ($A$), observed heterozygosity ($H_o$), Nei’s (1973) index of gene diversity averaged over loci ($H_s = 1 - \sum p_i^2$, where $p_i$ is the allele frequency), and Wright’s (1951) fixation index ($F_is$). The allelic frequencies for each population were calculated using the computer program FSTAT version 2.9.3 (Goudet 1995, 2001).

**Diversity of multilocus genotypes**

Single-locus genotypes were designated by letters representing the combination of bands amplified. The slowest migrating band received the letter ‘a’, the second slowest band ‘b’ and so on. Then, the number of multilocus genotypes ($G$) was computed and the frequency of each was calculated at each population and the total sample. The multilocus genotypic diversity ($H_g$) was estimated as $H_g = 1 - \sum g_i^2$, where $g_i$ is the frequency of the multilocus genotype $i$.

**Distribution of the genetic variation**

We used $F$-statistics (Weir & Cockerham 1984) to estimate the genetic variation within and among populations. $F_{is}$-values were estimated for each locus per population and their significance evaluated using $\chi^2 = F_{is}N (a -1)$ with df = $a (a-1)/2$, where $N$ is the total number of individuals sampled and $a$ is the number of alleles per locus (Li & Horvitz 1953). Genetic population differentiation ($F_{st}$) was calculated for the whole sample and between populations from different successional forest habitats using a pairwise comparison test (FSTAT; Goudet 1995, 2001). Tests of significance of the $F_{st}$-values were calculated by constructing a null distribution after 1000 permutations of multilocus genotypes between populations. A corrected p-value for multiple comparisons was obtained using a Bonferroni correction (nominal level $\alpha = 0.05$).

**Results**

**Genetic variation**

Populations of *T. fasciculata* displayed high levels of allelic richness, all three populations having high percentages (100%) of polymorphic microsatellite loci (Table 1). High levels
of heterozygosity ($H_o > 0.55$) were found in all populations. Microsatellite loci exhibited between two and six alleles per locus (Table 2 and Figs. 1 to 6), with an overall mean of $A = 4.33$. However, as expected, not all possible allelic combinations were observed (Table 3). In all cases, heterozygous individuals exhibited only two bands, and three loci displayed fixed heterozygosity: CT-5, e6b, and p2p19 (Fig. 2, 5 and 6).

### Table 1. Summary of genetic diversity in populations of *Tillandsia fasciculata* in successional premontane forests, Costa Rica. $N$ is the number of individuals analyzed. Estimates are presented for the proportion of polymorphic loci ($L_p$), mean number of alleles per locus ($A$), number of multilocus genotypes ($G$), multilocus genotypic diversity ($H_g$), observed heterozygosity ($H_o$), and Nei's (1973) index of gene diversity ($H_s$).

<table>
<thead>
<tr>
<th>Population</th>
<th>$N$</th>
<th>$L_p$</th>
<th>$A$</th>
<th>$G$</th>
<th>$H_g$</th>
<th>$H_o$</th>
<th>$H_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early succession</td>
<td>99</td>
<td>100.0</td>
<td>3.33</td>
<td>8</td>
<td>0.401</td>
<td>0.776</td>
<td>0.519</td>
</tr>
<tr>
<td>Mid-succession</td>
<td>106</td>
<td>100.0</td>
<td>3.33</td>
<td>4</td>
<td>0.447</td>
<td>0.731</td>
<td>0.563</td>
</tr>
<tr>
<td>Mature forest</td>
<td>141</td>
<td>100.0</td>
<td>3.50</td>
<td>6</td>
<td>0.330</td>
<td>0.583</td>
<td>0.399</td>
</tr>
<tr>
<td>Pooled</td>
<td>346</td>
<td>100.0</td>
<td>4.33</td>
<td>14</td>
<td>0.600</td>
<td>0.683</td>
<td>0.589</td>
</tr>
</tbody>
</table>

The allele frequencies of populations in early and mid-successional forest conditions were more similar between each other than compared to the mature forest population (Table 2). Private alleles (Slatkin 1985b) were found in all three populations and for all microsatellite loci, except CA-21 (Table 2). The populations in early and mid-successional forest habitats shared alleles for locus CT-5 (allele ‘a’), e6 (allele ‘d’), e6b (allele ‘b’), and p2p19 (alleles ‘d’ and ‘e’), which were absent from the mature forest. Conversely, in the mature forest population, alleles for locus CT-5 (allele ‘c’), e19 (allele ‘c’), e6 (allele ‘a’), e6b (alleles ‘a’ and ‘e’), and p2p19 (allele ‘b’) were absent from both early and mid-successional forest populations.
Table 2. Gene frequencies of six microsatellite loci in populations of the epiphytic bromeliad Tillandsia fasciculata in three successional forests in a premontane area, Costa Rica

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Early succession</th>
<th>Mid-succession</th>
<th>Mature forest</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-21</td>
<td>a</td>
<td>0.581</td>
<td>0.651</td>
<td>0.908</td>
<td>0.736</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.419</td>
<td>0.349</td>
<td>0.092</td>
<td>0.264</td>
</tr>
<tr>
<td>CT-5</td>
<td>a</td>
<td>0.404</td>
<td>0.344</td>
<td>0</td>
<td>0.221</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.414</td>
<td>0.349</td>
<td>0.078</td>
<td>0.257</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0</td>
<td>0</td>
<td>0.014</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>0.010</td>
<td>0.005</td>
<td>0.092</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>e</td>
<td>0.086</td>
<td>0.151</td>
<td>0.408</td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>0.086</td>
<td>0.151</td>
<td>0.408</td>
<td>0.237</td>
</tr>
<tr>
<td>e19</td>
<td>a</td>
<td>0.152</td>
<td>0.302</td>
<td>0.918</td>
<td>0.510</td>
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</tr>
<tr>
<td></td>
<td>c</td>
<td>0</td>
<td>0</td>
<td>0.068</td>
<td>0.027</td>
</tr>
<tr>
<td>e6</td>
<td>a</td>
<td>0</td>
<td>0</td>
<td>0.004</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>b</td>
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<td>0.005</td>
<td>0.106</td>
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<tr>
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<tr>
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<td>0.419</td>
<td>0.349</td>
<td>0</td>
<td>0.227</td>
</tr>
<tr>
<td>e6b</td>
<td>a</td>
<td>0</td>
<td>0</td>
<td>0.078</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>b</td>
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<td>0.344</td>
<td>0</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>0.419</td>
<td>0.354</td>
<td>0.078</td>
<td>0.260</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>0.081</td>
<td>0.151</td>
<td>0.408</td>
<td>0.236</td>
</tr>
<tr>
<td></td>
<td>e</td>
<td>0</td>
<td>0</td>
<td>0.028</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>0.081</td>
<td>0.151</td>
<td>0.408</td>
<td>0.236</td>
</tr>
<tr>
<td>p2p19</td>
<td>a</td>
<td>0.081</td>
<td>0.151</td>
<td>0.486</td>
<td>0.267</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0</td>
<td>0</td>
<td>0.078</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>c</td>
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<td>0.505</td>
<td>0.436</td>
<td>0.477</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>0.394</td>
<td>0.339</td>
<td>0</td>
<td>0.217</td>
</tr>
<tr>
<td></td>
<td>e</td>
<td>0.020</td>
<td>0.005</td>
<td>0</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Figure 1. Microsatellite locus CA-21 banding patterns in Tillandsia fasciculata. Letters designate two of the three individual phenotypes recorded. Phenotypes A and B were the most common, representing 47% and 52% of the total sample (N = 346). m = marker

Figure 2. Microsatellite locus CT-5 banding patterns in Tillandsia fasciculata. Letters designate three of the four individual phenotypes recorded. Phenotypes A and B were the most common, representing 47% and 44% of the total sample (N = 346)

Figure 3. Microsatellite locus e19 banding patterns in Tillandsia fasciculata. Letters designate two of the five individual phenotypes recorded. Phenotypes A and B were the most common, representing 48% and 46% of the total sample (N = 346). m = marker

Figure 4. Microsatellite locus e6 banding patterns in Tillandsia fasciculata. Letters designate three of the six individual phenotypes recorded. The most common phenotypes, A and B, represented 47% and 45% of the total sample (N = 346). m = marker

Figure 5. Microsatellite locus e6b banding pattern in Tillandsia fasciculata. Letters designate four of the six individual phenotypes recorded. Phenotypes A and B were the most common, representing 47% and 45% of the total sample (N = 346). m = marker

Figure 6. Microsatellite locus p2p19 banding patterns in Tillandsia fasciculata. Letters designate three of the six individual phenotypes recorded. Phenotypes A and B were the most common, representing 48% and 43% of the total sample (N = 346). m = marker
Multilocus genotypic diversity

Single-locus genotype frequencies were skewed toward a few abundant genotypes per locus (Table 3). The multilocus genotype diversities were similar between populations in young and mid-successional habitats ($H_g = 0.401$ and $H_g = 0.447$, respectively) and lower in the mature forest ($H_g = 0.330$). In total, 14 multilocus genotypes were recorded and the sampled individuals were over represented by genotype ‘1’ (161 out of a total of 346 assayed individuals) and genotype ‘2’ (147 out of 346) (Fig. 7). There was a clear non-random distribution of multilocus genotypes among populations (Fig. 8). In the mature forest, it was dominated by individuals of genotype 1 (114/146), whereas in the young and mid-successional populations genotype 2 was the most abundant (75/99 and 72/106, respectively). Interestingly, the latter multilocus genotype was absent from the mature forest sample, while the second most common multilocus genotype in that population (genotype 3) was absent from the other two populations (Fig. 8).

Table 3. Frequency of single-locus genotypes recorded for six microsatellite markers in *Tillandsia fasciculata* in a premontane area, Costa Rica. Letters designated the amplified bands (alleles) per locus and next to them (in parenthesis) the number of individuals.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Single locus phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>CA-21</td>
<td>aa (164)</td>
</tr>
<tr>
<td>CT-5</td>
<td>ef (164)</td>
</tr>
<tr>
<td>e6</td>
<td>cc (162)</td>
</tr>
<tr>
<td>e6b</td>
<td>df (163)</td>
</tr>
<tr>
<td>e19</td>
<td>aa (166)</td>
</tr>
<tr>
<td>p2p19</td>
<td>ac (157)</td>
</tr>
</tbody>
</table>

Figure 8. Distribution of multilocus genotypes among populations of Tillandsia fasciculata in successional premontane forests in Costa Rica. Six microsatellite loci: CA-21, CT-5, e19, e6, e6b and p2p19. Sample sizes are 99, 106, and 141 for population in early and mid-successional and mature forest habitats respectively.
Genetic variation within and among populations

*Tillandsia fasciculata* exhibited an excess of heterozygous individuals in each population, as indicated by the negative pooled values of the inbreeding coefficient ($F_{is}$) (Table 4). Within each population, all loci showed negative $F_{is}$-values, except for $e19$, which had a completely opposite pattern of heterozygote deficiency ($F_{is} = 1.000$) in the young and mid-successional populations, and a lower but still positive value in the mature forest population ($F_{is} = 0.022$). The $e6$ locus also showed a significant heterozygote deficiency in the mature forest population ($F_{is} = 0.173$). The chi-square tests indicated that the inbreeding coefficients were significantly different from zero for all loci in the young and mid-successional population, while for the mature forest population, tests for the loci CA-21 and $e19$ were not significant (Table 4).

Genetic differentiation among the studied populations of *T. fasciculata* was high ($F_{st} = 0.247$). The allele frequencies in Table 2 suggested that differentiation was more noticeable between the mature forest population and those in the young and mid-successional habitat. The pairwise tests of differentiation confirmed this observation. Populations in young and mid-successional forest habitat were not genetically differentiated from one another ($F_{st} = 0.014$, $P > 0.05$); however, each of them showed a significant differentiation with respect to the mature forest population ($F_{st} = 0.366$ and 0.268, respectively; $P<0.01$).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Early succession</th>
<th>Mid-succession</th>
<th>Mature forest</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-21</td>
<td>-0.678 ***</td>
<td>-0.533 ***</td>
<td>-0.098</td>
<td>-0.505</td>
</tr>
<tr>
<td>CT-5</td>
<td>-0.534 ***</td>
<td>-0.397 ***</td>
<td>-0.530 ***</td>
<td>-0.487</td>
</tr>
<tr>
<td>e19</td>
<td>1.000 ***</td>
<td>1.000 ***</td>
<td>0.022</td>
<td>0.772</td>
</tr>
<tr>
<td>e6</td>
<td>-0.699 ***</td>
<td>-0.512 ***</td>
<td>0.173 *</td>
<td>-0.433</td>
</tr>
<tr>
<td>e6b</td>
<td>-0.570 ***</td>
<td>-0.390 ***</td>
<td>-0.526 ***</td>
<td>-0.493</td>
</tr>
<tr>
<td>p2p19</td>
<td>-0.696 ***</td>
<td>-0.629 ***</td>
<td>-0.760 ***</td>
<td>-0.700</td>
</tr>
<tr>
<td>Pooled</td>
<td>-0.492</td>
<td>-0.295</td>
<td>-0.460</td>
<td>-0.411</td>
</tr>
</tbody>
</table>

Significance of p-values after a chi-square test: * $0.01 < P < 0.05$, *** $P < 0.001$
Discussion

Genetic variation
The present study is one of the first reports on genetic variation of an epiphytic plant using microsatellite markers. The previous reports in the literature of vascular epiphytes are based on isozymes (see: Soltis et al. 1987, Hooper & Haufler 1997, Ackerman & Ward 1999, Tremblay & Ackerman 2001, González-Astorga et al. 2004), thus a direct comparison is not appropriate. Comparing the results with those from a similar study on the epiphytic bromeliad Guzmania monostachia (Chapter 8), the high allelic richness found in T. fasciculata is striking, respect to the proportion of polymorphic loci ($L_p = 66.7\%$ vs. $100.0\%$), mean number of alleles per locus ($A = 1.83$ vs. $4.33$), gene diversity ($H_s = 0.271$ vs. $0.589$) and observed heterozygosity ($H_o = 0.028$ vs. $0.683$). Both species showed a mating system nearly indistinguishable from complete selfing (Chapter 6). The low genetic diversity in G. monostachia was mainly associated with its high inbreeding ($t_m = 0.007$). For T. fasciculata, however, the high levels of allelic richness reported here are not in accordance with its low rate of outcrossing ($t_m = 0.028$). Concomitantly, the maintenance of significant levels of heterozygosity in T. fasciculata, as indicated by the negative values of the inbreeding coefficients (Table 4), is not likely under the influence of normal sexual recombination.

An explanation of the high levels of heterozygosity in T. fasciculata is the fixed heterozygosity or duplicated loci discovered in several of the studied microsatellite markers during the analysis of its mating system (Chapter 6). Fixed heterozygosity, a condition associated with polyploidy, can maintain high levels of heterozygosity due to the preferential pairing of homologous chromosomes causing disomic inheritance during meiosis (Roose & Gottlieb 1976, Barrett & Shore 1989), by which the heterozygous condition of the mother is transferred to the offspring. The existence of polyploid species within the Bromeliaceae family is recognized, particularly in the subfamily Tillandsioideae: T. capillaris, T. tricholepis (Brown & Gilmartin 1989) and T. butzii (Brown & Palaci 1997). For T. fasciculata, chromosome counts have yielded variable results (Benzing 2000 and references therein). Its wide geographical distribution and the morphological variation of the species, which is represented by at least 10 recognized taxonomical varieties (Smith & Downs 1977), suggests that the species might have polyploid as well as diploid populations. In general, data from molecular analyses that supports polyploidy within the Bromeliaceae are surprisingly lacking.

Population genetic differentiation
Genetic heterogeneity among populations of vascular plants has been generally explained by the combination of particular life-history traits, from which life form and breeding
system is the most important and followed by the breeding system and dispersal mechanism (Hamrick & Godt 1996). *Tillandsia fasciculata* exhibited general levels of genetic differentiation ($F_{st} = 0.247$) that are below reported values for species sharing the same combination of traits: selfing and wind-dispersed perennial herbs (Hamrick & Godt 1996). For some epiphytic bromeliads, the mating system or indirectly the restricted gene flow is held responsible for the population genetic subdivision. In *T. recurvata* and *T. ionantha* the contrasting values of genetic differentiation in populations separated by 50 km ($F_{st} = 0.906$ and 0.043, respectively) were explained by the obligate selfing and outcrossing of the species, respectively (Soltis *et al.* 1987). In *T. achrostachys*, genetic subdivision ($F_{st} = 0.391$) among populations separated from 2.6 to 25.4 km was attributed to a reduction of gene flow by forest fragmentation (González-Astorga *et al.* 2004). Inbreeding, limited dispersal and factors related to colonization history as discussed below, best explain the local population genetic subdivision of *T. fasciculata*.

The extent of genetic differentiation between populations of *T. fasciculata* in secondary and mature forest was not consistent with the general pattern and unveils the potential bias when pooling data from populations with different histories of colonization. Populations in secondary forests (early and mid-succession) showed similar allele frequencies ($F_{st} = 0.014$) but when compared to the mature forest population, the differences were significantly larger ($F_{st} = 0.366$ and 0.268, respectively). Genetic differentiation among populations of terrestrial plants on a local scale has been attributed to natural selection operating through variation in abiotic conditions of the habitat or interactions with other plant species, predators or pathogens (Linhart & Grant 1996). Subtle selective differences among secondary and mature forest, e. g. related to microclimatic differences, might account for the observed differences in allele frequencies in *T. fasciculata*. This assumption needs to be properly tested by means of reciprocal transplantation experiments, though. As previously mentioned, genetic subdivision at different geographical scales has also been attributed to the breeding system and dispersal mode (Loveless & Hamrick 1984, Hamrick & Godt 1989, 1996). In *T. fasciculata*, events occurring during colonization may have left their imprints on the distribution of the genetic variation among populations in secondary and mature forest. In the terrestrial bromeliad *Aechmea magdalenae*, founder effects together with its patchy distribution are held responsible for the genetic heterogeneity among populations (Murawski & Hamrick 1990). The negative and positive values of the fixation indices for different loci within populations and variation for the same locus across populations (Table 4) have been related to departures from random mating but also to founder effects (Murawski & Hamrick 1990).
The small number of multilocus genotypes and uneven distribution found in *T. fasciculata* can be the result of colonization by a small number of individuals, followed by restricted gene flow and inbreeding, which tend to preserve the specific allelic combinations in each population (Barrett & Shore 1989). The low diversity of multilocus genotypes recorded in *T. fasciculata* \( H_g = 0.60, \ G = 14 \) is likely the result of continuous selfing following the foundation of the population by a few propagules. However, it might also mean that source populations have also low diversity as well. High inbreeding or low sexual recombination has probably reduced the number of multilocus genotype combinations in *T. fasciculata* (Table 3, Fig. 1 to 6). The population exhibited up to six alleles per microsatellite locus (Table 2), which were nevertheless only found in few of the many possible combinations.

The non-random distribution of multilocus genotypes in *T. fasciculata* (Fig. 8) emphasizes the limited dispersal at a local scale. The dominant genotypes in the secondary and mature forest populations differed for all six microsatellite loci analyzed, differences that can not be ascribed to mutation processes but suggest different sources of colonization and limited dispersal. For epiphytic bromeliads, the available evidence of seed dispersal range indicates that propagules movement is limited to within forest patches (García-Franco & Rico-Gray 1988, Chapter 4 & 5). Using seed trapping experiments in the study site, we found a low number of seeds of species from nearby secondary forests in traps located inside the mature forest (Chapter 4). Similarly to *G. monostachia*, the limited seed dispersal and high selfing rate in *T. fasciculata* (Chapter 4 & 6) are probably responsible for the genetic similarity of individuals on the same host tree (data not shown).

Tropical montane forest areas constitute an important habitat for many epiphyte species; however, it is an ecosystem under continuous reduction and fragmentation (Sánchez-Azofeifa *et al.* 2001). The heterogeneity of the landscape of forest patches in different successional stages and intermixed with areas strongly affected by human activities is likely to disrupt the natural levels of gene flow among populations of epiphytic plants. In that context, the successional processes associated with the colonization of secondary forest patches are expected to exert an additional effect on the extent and distribution of the genetic variation among populations. Factors related to the species’ ploidy level can increase or mitigate the effects of other life-history traits on the genetic variation.

Our study made clear that a good reference data set on genetic variation in bromeliads with different breeding systems is necessary to be able to interpret the outcome of the effects of habitat fragmentation on population differentiation, particularly when the breeding system and inheritance patterns of alleles are unknown. Inferring the breeding system from the flower morphology (González-Astorga *et al.* 2004) is apparently risky. Although *T.*
fasciculata shows all traits of a predominantly outcrossing species, having a large, colourful inflorescence, a long flowering time with few flowers open at any day, and flowers with a long corolla tube and exerted anthers and stigma, it is without doubt a predominantly selfing species in the study area (Chapter 6). This unexpected result also sheds another light on the classification of pollination syndromes in Tillandsia by Gardner (1986). Our field observations show, for example, that the exerted anthers and stigma of T. fasciculata, which suggest bird-mediated pollination already open and become receptive during night time. Moreover, they touch each other easily, which apparently leads to rapid autonomous self pollination (ACM pers. observ.). The normal behaviour of a self-compatible species, i.e. first exposing the stigma to cross-pollination and promoting delayed selfing by ensuring anther-stigma contact in a late stage of anthesis as mechanism of reproductive assurance, has apparently been reversed in this species. Perhaps the fixed heterozygosity of this polyploid species has effectively removed the disadvantages of inbreeding depression associated with high selfing rates.

Acknowledgments

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Chapter 10

Conclusions

Are epiphytic bromeliad communities seed dispersal limited?

There is a growing consensus that epiphytic vascular plant communities in secondary forests are different from those in nearby mature or old-growth forests (Dunn 2000, Barthlott et al. 2001, Krömer & Gradstein 2003, Chapter 2). The most relevant difference concerns the loss of diversity in the successional vegetation, which usually represents a subset of the mature or more stable communities in old-growth forests. Nonetheless, in some epiphyte groups the emergent community seems to differ in its composition from the mature forest community, as exemplified by the relative higher abundances and the presence of bromeliad species specific to the successional habitats (Dunn 2000, Barthlott et al. 2001, Chapter 2).

The composition of successional epiphyte communities of tropical forests has been mainly explained under the niche assembly perspective. Accordingly, the vegetation of secondary forests represents a less heterogeneous environment created by a homogeneous forest structure and lower diversity of trees (Brown & Lugo 1990). Consequently, the microclimatic conditions in the canopy are less diverse in comparison to the more heterogeneous biotic and abiotic conditions in old-growth forests. This variation in the availability and quality of substrates (host trees), as well as in light and humidity conditions that distinguish early successional forest from mature forest habitats, are held responsible for the composition of epiphytic communities (Barthlott et al. 2001, Krömer & Gradstein 2003).

Those studies are mainly correlative and descriptive and from their explanations alternative factors are missing that can help present a more complete picture, such as (1) dispersal: establishment can be hampered by an inefficient dispersal of propagules into the empty sites, even when the species are capable of dealing with the conditions in the successional habitats. Apart from abiotic factors controlling the movement of seeds, dispersability can be viewed as depending on the availability of sources in the surroundings, being the latter a combination of the presence of a species pool and the reproductive capacity of the species. Many important vascular epiphytes (orchids, bromeliads and ferns) have easily wind-dispersed propagules which are potentially able to travel long distances, giving
the perhaps wrong impression that dispersal is not a limiting factor during colonization—an assumption that needs to be proven; (2) time for colonization: old growth-forests have been there for probably several hundreds of years, thus increasing the probability of arrival of propagules from the surrounding sources and the establishment of a stable population. On the contrary, in many cases the relative short existence of the successional forests may have been not enough for achieving a successful establishment and constitution of a stable population. The latter is also affected by dispersal and is influenced by the long time some epiphyte species need to reach the reproductive stage.

The experimental approach utilized in this project provided evidence that seed dispersal limitation plays an important role in the development of epiphytic bromeliad communities in successional premontane forests. The contribution of life-history traits related to growth and reproduction also influenced that process and will be discussed later. The studied successional forest habitats exhibited the expected differences in physiognomy and tree species composition (Chapter 2).

Contrary to expectations derived from the niche-assembly hypothesis, we did not find differences in seed germination or seedling establishment after 2 y that could be associated to the particular habitat conditions or physiological and morphological traits of the studied species (Chapter 3). Complementary results from the seedling transplantation experiment (Chapter 5) supported the previous findings. Seedlings had the same survival chance and growth after 1 y, regardless of the forest type or the micro-climatic differences in canopy temperature and relative humidity. Interestingly, a species presumed to be adapted to water stress and high light exposure (*Tillandsia fasciculata*) performed better than the other species inside the forest as well as in forest edges and isolated trees (Chapter 5). The latter underscores the potential pitfall in predicting species composition based solely on physiological traits and suggests the need for more research as to the relation between the ecophysiological performance of the species and the successional forest stage.

The seeds of Tillandsioid species in the Bromeliaceae family are small and with a hairy appendage or coma (Smith & Downs 1974, 1977) that promotes air buoyancy and is likely to increase the distance traveled by the seeds. However, the results of several correlative studies and the seed dispersal experiments undertaken in this project suggested an opposite tendency. The spatial distribution of plants of different age (adults and seedlings) has been shown to exhibit an aggregated pattern within host trees, indicating short-distance range of seed dispersal, which causes preferential seedling recruitment in the vicinity of the mother plant, a consistent pattern in secondary and mature forest communities (Benzing 1978, Hietz & Hietz-Seifert 1995, Zotz 1997, Bader et al. 2000, Chapter 2). This happens when
most of the seeds fall from the ripe pods in the vicinity of the fruiting plant in the absence of strong winds.

Seed trapping experiments intended to measure the seed dispersal pattern in epiphytic bromeliads report a tendency of seeds to travel short distances mainly confined to vegetation patches (García-Franco & Rico-Gray 1988, Chapter 4 and 5). The frequent association between the presence of nearby reproductive plants and the number of dispersing seeds captured on traps (Chapter 4 and 5) is in accordance with the documented pattern of spatial distribution of seedlings and adult plants. Although long distance dispersal is also likely to occur at a local scale, the low amount of con-specific seeds arriving at a single point in time and space in the three-dimensional canopy (Chapter 5) is probably not enough to overcome the constrains related to attachment to the bark surface, germination and successful establishment. In most of the studied species, the number of seeds recorded in the majority of traps did not exceed five (Chapter 5) and the survival probability recorded from seed arrival to the 2 y seedling stage ranged from 3.8 – 5.7% in secondary and mature forests (Chapter 3). These results support the hypothesis that the amount of seeds dispersed and the probability of landing in a “safe site” represent a major bottleneck in the establishment of epiphytes (Hietz et al. 2002). We lack data on plant survival beyond the seedling stage; however, results from *Tillandsia* and *Catopsis* species in Mexico suggest that survival of epiphytic bromeliads older than 2 y is in general high: 0.90-0.96 (Hietz et al. 2002). An unexpected result that could not be explained was the lower density of adult bromeliad inside the forest compared with some successional habitats. A long-term monitoring project might shed some light on the factors affecting growth and reproduction of epiphytic bromeliads in forest interiors.

The differences in propagule availability are important in determining the dispersal success of the species and will be discussed next. An aspect of dispersal not explored in this thesis was the influence of local patterns of wind currents, the topography of the terrain and the distribution of the forest in the landscape. These variables are likely to determine specific patterns of dispersal in wind-borne propagules.

**Dispersal is also regulated by propagule availability**

The effectiveness of seed dispersal depends on the availability of propagule sources (species pool) in the area, as well as on the size of the reproductive population and the reproductive capacity of the species. Ackerman *et al.* (1986) demonstrated the importance of a seed source in the recruitment levels of the epiphytic orchid *Tolumnia variegata* in Puerto Rico.
by increasing the availability of seeds to be dispersed. In accordance with those results, the seed trapping experiments (Chapter 4 and 5) showed an association between the number of seeds caught in the traps and the abundance of adult plants in their vicinity. On a local scale, the additional influence of wind currents or the vegetation may limit dispersal even in the presence of a source as confirmed by the low number of seeds from the secondary forest species that landed on traps inside the nearby mature forest (Chapter 4).

The rate of population recruitment and the species’ colonizing ability is also dependent on the amount of seeds produced. The reproductive capacity of the species is affected by life-history traits expressed during the pre-dispersal phase. The differences recorded in life-history traits were highly correlated with the rate of seedling recruitment (Chapter 6). Species with a high reproductive capacity or number of seeds produced displayed a concomitant high number of seedlings in their population structure. The fast-growing Guzmania monostachia exhibited reproductive characteristics of a colonizing species in accordance with its status as the most abundant species in secondary forests (Chapter 2). Contrary to Tillandsia fasciculata, which showed low rates of recruitment, G. monostachia showed a reproductive behaviour that assures a steady and higher availability of propagules for colonization. The remarkable differences in growth rate, flowering and fruit maturation time accounted for most of the differences in seed production between the two species rather than differences in their breeding system, which in both cases showed high rates of inbreeding and autogamous seed production (Chapter 6). The results about the mating system add to the increasing evidence of self-compatibility and autogamous seed production in epiphytic bromeliads (Bush & Beach 1986, Martinelli 1994, Chapter 6 and 7).

Basic research on the reproductive biology and mating system of the species can provide important complementary information to understand the distribution and abundance of epiphytic species. Predictions about the mating system of a species based solely on floral morphology and flowering behaviour may be misleading. Most Tillandsioid species are presumed to be hummingbird- and bat-pollinated (Vogel 1969, Gardner 1986, Sazima et al. 1995). These pollination syndromes are expected to enhance outcrossing; however, the appropriate estimation of their mating system requires the use of molecular markers.

The genetic consequences of dispersal and reproduction mode of the studied bromeliads are discussed in the light of the population genetic variation and its distribution in the successional habitats.
The genetics of epiphytic bromeliads in successional forests

The studies on the genetic variation and distribution of epiphytic plants are not as abundant as in their terrestrial counterparts and there is a particular lack of information about the genetic constitution of the species during forest recovery. Therefore, this project presents novel information about the genetics of epiphytic plants in successional habitats.

The contrasting levels of genetic variation between species of seemingly different habitat preference regarding the forest successional stage were explained by their breeding system and ploidy level (Chapter 8 and 9). The most abundant species in secondary vegetation, *G. monostachia*, showed a low genetic diversity and high frequency of homozygous genotypes as expected for a colonizing species (Barrett & Shore 1989, Chapter 8). The latter correlated well with the very low rate of outcrossing detected for this species (Chapter 6). On the contrary, *T. fasciculata* the most common species in old-growth forest showed higher levels of genetic diversity and a higher frequency of heterozygous genotypes which did not coincide with its also low outcrossing rate (Chapter 9). The “fixed heterozygosity” mechanism detected in the latter species (Chapter 6), which is associated to polyploidy, and being the consequence of non-pairing of both parental chromosome sets, is probably maintaining the high levels of (‘pseudo’-) heterozygosity. *Tillandsia fasciculata* has a wide range of geographic distribution and a significant morphological variation represented by some 10 taxonomical varieties (Smith & Downs 1977). Thus, it is likely that this species is composed by a complex of populations with different ploidy levels across its geographic distribution. A study of the occurrence and distribution of polyploidy in the taxon is lacking, however.

The results of this thesis underscore the potential for significant genetic subdivision among local populations of epiphytic plants in successional habitats. Previous studies have reported varying degrees of population subdivision in epiphytic bromeliads in a regional scale where restrictions to gene flow are more likely to occur (i.e. Soltis *et al.* 1987, González-Astorga *et al.* 2004). The relatively high levels of population genetic differentiation recorded in this study were associated to the dispersal mode and mating system of the studied species (Chapter 8 and 9). The results also supported previous hypotheses about the effect of colonization-related events, founder effects and drift on the genetic structure of colonizing species.

In the literature of plants, two major life-history traits have been suggested to influence the distribution of genetic variation: breeding system and dispersal (Loveless & Hamrick 1984, Hamrick & Godt 1989, 1996). Accordingly, the high levels of population genetic
differentiation in the successional habitats were explained by the high selfing and limited seed dispersal range, factors that are likely to reduce gene exchange (Chapter 8 and 9). Genetically homogeneous progeny, produced by the interaction of self compatibility and a floral morphology that enhances autonomous pollination (Chapter 6), disperse in the vicinity of the mother plants creating a neighborhood that is likely to become genetically isolated from other bromeliad groups.

In agreement with the described scenario, the individuals in the same subpopulations (groups of plants inhabiting the same host tree) showed a high degree of genetic similarity. In *G. monostachia*, the degree of genetic relatedness between individuals was a function of the separation distance. Plants located in the same population or vegetation patch showed a higher coefficient of genetic relatedness but this decreased as the distance between individuals increased, especially when individuals were located in different populations or vegetation patches (Chapter 8).

Events related to colonization and founder effects augmented the genetic differences between recently established populations (Chapter 8). This is possibly associated with colonization by a few propagules and random demographic processes acting at each population. As time goes by and populations approach to an equilibrium stage, there is more chance for gene flow to reduce the differences among populations. The inclusion of populations in different stages of forest succession may introduce a bias in the general assessment of population genetic subdivision.

An implication of the genetic results is that populations of epiphytic bromeliads that extend over large areas of forest are probably composed of genetically discrete subpopulations. The well-documented aggregated distribution of epiphytic plants inside the forest canopy is likely to augment the genetic differentiation due to a lower gene flow among groups on separated host trees. For epiphytic bromeliads with a distribution range over large geographical areas, as in the case of the studied species: *Guzmania monostachia* and *Tillandsia fasciculata*, the level of genetic subdivision across their geographic distribution is expected to be significantly high, owing to restricted gene flow and the additive effect of population isolation caused by forest fragmentation.

**Conservation of epiphytic bromeliads in successional forests**

As mentioned earlier, the fate of epiphytic plants in secondary or successional tropical forests remains an open field for research and it might well represent an important topic
in the near future due to the increase in secondary forest areas. The results reported in this thesis emphasize the potential detrimental effect of forest fragmentation on the survival and genetics of local populations of epiphytic plants. The geographic isolation among remnant forests caused by the steady reduction of continuous forest stands or complete elimination of existing forest fragments is likely to increase the genetic separation between epiphyte populations by breaking the effective gene flow (i.e. González-Astorga et al. 2004). Stochastic processes may also reduce the population size of epiphytic plants and menace their survival in forest fragments while affecting the local gene pool. As a consequence of fragmentation, the probability of tree-falling increases as a result of an increment of wind currents (Lovejoy et al. 1986, Saunders et al. 1991), in addition to the effect of the frequent heavy rains and tropical storms. Epiphytes subpopulations on single trees that have fallen to the ground have little chance of survival (Matelson et al. 1993). Thus, considering the aggregated distribution of epiphytes in a few host trees, populations in small forest remnants are more prone to extinction because of stochastic effects.

From a conservation standpoint, the presence of a local source of propagules is important for the colonization of nearby successional habitats. The more distant a source is from the new empty habitat the lower the chance that colonization occurs in an immediate way due to factors associated to limited dispersal. Therefore, the current patterns of forest fragmentation may slow down or hamper the recovery of epiphytic populations in some successional habitats. As determined in this project, seed dispersal of epiphytic bromeliad rarely occurs at long distances and is influenced by the availability of propagules in the area and probably by the local pattern of wind currents. Moreover, the slow growth rates documented for several epiphyte species (Zotz 1995, Hietz et al. 2002, Chapter 6) augments the time needed for the establishment of a stable population in a successional forest. The practice of leaving some spare-trees as seed sources after logging may contribute to the processes of forest recovery (Wolf 2005). A more practical approach to the recovery of epiphytic populations in altered or successional habitat is to overcome the effect of limited dispersal in natural conditions by introducing fruiting individuals, following the results of Ackerman et al. (1996). By doing so, the genetic constitution of the new population might be manipulated to create a more diverse array by introducing plants from different populations while lessening the effect of founder events and drift on the genetic variation of the new population.
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**SUMMARY**

**Why study epiphytes?**
Epiphytic plants constitute an important element of tropical forests around the world and reach their highest diversity in the Neotropics. Some families of vascular epiphytes are restricted to the new tropics, such as bromeliads (members of the pineapple family) and cacti, while other groups with a worldwide distribution have experienced a burst of speciation on this continent, such as the orchids. The permanence of epiphyte populations in tropical forests is threatened by the continuous reduction and fragmentation of their natural habitat. Uncontrolled harvesting of species for commercial purposes may cause local extinctions. The recovery of plant communities has been well documented for ground-rooted species; however, the processes that regulate the recovery of epiphyte communities during forest regeneration are still elusive.

**Aims**
The main purpose of this dissertation was to investigate the community composition and population structure of epiphytic bromeliads (subfamily Tillandsioidae) in relation to differences in colonization ability, life history strategy and breeding system among local species. The project took place in a tropical premontane forest in the region of Monteverde, province of Puntarenas, Costa Rica (latitude: 10° 17′ 10″ N, longitude: 84° 47′ 40″ W, 1050 m a.s.l.). As a starting point, the composition of communities in secondary and mature forest was studied. Life-history traits related to seed dispersal, seed germination, plant growth and reproduction were examined in selected species with seemingly different habitat preferences in secondary and mature forest. An analysis of the breeding system, the genetic variation and its distribution among populations in different successional habitats was undertaken by using microsatellite primers especially designed for the targeted species.

**Theory**
The results are examined into the context of the current hypotheses about plant community assembly, which predict that differences in seed availability and dispersal are most important in determining the development and structuring of communities during secondary succession. The alternative view suggests that differences in the ability to exploit the conditions in a particular habitat and competitive exclusion interactions among species are more important in shaping the community composition. Both ideas are usually referred to as the ‘dispersal’ and ‘niche’ perspective, respectively. In epiphytic plants, some correlative studies have suggested that factors affecting dispersal are more important in determining the identity of
the species that constitute the communities in a local scale. The later assumption implies that most epiphytic species are ecological (near) equivalents, but this remains to be tested.

**Hypotheses**
Following the previous ideas, epiphytic species inhabiting early (*i.e.* pioneer species) and later stages of forest succession (*i.e.* late-successional species) are expected to exhibit differences in their ecology and breeding system according to the ‘niche assembly’ perspective. It is expected that colonizer or pioneer species are short-lived, light-demanding, and have a high demographic turnover. They most likely have a breeding system that promotes the rapid production of many propagules by autonomous means to colonize new empty sites, which involves self-compatibility and self-pollination mechanisms. As a consequence of this mating system, the levels of genetic variation will be lower in colonizer species due to the higher selfing rates, and a significant genetic differentiation among populations is likely, due to limited gene flow through means of pollen vectors. In comparison, late-successional species are expected to be long-lived, slow-growing, more competitive and shade-tolerant. They putatively have a breeding system that promotes outcrossing by pollinators and produce fewer, larger seeds.

On the other hand, the ‘dispersal assembly perspective’ model predicts that species occurring in dissimilar successional forest habitats do not significantly differ in their ecology. That is, they are ecologically (near) equivalents, and seed dispersal efficiency determines their presence or absence in a particular habitat. In this regard, the dispersal success of a species is under the control of factors related to the presence and magnitude of local seed sources, the dispersal mode and the species’ fecundity (which involves several factors, such as the rate of flowering, the pollination mode, breeding system, and flower and fruit production).

**Chapter 2. Community structure**
The differences in species composition, spatial distribution and population structure between epiphytic bromeliad communities in secondary and mature forests were examined in Chapter 2. In the less diverse secondary forests of early (8-12 y) and mid-successional (35-40 y) stages, the community was dominated by *Guzmania monostachia* and *Catopsis nutans*, which exhibited high rates of seedling recruitment. In the more diverse mature forest (> 100 y), *Tillandsia fasciculata* and *T. tricolor* were the dominant species, albeit that a low rate of seedling recruitment was recorded. Overall, the density of epiphytic bromeliads was higher in the secondary than in the mature forests. Bromeliads were aggregated in a few host trees in both forest conditions. Similarly, adults and juveniles were growing at similar heights and substrate types (*i.e.* trunk, primary-, secondary- and tertiary branches).
Chapter 3. Germination and establishment

In order to determine whether the observed differences in composition are established during early phases of colonization, the probability of establishment in secondary and mature forest was examined by carrying out an experiment on seed germination and early seedling survival (Chapter 3). The germination rate of seeds from four species that were artificially affixed to the bark of several host trees was similar among forests conditions and the species showed no preference for a specific habitat. Similarly, the survival probability after 2 y of germination was not affected by the forest type, except for *Werauhia gladioliflora* (survival) and *Catopsis nutans* (growth), for which values were higher in the early-successional habitat. Contrary to our expectations, there was no association between survival and specific ecophysiological (e.g. CAM vs. C3) adaptations of the species. The previous results were confirmed in a survival experiment with transplanted one-year old seedlings into three different forest habitats: forest interiors, edges and isolated trees (Chapter 5). These results suggest that the studied species are ecologically near-equivalent and that their seemingly contrasting habitat preferences are not (or at least not exclusively) the result of species-specific habitat requirements.

Chapters 4 & 5. Seed dispersal

Simultaneous studies on seed dispersal revealed that bromeliad seeds are mainly dispersed over a short distance range (Chapters 4 and 5). The proximity of dispersing plants was correlated with the amount of seeds found on traps located in the canopy of secondary and mature forest, demonstrating the importance of the presence of a nearby seed source for colonization. Long distance dispersal events were recorded, but in a very low frequency. Hence, it is likely that most seeds land in the vicinity of the mother plants, which is supported by the documented pattern of aggregation of seedlings near adult plants. The low number of seeds from secondary forest species caught inside the mature forest further suggests that seed movement between forest habitats is low. In all, the previous findings demonstrated that epiphytic bromeliads were capable of establishing in different successional habitats, but that their presence was likely dispersal-limited.

Chapters 6 & 7. Life history, mating system and pollination

The analysis of life-history traits in species with contrasting colonizing strategies showed that the dispersal efficiency and the colonizing ability can be influenced by differences in reproductive success (Chapter 6). The differences were associated with differences in the number of seeds available to colonize new sites. For the most common species of secondary forests, *G. monostachia*, a growth model estimated that it will on average take 6 y to reach the reproductive stage after germination, as compared to 13 y in *T. fasciculata*. Moreover, the reproductive period, from flowering to dispersal, was shorter in the former species (8-
9 versus 16-20 months) which assures a continuous reproduction and seed availability. The previous situation was enhanced by the faster growth of side-rosettes, which become reproductive in a shorter time (ca. 1 y) in *G. monostachia*. Additional differences in fruit set and seed production agreed with the higher rate of seedling recruitment recorded for *G. monostachia* in secondary forests.

The mating system analysis based on progeny arrays showed very low rates of outcrossing in both *G. monostachia* and *T. fasciculata* (Chapter 6). That result was not congruent with the flower morphology and flowering behaviour of the latter species, which have been interpreted as adaptations to promote cross-pollination. An additional species examined, *Werauhia gladioliflora*, showed high levels of seed production in bagged inflorescences, indicating a high ability for self-pollination, although it was shown to be visited by small bats during the night (Chapter 7). However, this species was absent from nearby successional forests where our experiments showed that it was capable of establishing. Again, this suggests that its occurrence is dispersal-limited.

**Chapters 8 & 9. Population genetic structure**

The low genetic variation and high homozygosity in populations of *G. monostachia* agreed quite well with its inbreeding condition (Chapter 8). However, in *T. fasciculata* the higher genetic variation and heterozygosity did not concur with its low rate of outcrossing (Chapter 9). We argued that fixed heterozygosity, a condition associated with the species’ polyploidy, is responsible for the maintenance of high levels of (“pseudo-“) heterozygosity. Although selfing normally results in homozygosity, the independent pairing of the chromosomes of the different constituting genomes of a polyploid species can result in heterozygosity that may become fixed in the population despite high selfing rates.

The degree of genetic differentiation among populations in different stages of forest succession was significant and it was ascribed to the high levels of selfing in combination with the limited seed dispersal, factors that highly reduce the genetic exchange among plant groups (Chapter 8 and 9). Additional factors, related to colonization and founder events, were also presumed to influence the genetic differentiation among populations. Young populations of *G. monostachia* in early successional vegetation patches had higher levels of differentiation than older populations in mid-successional vegetation patches, probably resulting from random processes associated with colonization (Chapter 8). Similarly, different *T. fasciculata* populations in secondary forests were more genetically similar between one another than when compared to the mature forest (Chapter 9).
Summary

Conclusions
This thesis has shown that epiphytic bromeliads have a limited seed dispersal range that place restrictions on the number of local habitats that can be colonized by the species. The dispersal limitation in this community was influenced by the spatial distribution of the local species pool, which is partially responsible for the heterogeneous seed rain recorded in the studied habitats, other factors being the effect of the prevailing wind currents and turbulence, which were not addressed in this study. In addition, the dispersal capacity of the species depends on the fecundity of the species through differences in flowering and fruit maturation. The latter also explain the observed rates of seedling recruitment, as exemplified by the comparison between Guzmania monostachia and Tillandsia fasciculata.

The studied bromeliad species did not exhibit exclusive abilities to establish in the habitat where they were preferentially occurring in the studied site. Instead, they were also able to germinate and survive to the seedling stage in habitats where they were scarce or absent. Altogether, these findings suggest that dispersal limitation, rather than niche differences, is the most important factor driving the composition of epiphytic bromeliad communities. Nevertheless, the relatively low abundance of epiphytic bromeliads in mature forest habitats in the studied site are not fully explained by these results and deserves further studies. Perhaps the mortality rates are higher during later stages of the plant life cycle or when the canopy closes during succession.

Regarding the breeding system and genetic variation, as expected, the most common species in early successional habitats - Guzmania monostachia - showed the reproductive characteristics associated with a pioneer species, with high selfing rates that account for the low genetic variation and significant genetic differentiation among populations in spatially separated forest patches. The higher genetic variation in the apparently late-successional species, Tillandsia fasciculata, was not associated with a higher outcrossing rate, but was rather the effect of fixed heterozygosity in this polyploid species combined with high selfing. The latter result challenges the common practice of deducing the mating system of a species from its floral morphology and flowering behaviour. As in the early successional species, T. fasciculata exhibited high genetic differentiation among populations in different successional forest patches, which emphasizes the limited gene flow via seed dispersal.
SAMENVATTING

Waarom onderzoek aan epifyten?
Epifyten bereiken hun hoogste diversiteit in tropisch Amerika, waar deze levensvorm een belangrijk bestanddeel van bossen vormt. Bromelia’s, cactussen en enkele andere families van vaatplanten komen uitsluitend in dit deel van de wereld als epifyt voor. Tegelijkertijd heeft de soortvorming in orchideeën hier een enorme vlucht genomen.

In heel de tropische zone worden epifytische vegetaties bedreigd door houtkap en fragmentatie van het bos. Daarnaast heeft plaatselijk een ongecontroleerde oogst van natuurlijke epifytenpopulaties voor verkoop als sierplanten tot uitsterven geleid. Het herstel van in de bodem wortelende vegetaties heeft de volle aandacht van natuurbeschermers en wetenschappers, maar er is nog weinig bekend over de processen die een rol spelen bij het herstel van de epifytische vegetatie in regenererende bossen.

Doel van dit proefschrift
Het hoofddoel van deze dissertatie is inzicht te verschaffen in the demografie en samenstelling van, respectievelijk, bromeliapopulaties en -gemeenschappen in relatie tot differentiatie in kolonisatiecapaciteit, levenscyclus en voortplantingssysteem van de verschillende soorten. Het onderzoek vond plaats in de tropisch bergbossen (800-1000 m) in de Monteverde-regio, Costa Rica (10° 17’ 10” N, 84° 47’ 40” W). Om te beginnen is de soortensamenstelling, ruimtelijke verdeling en populatiestructuur van bromelia-gemeenschappen in zowel onverstoorde bossen als secundaire bossen van verschillende leeftijd bepaald. Van enkele soorten bromelia’s met een duidelijk hogere abundantie in één van de bovenstaande bostypen is vervolgens de zaadverspreiding, zaadkieming, groei en de voortplanting onderzocht. De genetische variatie tussen populaties in bossen van verschillende leeftijd werd onderzocht met behulp van speciaal daartoe ontwikkelde microsatelliet primers.

Theorie
De resultaten zijn verzameld om theorieën te toetsen die betrekking hebben op de ontwikkeling van plantengemeenschappen. De nieuwste theorie voorspelt dat gedurende successie de structuur en samenstelling van plantengemeenschappen in hoge mate bepaald wordt door de beschikbaarheid en verspreiding van zaden. De meer klassieke hypotheses nemen aan dat de samenstelling vooral bepaald wordt door biotische en abiotische habitatverschillen, waarbij de ecologisch het best aan deze habitats aangepaste soorten andere verdringen. Deze verschillende uitgangspunten worden respectievelijk de ‘dispersal assembly’ en de ‘niche assembly’ theorie genoemd. Met betrekking tot epifytische planten suggereren verschillende
studies dat op lokaal niveau, binnen waardbomen en in bospercelen, de samenstelling van de
vegetaties op takken vooral bepaald wordt door de verspreiding, lokale beschikbaarheid en
soortensamenstelling van de zaadregen. Dit veronderstelt dat epifytische planten ecologisch
weinig van elkaar verschillen, een aannemer waarvoor het bewijs vooralsnog grotendeels
ontbreekt.

De ‘niche assembly’ theorie voorspelt dat de vroege kolonisten of pioniersoorten in
ecologie en voortplantingsstrategie zullen verschillen van soorten die deel uit maken
van oudere vegetaties. Pioniersoorten zullen naar verwachting afhankelijk zijn van licht,
droogtetolerant zijn, kort leven, snel groeien en bloeien en veel zaad produceren, terwijl de
soorten van latere successiestadia schaduwtoleranter, maar vochtafhankelijker zullen zijn,
langzaam groeien, en relatief minder vaak bloeien en zaad produceren. Zelfbestuiving, die
soorten voor hun zaadproductie onafhankelijk maakt van bloemzoekers, is waarschijnlijk
een belangrijke aanpassing van pioniersoorten. De laat-successionele soorten zijn naar
verwachting juist kruisbestuivers. Dat vergroot weliswaar het risico’s op een mislukte
zaadproductie, maar de kwaliteit van het zaad (en dus de concurrentiekracht) zal door de
geringere inteeltdepressie toenemen. Met betrekking tot de genetische structuur zullen de
pioniersoorten door de hogere zelfbestuiving minder variatie binnen populaties, en een
hogere differentiatie tussen populaties laten zien dan de laatkomende soorten.

Het ‘dispersal assembly perspective’ model veronderstelt dat epifytische pioniersoorten
in hun ecologie nauwelijks zullen verschillen van de laatkomende soorten in oudere bossen
(ecologische equivalentie), en dat de aanwezigheid van soorten in een bepaalde habitat voor
een groot deel bepaald wordt door de efficiëntie van de zaadverspreiding. Zaaddepositie
is afhankelijk van de afstand tot een zaadbron, het verspreidings-mechanisme en de
zaadproductie. De zaadproductie wordt daarbij mogelijk voor een belangrijk deel bepaald
door de snelheid waarmee individuen reproductief worden en het voortplantingssysteem.

Hoofdstuk 2. Structuur van de bromeliagemeenschap

In hoofdstuk 2 worden de bromeliavegetaties in bossen van verschillende leeftijd beschreven.
In het jongste (8-12 jaar) en oudere bos (35-40 jaar) waren *Guzmania monostachia* en
*Catopsis nutans* het meest algemeen, en vertoonden een hoge mate van kieming en vestiging.
In het meer boomsoortenrijke oude bos (> 100 jaar) werden voornamelijk *Tillandsia fasciculata* en *T. tricolor* aangetroffen, ofschoon het aantal zaailingen van beide soorten
gerings was. Het oude bos had ook een geringere populatiedichtheid en -grootte. In alle
bostypes bleken de bromelia’s niet ruimtelijk homgeeen verdeeld, maar geconcentreerd op
enkele waardbomen. Volwassen en jonge planten hadden dezelfde voorkeur voor bepaalde
posities binnen de boom (stam, primaire, secundaire en tertiaire takken).
Hoofdstuk 3. Kieming en vestiging

Met het onderzoek dat wordt gepresenteerd in hoofdstuk 3 is experimenteel nagegaan of bovengenoemde patronen in de bromeliagemeenschap reeds gedurende de eerste fase in de kolonisatie worden bepaald. Het kiemingspercentage van vastgeplakte zaden van vier soorten bromelia’s was gelijk in de diverse bostypes en ook habitatverschillen binnen takken hadden geen invloed op de kieming. De overlevingskans en groei van tweejarige zaailingen was evenmin afhankelijk van het bostype, behalve voor *Werauhia gladioliflora* (overleving) en *Catopsis nutans* (groei) welke hogere waarden lieten zien in het jongste bos. In tegenstelling tot de verwachting bestond er geen relatie tussen de overlevingskans van soorten en hun ecofysiologische aanpassingen (CAM- of C3-metabolisme). De voorgaande resultaten werden bevestigd in een ander overlevingsexperiment waarbij zaailingen werden overgeplaatst naar alleenstaande bomen, bosranden en in de kern van bosfragmenten (Hoofdstuk 5). Voorgaande resultaten suggereren dat de onderzochte bromeliasoorten ecologisch slechts weinig van elkaar verschillen en dat hun schijnbare voorkeur voor een bepaalde habitat niet uitsluitend kan worden toegeschreven aan specifieke aanpassingen aan die habitat.

Hoofdstuk 4 & 5. Zaadverspreiding

Tegelijkertijd heeft onderzoek aan de verspreiding van zaden aangetoond dat de meeste zaden slechts korte afstanden afleggen (Hoofdstuk 4 en 5). Het aantal zaden dat terecht komt op zaadvallen in de boomkroon van jonge en oude bossen was afhankelijk van de afstand tot zaadverspreidende planten. De verspreiding van zaden over grotere afstand werd zelden waargenomen en de meeste zaden leken in de nabijheid van de moederplant terecht te komen. Dit wordt bevestigd door het gegroepeerd verspreidingspatroon van zaailingen rondom moederplanten. Het geringe aantal zaden van soorten uit het jonge bos in het oude bos duidt eveneens op een beperkte verspreiding van zaden tussen de bosfragmenten. Het totale beeld van de voorafgaande waarnemingen is dat epifytische bromelia’s zich weliswaar goed kunnen vestigen in bosfragmenten van verschillende leeftijd, maar dat hun voorkomen in die bossen lijkt af te hangen van het lokale aanbod van zaden.

Hoofdstuk 6 & 7. Levenstrategie, voortplantingssysteem en bestuiving

Een analyse van de eigenschappen van soorten met uiteenlopende kolonisatie-strategieën (Hoofdstuk 6) suggereert dat de doelmatigheid van de zaadverspreiding en de kolonisatie beïnvloed worden door het succes van de voortplanting. Dit laatste kan in verband gebracht worden met verschillen in het aantal zaden dat vrije plaatsen kan koloniseren. Een groeimodel van de meest algemene soort van regenererende bos, *G. monostachia*, voorspelt dat bloeiende planten gemiddeld zes jaar oud zijn, in vergelijking tot 13 jaar voor *T. fasciculata*.

Samenvatting
Bovendien verliep de rijping van het zaad in de eerstgenoemde soort veel sneller, namelijk in slechts 8-9 in plaats van 16-20 maanden in *T. fasciculata*. De zaadproductie per tijdseenheid werd in *G. monostachia* ook bevorderd door sneller groeiende en al na ca. 1 jaar bloeiende zijrozetten.

Binnen *G. monostachia* en *T. fasciculata* bleek nauwelijks kruisbestuiving plaats te vinden (Hoofdstuk 6). Voor *T. fasciculata* was dit geheel onverwacht, aangezien de morfologie en het gedrag van de bloemen gericht lijken op het bevorderen van kruisbestuiving. Een andere soort, *Werauhia gladioliflora*, werd `s nachts bezocht door vleermuizen, maar produceerde ook veel zaden wanneer bloeitijden waren ingehuld om bloembezoek te voorkomen (Hoofdstuk 7). Toch was deze soort afwezig in nabijgelegen regenererend bos met een voor deze soort geschikte habitat, wat opnieuw suggereert dat het ontbreken van *Werauhia gladioliflora* in de zaadregen een rol speelde.

**Hoofdstuk 8 & 9. Populatiegenetische structuur**

De geringe genetische variatie binnen populaties en de hoge mate van homozygotie in *G. monostachia* kwamen veelal goed overeen met de eerder gevonden hoge mate van zelfbestuiving (Hoofdstuk 8). *T. fasciculata* liet echter een hogere genetische variatie en heterozygotie zien dan op grond van het waargenomen lage percentage kruisbestuiving verwacht kon worden (Hoofdstuk 9). Waarschijnlijk leidt polyploïdie in deze soort tot een fixatie van de heterozygote toestand (=pseudo-heterozygotie). In het algemeen leidt zelfbestuiving tot homozygotie, maar in polyploïde soorten kan de heterozygotie blijven voortbestaan doordat chromosomen van naast elkaar aanwezige genomen geen gemengde paren vormen.

Er was een significant verschil in de genetische variabiliteit in populaties tussen bosfragmenten van verschillende leeftijd. Dit schrijven we toe aan een combinatie van een hoge mate van zelfbestuiving en geringe zaadverspreiding. Beide factoren beperken de genetische uitwisseling tussen groepen planten (Hoofdstuk 8 en 9). De genetische differentiatie tussen populaties wordt bovendien waarschijnlijk beïnvloed door de aard van kolonisatie en vestiging. Jonge populaties van *G. monostachia* in vroege successiestadia van het bos vertoonden onderling meer genetische differentiatie dan oudere populaties in intermediaire stadia van de successie. Dit wordt waarschijnlijk veroorzaakt doordat de kolonisatie plaatsvindt door zaden uit verschillende, willekeurige bronpopulaties (Hoofdstuk 8). *T. fasciculata*-populaties in diverse secundaire bosfragmenten verschillen genetisch minder van elkaar dan van populaties in oude bossen (Hoofdstuk 9).
Conclusies
De resultaten van deze dissertatie laten zien dat het aantal geschikte habitats dat gekoloniseerd kan worden door epifytische bromelia’s ondanks hun lichte pluszaden beperkt wordt door hun geringe ruimtelijke verspreidingsvermogen. De zaadverspreiding werd beperkt door de ruimtelijke verdeling van de soorten ter plaatse, die deels ook de heterogene depositie van zaden in de onderzochte habitats verklaart. Andere factoren zijn de dominerende windrichting en turbulentie, die buiten het blikveld van deze studie vallen. De vergelijkende studie aan *Guzmania monostachia* en *Tillandsia fasciculata* liet zien dat de zaaddepositie bovendien afhangt van de hoeveelheid per tijdseenheid geproduceerd zaad, die was gerelateerd aan de tijd die nodig was om vanuit zaad of zijscheut uit te groeien tot een bloeiende plant en de rijpingstijd van de vruchten.

De bestudeerde bromeliasoorten konden zich ook buiten de habitat waar ze vooral werden waargenomen vestigen. Transplantatie-experimenten lieten zien dat op plaatsen waar de soorten van nature zeldzaam waren de kiemingspercentages en zaailingsterfte van vergelijkbaar waren aan de waarden op de plekken waar ze veel voorkwamen. Deze resultaten suggereren dat de (beperkende) zaadverspreiding de samenstelling van epifytengemeenschappen meer bepaalt dan verschillen in ecologie (niche-differentiatie). Desalniettemin kan het beperkte aanbod van zaden niet volledig het geringe aantal bromelia’s in het oudste bostype verklaren. De mogelijk hogere sterfte van volwassen planten in dit bostype verdient verdere aandacht.

Wat betreft de voortplanting had de dominante bromelia in de jongste bossen, *Guzmania monostachia*, genetische eigenschappen die karakteristiek zijn voor pioniersoorten met een hoge mate van zelfbevruchting. De genetische variatie binnen populaties was gering en de genetische differentiatie tussen populaties in verschillende bosfragmenten was aanzienlijk. De relatief hoge genetische variatie binnen *Tillandsia fasciculata*, een soort van oudere bossen, was niet het gevolg van een hogere mate van kruisbestuiving, maar wordt in deze polyploïde soort toegeschreven aan een combinatie van gefixeerd heterozygotie en zelfbestuiving. Omdat *T. fasciculata* wat betreft de morfologie en het gedrag van de bloemen een typische kruisbestuiver leek ondermijnt het resultaat van ons onderzoek de gebruikelijke praktijk om het voortplantingssyndroom van soorten rechtstreeks van dit soort uiterlijke kenmerken af te leiden. Evenals in de pioniersoort liet ook *T. fasciculata* een hoge mate van genetische differentiatie zien tussen populaties in bosfragmenten van verschillende ouderdom. Dit wijst op een geringe uitwisseling van genetisch materiaal via zaadverspreiding.
Porqué estudiar las epífitas?

Las plantas epífitas constituyen un elemento importante de los bosques tropicales alrededor del mundo, alcanzando su mayor diversidad en el Neotrópico. Algunas familias de epífitas vasculares están restringidas a los trópicos del Nuevo Mundo, tales como las bromelias (miembros de la familia de la piña) y los cactus; mientras que algunos grupos de distribución mundial han experimentando una explosión de especies en el continente americano, como son las orquídeas. Hoy en día, la permanencia de las comunidades de plantas epífitas en los bosques tropicales está siendo amenazada por la reducción y fragmentación de su hábitat natural. Las extracción no controlada de especies con propósitos comerciales puede causar extinciones locales. En general, la recuperación de las comunidades de plantas ha sido bien documentada para especies terrestre, no obstante, los procesos que regulan la recuperación de comunidades de epífitas durante la regeneración del bosque siguen poco conocidos.

Objetivo

El propósito principal de este proyecto de tesis fue la investigación de la composición de las comunidades y la estructura poblacional de bromelias epífitas de la sub-familia Tillandsioideae en relación con diferencias en su habilidad colonizadora, las estrategias de vida y el sistema reproductivo de las mismas en un contexto local. El proyecto se realizó en un bosque premontano tropical en la región de Monteverde, provincia de Puntarenas, Costa Rica (latitud: 10° 17’ 10” N, longitud: 84° 47’ 40” O, 1050 m). Como punto de partida, se determinó la composición de las comunidades de bromelias epífitas en bosques secundarios y primarios. Las características de la historia de vida de las especies relacionadas con la dispersión de semillas, la germinación de las mismas, el crecimiento y la reproducción fueron examinados en especies seleccionadas cuya preferencia por bosques secundarios y maduros mostraron diferencias aparentes. Se realizó un análisis del sistema reproductivo, la variación y distribución de la diversidad genética entre poblaciones en diferentes hábitats sucesionales, utilizando imprimadores de microsatélites diseñados especialmente para las especies de estudio.

Marco teórico

Los resultados son examinados en el contexto de las hipótesis actuales sobre la conformación de las comunidades de plantas, las cuales predicen que diferencias en la disponibilidad de semillas y su dispersión son importantes en la determinación de la composición y desarrollo de las comunidades vegetales durante la sucesión secundaria. La hipótesis
alternativa propone que las diferencias en la habilidad para explotar las condiciones de un hábitat particular y las interacciones de exclusión competitiva entre las especies son más importantes en la conformación de las comunidades. Ambas ideas son usualmente nombradas como la ‘perspectiva de dispersión’ y la ‘perspectiva de nichos’, respectivamente. En el caso de plantas epífitas, algunos estudios correlativos han sugerido que los factores que afectan la dispersión son más importantes en determinar la identidad de las especies que constituyen las comunidades en el nivel local. La anterior suposición implica que la mayoría de especies epifíticas son (casi) equivalentes ecológicos, sin embargo, éstas ideas siguen sin ser examinadas.

Hipótesis

Derivado de las ideas anteriores, y en concordancia con la ‘perspectiva de nichos’, se espera que las especies epífitas que habitan los estadios tempranos (i.e. especies pioneras) y los más avanzados de la sucesión del bosque (i.e. especies de sucesión tardía) exhiban diferencias en su ecología y sistema reproductivo. Las especies pioneras o colonizadores se espera que sean de vida corta, que requieran mayor luz y con una tasa mayor de recambio demográfico. Estas especies posiblemente tendrán un sistema reproductivo que facilite la reproducción rápida de muchos propágulos por medios autónomos, para la colonización de nuevos sitios vacíos, lo cual incluye mecanismos de auto-compatibilidad y auto-polinización. Como consecuencia de este sistema reproductivo, los niveles de variación genética serán más bajos en especies colonizadoras debido a una mayor tasa de auto-cruzamiento; además, es probable que exista una diferenciación genética significativa entre poblaciones debido a limitaciones en el flujo génico por medio de vectores de polen. En comparación, las especies de sucesión tardía se espera que tengan una longevidad mayor, que sean de crecimiento lento, más competitivas y tolerantes a las condiciones de sombra. Se presume que estas especies tendrán un sistema reproductivo que promueva el exo-cruzamiento por medio de polinizadores, produciendo pocas semillas de mayor tamaño.

Por el contrario, la ‘perspectiva de dispersión’ predice que las especies que habitan en bosque en estadios sucesionales diferentes no diferirán significativamente en su ecología. En otras palabras, las especies son (casi) equivalentes ecológicos, y que son las diferencias en la eficiencia de dispersión de sus propágulos lo que determina su presencia o ausencia en un hábitat particular. En este sentido, el éxito en la dispersión de una especie está determinado por factores relacionados con la presencia y magnitud de fuentes de semillas en los alrededores, el modo específico de dispersión y la fecundidad de la especie, la cual involucra a su vez la tasa de floración, el tipo de polinización, el sistema reproductivo y la producción de flores y frutos.
Capítulo 2. Estructura de las comunidades
Las diferencias en la composición de especies, la distribución espacial y la estructura poblacional entre comunidades de bromelias epífitas en bosque secundario y bosque maduro se examinaron en el capítulo 2. En los bosques de menor diversidad de bromelias, en estadios de sucesión secundaria temprana (8-12 años) e intermedia (35-50 años), la comunidad estuvo dominada por Guzmania monostachia y Catopsis nutan, las cuales mostraron tasas altas de reclutamiento de plántulas. En el bosque maduro (>100 años), más diverso en especies de bromelias, Tillandsia fasciculata y T. tricolor, fueron las especies dominantes aunque mostraron un tasa de reclutamiento bajo. En general, la densidad de bromelias epífitas fue mayor en bosques secundarios que en el bosque maduro. Las bromelias se encontraron agrupadas en unos pocos árboles hospederos en ambos tipos de bosque. Igualmente, los individuos juveniles y adultos estaban creciendo en alturas y tipos de sustratos similares (i.e. tallos o troncos, ramas primarias, secundarias y terciarias).

Capítulo 3. Germinación y establecimiento
Con el propósito de determinar si las diferencias en la composición de la comunidad son establecidas durante las fases tempranas de colonización, se examinó la probabilidad de establecimiento de plántulas en bosques secundarios y maduros por medio de un experimento de germinación de semillas y sobrevivencia (Cap. 3). La tasa de germinación de cuatro especies de bromelias, artificialmente adheridas a la corteza de varios árboles hospederos, fue similar entre los hábitat sucesionales estudiados y las especies no mostraron preferencia por un hábitat específico. Igualmente, la probabilidad de sobrevivencia después de 2 años de haber germinado no fue afectada por el tipo de bosque, excepto en Werauhia gladioliflora (sobrevivencia) y en Catopsis nutans (crecimiento), cuyos valores fueron mayores en el hábitat sucesional temprano. En contra de nuestras suposiciones, no se encontró una asociación entre el éxito de sobrevivencia y las adaptaciones eco-fisiológicas específicas de las especies (i.e. CAM vs. C3). Los resultados anteriores fueron confirmados en un experimento de sobrevivencia a lo largo de un año, de plántulas transplantadas a tres hábitat boscosos: interiores de bosque, bordes de bosque y árboles aislados en potreros (Cap. 5). Los resultados anteriores sugieren que las especies estudiadas son ecológicamente casi-equivalentes y que sus aparentes preferencias por hábitats diferentes no son, al menos exclusivamente, el resultado de requerimientos específicos de las mismas por un hábitat particular.

Capítulo 4 & 5. Dispersión de semillas
Estudios simultáneos sobre la dispersión de semillas revelaron que las semillas de bromelias son dispersadas principalmente a distancias cortas (Cap. 4 y 5). La cercanía de las plantas reproductoras estuvo correlacionada con la cantidad de semillas halladas en trampas.
colocadas en el dosel de bosques secundarios y maduros, demostrando la importancia de la presencia de fuentes cercanas de propágulos durante la colonización. Se registraron eventos de dispersión de semillas en distancias largas, pero en muy baja frecuencia. Por lo tanto, es probable que la mayoría de semillas aterricen en los alrededores de las plantas madres, lo cual es corroborado por el patrón documentado de agregación de plantas juveniles cerca de individuos adultos. El bajo número de semillas de las especies de bromelias de bosque secundario que se capturaron en el interior del bosque maduro sugiere, adicionalmente, que el movimiento de semillas entre hábitats boscosos es bajo. En conjunto los hallazgos anteriores demuestran que las bromelias epífitas fueron capaces de establecerse en diferentes hábitats sucesionales, pero que su presencia está probablemente limitada por dispersión.

Capítulo 6 & 7. Historia de vida, sistema reproductivo y polinización

El análisis de los rasgos de historia de vida en especies con estrategias de colonización diferentes demostró que la eficiencia en la dispersión y la habilidad colonizadora puede estar influenciada por diferencias en el éxito reproductivo (Cap. 6). Estas diferencias estuvieron asociadas con el número de semillas disponibles para colonizar sitios nuevos. Para la especie más común de bosque secundario, *G. monostachia*, el modelo de crecimiento estimó un promedio de 6 años, después de la germinación, para alcanzar el estado reproductivo, en comparación con 13 años en *T. fasciculata*. Además, el período reproductivo (desde la floración hasta la dispersión de las semillas) fue más corto en la primera especie: 8-9 versus 16-20 meses, respectivamente, lo cual le asegura una producción continua y mayor disponibilidad de semillas. La situación anterior se acrecenta debido al crecimiento más rápido de los rametes asexuales, los cuales se tornan reproductivos en un período más corto (ca. 1 año) en *G. monostachia*. Diferencias adicionales en la producción de frutos y semillas están en concordancia con la mayor tasa de reclutamiento de plántulas documentado para *G. monostachia* en bosques secundarios.

El análisis del sistema reproductivo con base en cohortes demostró muy bajas tasas de exo-cruzamiento en ambas especies: *G. monostachia* y *T. fasciculata* (Cap. 6). Este resultado no concuerda con la morfología floral y el comportamiento de floración de *T. fasciculata*, el cual ha sido interpretado como una adaptación para promover la polinización cruzada. Una especie adicional analizada, *Werauhia gladioliflora*, mostró niveles altos de producción de semillas en inflorescencias embolsadas, indicando una habilidad alta de auto-polinización, aunque se demostró que es visitada por pequeños murciélagos nocturnos (Cap. 7). Sin embargo, esta especie está ausente en los bosques sucesionales cercanos donde nuestros experimentos mostraron que era capaz de establecerse. De nuevo, esto sugiere que su presencia está limitada por dispersión.
Resumen

Capítulo 8 & 9. Estructura genética poblacional
La baja variación genética y la alta homocigosidad en las poblaciones de *G. monostachia* concuerdan bien con su condición endogámica (Cap. 8). Sin embargo, en *T. fasciculata* la mayor variación genética y heterocigosidad no concordaron con su baja tasa de exocruzamiento (Cap. 9). Consideramos que la heterocigosidad fija, una condición asociada con especies poliploides, es la responsable por el mantenimiento de niveles altos de (‘seudo’) heterocigosidad. Aunque el auto-cruzamiento normalmente resulta en homocigosidad, la unión independiente de los cromosomas de los diferentes genomas que constituyen una especie poliploide puede resultar en heterocigosidad que puede volverse fija en la población, a pesar de las altas tasas de auto-cruzamiento.

El grado de diferenciación genética entre poblaciones en diferentes estadios sucesionales del bosque fue significativo y se debió a los niveles altos de auto-cruzamiento en combinación con la dispersión limitada de semillas, factores que reducen grandemente el intercambio genético entre grupos de plantas (Cap. 8 & 9). Factores adicionales relacionados con colonización y eventos fundadores se presume que también influencian la diferenciación genética entre poblaciones de bromelias epífitas. Las poblaciones jóvenes de *G. monostachia* en parches de vegetación sucesional temprana tuvieron mayores niveles de diferenciación entre sí que poblaciones más viejas en parches sucesionales intermedios, probablemente como resultado de procesos aleatorios asociados con colonización (Cap. 8). De forma similar, las poblaciones de *T. fasciculata* en bosques secundarios estuvieron genéticamente más relacionadas que cuando se compararon con la población del bosque maduro (Cap. 9).

Conclusiones
Esta tesis ha demostrado que las bromelias epífitas tienen un ámbito limitado de dispersión de semillas que pone restricciones al número de hábitats locales que pueden ser colonizados por la especie. La limitación de dispersión en esta comunidad estuvo influenciada por la distribución espacial del ‘pool’ local de especies, el cual es parcialmente responsable por la heterogeneidad en la lluvia de semillas documentada en el sitio de estudio, siendo otros factores adicionales a considerar: el efecto local de las corrientes de viento y la turbulencia, las cuales no fueron contempladas aquí. Adicionalmente, la capacidad de dispersión de las especies depende de la fecundidad de las mismas, a través de diferencias en la floración y la maduración de frutos. Lo anterior también explica las tasas de reclutamiento de plántulas observadas, como se ejemplificó al comparar *Guzmania monostachia* y *Tillandsia fasciculata*.

Las especies de bromelias estudiadas no mostraron habilidades exclusivas para establecerse en el hábitat donde se encontraban creciendo en mayor abundancia en el sitio...
de estudio. En su lugar, las mismas especies fueron capaces de germinar y sobrevivir hasta el estadio de plántula en hábitats donde eran escasas o estaban ausentes. En conjunto, estos resultados sugieren que la limitación de dispersión, más que diferencias de nicho, es el factor más importante que determina la composición de las comunidades de bromelias epífitas. No obstante, la abundancia relativamente baja de bromelias epífitas en el interior del bosque maduro estudiado no se puede explicar por completo y merece estudios adicionales. Quizás, la mortalidad es mayor en estadios más avanzado del ciclo de vida de las plantas o cuando el dosel del bosque se cierra durante el proceso de sucesión.

Con respecto al sistema reproductivo y la variación genética, como se esperaba, la especie más común en hábitats sucesionales tempranos, *Guzmania monostachia*, mostró características reproductivas de una especie pionera con una tasa alta de auto-cruzamiento que explica la baja variabilidad genética y la diferenciación genética significativa entre poblaciones en parches de bosque separados espacialmente. La mayor variación genética en la especie aparentemente de estadios sucesionales tardíos, *Tillandsia fasciculata*, no estuvo asociada con una tasa mayor de exo-cruzamiento, sino que fue el efecto de heterocigosisidad fija en esta especie poliploide, en combinación con un auto-cruzamiento alto. Este resultado cuestiona la práctica común de deducir el sistema reproductivo de una especie a partir de su morfología floral y comportamiento de floración. De igual forma que la especie de sucesión temprana, *T. fasciculata*, mostró una diferenciación genética alta entre poblaciones en diferentes parches de bosques sucesionales, lo cual enfatiza el limitado flujo génico por medio de dispersión de semillas.
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APPENDIX

First plate

1. View of the canopy’s interior in a mid-successional forest patch
2. Flowering ramet of *Tillandsia fasciculata*
3. Flowers of *T. fasciculata* (left) and *G. monostachia* (right), scale=1 cm long
4. Flowering ramet of *Guzmania monostachia*
5. Flowering genet of *Tillandsia tricolor*
6. View of the Upper San Luis River Valley with the Monteverde Cloud Forest Preserve at the mountains in the background
7. Flowering ramet of *Werauhia gladioliflora*
8. The project’s headquarters: the ‘casita bromelias’ at the Ecolodge San Luis
9. One of the remnant trees from the former forest left alone in a pasture area
10. Flowering ramet of *Catopsis nutans*
11. A little wasp of *Eurytoma werauhia* (Eurytomidae) trapped in the sticky mucilage of a flower bud of *W. gladioliflora*. This new wasp species was discovered in this project and is a flower predator of that bromeliad
12. A general view of the San Luis Valley
13. Partial view of the Ecolodge San Luis property: buildings surrounded by early successional vegetation in first plane and the mountains of the Monververde Cloud Forest Preserve in the background
Second plate

1. A mixture of wind-dispersed seed of some genera of epiphytic bromeliads (subfamily Tillandsioideae): *Catopsis* (lower half), *Guzmania, Tillandsia,* and *Werauhia* (upper half)
2. Seeds of epiphytic bromeliads that landed on an unsuitable place for establishment: a fern’s leaf
3. A genet of *Catopsis nutans* dispersing seeds. Notice the seeds’ plume highlighted by the sun light and how several seeds were intercepted by a nearby branch
4. Seedlings (ca. 14 mo) of *Guzmania monostachia,* whose seeds were artificially affixed to the host-tree bark
5. A genet of *G. monostachia* dispersing seeds
6. An infructescence of *C. nutans* releasing seeds
7. A seed-trap located inside the forest canopy during the seed-dispersal experiment and at its left side a plastic case containing the dataloggers for the microclimatic variables measurement
8. A piece of carpet with transplanted seedlings (ca.1 y old) in the experiment of survival
9. A post-dispersal infructescence of *G. monostachia* showing those seedlings that failed to disperse
10. An emergent side-rosette from a *G. monostachia* genet
11. An emergent side-rosette from a *T. fasciculata* genet
12. A developing side-rosette from a *C. nutans* genet
13. Established seedlings (ca. 18 mo) of *Werauhia gladioliflora*